Effects of Oxygen and Turmeric on the Formation of Oxidative Aldehydes in Fresh-Pack Dill Pickles

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Hexanal, pentanal, and heptanal were found to be normal components of commercially processed, fresh-pack dill pickles. Hexanal and pentanal were present at concentrations severalfold higher than their odor detection thresholds. These aldehydes were also found in pickles that were packed in the laboratory under anaerobic conditions. Injection of oxygen into pickles prepared in anaerobic conditions resulted in the production of increasing the amounts of hexanal, heptanal, and pentanal. Hexanal, pentanal, (E)-2-hexenal, and heptanal levels were negatively correlated with the addition of turmeric in dill pickles with oxygen injected into the jar, indicating that the curcumin present in turmeric is an effective antioxidant in this product. At commercial coloring levels (250 mg/L), turmeric addition maintained aldehyde levels near the concentrations found in commercial fresh-pack pickles packaged in glass containers when oxygen was added in amounts comparable to that which would enter a plastic container during a 1-year storage period. Therefore, the addition of turmeric appears to be an effective approach to minimize the formation of oxidative off-flavors in pasteurized dill pickles that may result from the oxygen permeability of plastic containers.

KEYWORDS: Cucumis sativus; Curcuma longa; antioxidant; cucumber; acidified; anaerobic

INTRODUCTION

Shelf-stable pickles sold directly to consumers are packaged almost exclusively in glass jars up to 3.8 L (1 gal), which are pasteurized to ensure microbial stability. However, there is an increasing use of plastic packaging in the industry due to factors such as dealing with broken glass in the plant, reduced weight of plastic containers, and consumer preference. Currently, plastic containers are under development to withstand the pasteurization processes required to ensure preservation of fresh-pack pickles. The food service industry presently uses institutional-size plastic containers, including pouches, gallon jars, and pails, which cannot withstand pasteurization for the packaging of nonheated, pickled vegetable products. These products are preserved with high acetic acid concentrations, low pH (≤3.3), and food preservatives. However, the shelf life of products in plastic containers is shorter than that of products in glass containers. An important factor in reduced shelf life is thought to be migration of oxygen through the plastic containers and closures, which can oxidize unsaturated lipids to produce off-flavors. Although by no means the only possible mechanism for product deterioration, lipid oxidation is often a major factor in quality loss for these products.

Both enzymatic and nonenzymatic lipid oxidation can form lipid hydroperoxides, which then degrade into various compounds, including aldehydes (1). These aldehydes are normally volatile and responsible for oxidative off-flavors. As a result, it is common to analyze aldehydes, particularly hexanal, which are formed to evaluate the progress of oxidation in foods (2–5). Although only ~0.1–0.14% of the wet weight of cucumbers is composed of lipids (6), a large fraction of the fatty acids in the lipids are unsaturated and, thus, susceptible to lipid oxidation. Zhou et al. (7) have shown that several aldehydes, including hexanal, (E)-2-pentenal, (E)-2-hexenal, (E)-2-heptenal, and (E)-2-octenal, form nonenzymatically when slurries of fermented cucumbers are exposed to oxygen. The formation of these aldehydes was shown by sensory analysis to correlate with an increase in oxidized odor.

Extension of the shelf life of products packaged in plastic containers would be possible by use of more expensive packaging materials and closures that have very low oxygen permeability. However, use of antioxidants in the products could be a more economical approach if an acceptable and effective antioxidant could be found. Turmeric oleoresin, extracted from the rhizomes of Curcuma longa, is commonly used as a natural yellow colorant in pickled cucumber products. Curcumin is the major compound responsible for the yellow color of turmeric. Peret-Almeida et al. (8) have recently characterized the spectral characteristics of curcumin and related compounds, including the molar absorptivity at the visible absorption maximum of..

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each curcuminoid. In addition to its color, curcumin has also been shown to have high levels of antioxidant activity (9–11). Zhou et al. (12) showed that concentrations of turmeric oleoresin were less than that used for coloring purposes in cucumber pickle products were effective in inhibiting the formation of oxidative aldehydes in fermented cucumber slurries exposed to oxygen. In contrast, FD&C yellow no. 5, which is also used commercially as a yellow coloring agent for cucumber pickles, did not inhibit oxidation.

The objectives of this project were to identify and measure the aldehydes present in commercial, fresh-pack pickle products during their normal shelf life, to investigate the relationship between oxygen added experimentally to fresh-pack pickles and during their normal shelf life, to investigate the relationship of oxidative aldehydes in commercial, fresh-pack pickle products, and to determine if the aldehydes present in commercial, fresh-pack pickle products were within their normal commercial shelf life. Three jars from each case were analyzed.

**Materials and Methods**

**Commercial, Fresh-Pack, Dill Pickle Samples.** Fresh-pack (pasteurized, nonrefrigerated) dill pickles in glass jars were provided by four processors distributed across the United States and Mexico. Each processor provided a case of product from a single product code (size, brand). Four jars (or processors) were dispensed from each code. The turmeric oleoresin used had a curcumin content of 8.5%. The dill spice was used at the recommended concentration of 0.57 mL of spice oil/L of cover brine solution. Cucumbers were refrigerated until they were cut into 6 mm thick slices.

**Chemicals.** All aldehyde standards and other analytical grade chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI).

**Anaerobic Filling of Jars with Cucumbers and Cover Solution.** The anaerobic hood (Coy Laboratory Products, Grass Lake, MI) was filled with a 5% 

\[ \text{H}_2/10\% \text{ CO}_2/85\% \text{ N}_2 \]

anaerobic gas mixture. The oxygen concentration was maintained at <1 mg/L with a palladium catalyst that catalyzed removal of residual \text{O}_2 by reaction with \text{H}_2 to form water. A heater inside the hood was used to maintain the temperature at 30 °C. Materials were introduced into the hood in an antechamber, where a vacuum was created and then released two times with \text{N}_2 gas, followed by one exchange with the anaerobic gas mixture to remove most of the oxygen before containers were transferred into the hood. The following procedure was used to remove the oxygen in jars of cucumbers as they were filled and closed. Oxygen was removed from cover brine solution by deaerating the solution under vacuum in an FS60 sonicator (Fisher Scientific, Pittsburgh, PA) for 1.5 h. The deaerated solution was distributed into Erlenmeyer flasks (240 g/flask) and then transferred into an anaerobic hood along with a flask that contained a concentrated solution of sodium benzoate. Cucumber slices (240 g) were packed into 480 mL jars and then transferred into the anaerobic chamber.

The cover brine, sodium benzoate solution, and cucumber slices were held inside the hood for 3 h. A flask of 240 mL of brine was poured into each jar with a few milliliters of sodium benzoate solution. The jars were tightly closed inside the hood using either standard commercial lids or, for those containers that were to be injected with oxygen after filling, lids that had a flanged rubber septum inserted (Fisher).

**Addition of Oxygen to Jars of Preserved Cucumbers.** A known volume of oxygen at 23 °C and 1.003 atm was injected into the jars through a rubber septum fitted into the lids. To be sure pure oxygen was injected, the needle of a gastight syringe with the plunger down was inserted through the wall of Tygon plastic tubing as oxygen was flowing through the tubing. The syringe was filled with oxygen and then locked to prevent further gas exchange. The needle was removed from the Tygon tubing and inserted into the jar septum, the syringe was unlocked, and the oxygen was injected into the jar. The septum was then caulked to minimize further gas transfer.

**Measurement of Dissolved Oxygen.** Dissolved oxygen was measured immediately after a jar of stored cucumbers had been opened. Dissolved oxygen in the brine was measured using an Oxi330i oxygen meter equipped with a StirrOxG galvanic oxygen probe (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

**Sampling, Identification, and Measurement of Volatile Aldehydes.** Measurement of volatile components was carried out by modification of the procedure of Zhou et al. (7). Samples for volatile component analysis were prepared by transfer of the entire contents of a 480 mL jar of pickles into a Waring blender. The cucumber tissue and brine were homogenized for 7 s. Slurry (10 g) was transferred to a 25 mL fritless sparger (Angel Inc., Panorama City, CA) and spiked with 1 mg/L with a palladium catalyst that catalyzed removal of residual \text{O}_2 by reaction with \text{H}_2 to form water. A heater inside the hood was used to maintain the temperature at 30 °C. Volatiles were adsorbed on a Tenax trap (Supelco Inc., Bellefonte, PA) held at 40 °C during purging. The Tenax trap was dried for 3 min to remove trapped water, heated at 180 °C for 6 min with a helium flow rate of 4.0 mL/min to desorb trapped volatiles, and baked at 250 °C for 5 min to clean the trap for the next sample.

Desorbed volatile compounds were delivered without splitting through a 30 m × 0.25 m i.d., 0.25 mm film thickness, HP-5MS capillary column (Hewlett-Packard, Palo Alto, CA). The GC-MS system consisted of an HP 5890 II chromatograph with an HP 5972 mass selective detector (MSD, Hewlett-Packard). The oven temperature was held at −20 °C during the 4.5 min volatile component desorption period with a 4.0 mL/min helium flow rate. The oven temperature was programmed to increase from −20 to 140 °C at 10 °C/min with a 1 min hold at 140 °C. The oven temperature then increased from 140 to 220 °C at 40 °C/min with a final hold of 3.5 min. Helium carrier gas was used at a constant flow rate of 1.0 mL/min. MSD settings were as follows: MS interface and ionization source temperature, 280 °C; electronic ionization voltage, 70 eV; scanning mass range, 35–350 Da. The electron multiplier voltage was set 200 V above the voltage selected by the "Maximum Autotuning" procedure.

Identification of aldehydes was based upon preliminary identification using the NIST/EPA/NIH Mass Spectral Library (2002) with HP 5100B/CA ChemStation software (version B.00.00, Hewlett-Packard). Identification was confirmed by matching both retention times and fragmentation patterns with standard aldehydes.

Concentrations of aldehydes were calculated on the basis of peak areas relative to the peak area of the internal standard. Standard curves were prepared for each aldehyde [hexanal, pentanal, (E)-2-hexenal, and heptanal] by making known additions of three concentrations of each aldehyde to a baseline cucumber slurry and calculating the linear regression equation for each analyte. The three concentrations of each analyte were chosen to encompass the range of aldehyde levels present in all samples.

**Effect of Oxygen Addition on Aldehyde Formation in Acidified Cucumbers.** Jars of acidified cucumbers were prepared anaerobically. Equal weights of cucumber slices and brine were packed so that the equilibrated components contained 0.12% CaCl₂, 0.57% glacial acetic

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**Table 1. Age (Time after Packing) of Commercial Fresh-Pack Dill Pickles Analyzed by GC-MS**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Age of Early Storage Samples (days)</th>
<th>Age of Late Storage Samples (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>31</td>
<td>407</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>448</td>
</tr>
<tr>
<td>C</td>
<td>41</td>
<td>344</td>
</tr>
<tr>
<td>D</td>
<td>38</td>
<td>393</td>
</tr>
</tbody>
</table>
acid, 2% NaCl, and 1.75% high fructose corn syrup. Three milliliters of 1.92 M sodium benzoate solution was added to each jar so that the equilibrated concentration was 12 mM. This prevented microbial growth in the containers during the duration of the experiment. A control treatment without added oxygen plus treatments with 2.5, 5.0, and 10 mL of oxygen added per jar were prepared. In addition, one set of jars was filled outside the anaerobic hood with no effort to exclude air from the containers. None of the jars were pasteurized. The jars were stored at 30 °C. Three replicate jars of each treatment were analyzed for dissolved oxygen and volatile components 4 and 28 days after the jars had been filled.

**Effect of Turmeric Concentration on Formation of Aldehydes in Pasteurized Cucumbers.** Cucumber slices were packed aerobically in 480 mL jars and covered with a brine solution so that the components equilibrated at 0.12% CaCl₂, 0.57% glacial acetic acid, 2% NaCl, and 1.75% high fructose corn syrup, a standard amount of dill spice, and turmeric oleoresin levels to equilibrate at 0, 16, 40, 100, and 250 mg/L. These jars were filled with 230 g of cucumber slices and 230 g of cover brine to allow an additional 20 mL of headspace in the jar for injection of oxygen. Two additional treatments were prepared with the same headspace in the jars. One treatment had neither dill spice nor turmeric added, and the second treatment had 250 mg/L turmeric added, but no dill spice. The jars were closed with lids that had a rubber septum to allow for oxygen injection. Finally, two treatments were prepared with minimal headspace by filling the jars with 240 g of cucumber slices and 240 g of cover brine solution. To one of these minimal headspace treatments, neither dill spice nor turmeric was added to the cover solution. The other cover solution contained the standard amount of dill spice and 250 mg/L turmeric. No oxygen was added to these two treatments, so the jars were closed with lids that did not have a septum. All jars were pasteurized to a center temperature of 74 °C for 15 min. After cooling, 30 mL of O₂ gas was injected into all jars with a 20 mL headspace. This amount of oxygen was selected to be similar to the amount of oxygen estimated to enter a moderately permeable, plastic container, such as a poly(ethylene terephthalate) (PET) container used for acid or acidified foods, and closure during a 1 year storage period (John Tobias, personal communication). All treatments were stored at 30 °C. Triplicate jars of the nine total treatments were analyzed for dissolved oxygen and volatile aldehydes at each sampling time. Analyses were done at 2, 6, and 10 weeks after packing. The experiment was duplicated with two different lots of cucumbers.

**Statistical Analysis.** All statistical analysis was carried out using SAS (version 8, SAS Inc., Cary, NC). Significant differences in commercial samples were calculated using Tukey’s multiple-means comparison. The ANOVA procedure in SAS was used to determine differences among treatments packed in the laboratory. For anaerobic treatments with oxygen added, the general linear model procedure was used with one class variable (time) at two levels, 4 and 28 days, regressed over the continuous variable, oxygen. The interaction term was the test for homogeneity of slopes.

**RESULTS**

Three aldehydes (hexanal, heptanal, and pentanal) were detected and quantified in all commercial pickle samples. Total aldehyde, hexanal, and heptanal concentrations in the short-term storage samples of commercial dill pickles were significantly higher than concentrations in the long-term storage samples (p ≤ 0.01). However, pentanal levels were not significantly different between the two storage times. Mean values of aldehydes across brands are shown in Table 2. Mean dissolved oxygen levels were very low, at 0.44 ± 0.9 and 0.46 ± 0.9 mg/L for short- and long-term storage samples, respectively. The dissolved oxygen levels were not significantly different between storage times.

Hexanal, pentanal, (E)-2-hexanal, and heptanal were identified in all experimental pickle samples, including pasteurized and nonpasteurized samples, treatments with and without dill spice and turmeric oleoresins, and treatments with various levels of oxygen. All four of these aldehydes were also present in pickle samples that had been prepared and stored in an anaerobic hood to strictly exclude oxygen.

**Table 2. Aldehyde Concentrations in Commercial Fresh-Pack Dill Pickles after Short- and Long-Term Storage**

<table>
<thead>
<tr>
<th>compound</th>
<th>short-term storage (µg/L)</th>
<th>SD</th>
<th>long-term storage (µg/L)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>hexanal</td>
<td>146</td>
<td>37</td>
<td>75</td>
<td>34</td>
</tr>
<tr>
<td>heptanal</td>
<td>11</td>
<td>13</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>pentanal</td>
<td>149</td>
<td>37</td>
<td>125</td>
<td>48</td>
</tr>
<tr>
<td>combined</td>
<td>305</td>
<td>74</td>
<td>202</td>
<td>69</td>
</tr>
</tbody>
</table>

*Triplicate jars were analyzed from each of four processors.

**Table 3. Aldehyde Concentrations in Fresh-Pack Pickle Samples Closed in Normal Atmospheric Conditions with No Dill Spices or Turmeric Added**

<table>
<thead>
<tr>
<th>compound</th>
<th>4 days (µg/L)</th>
<th>SD (µg/L)</th>
<th>10 days (µg/L)</th>
<th>SD (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hexanal</td>
<td>379.4</td>
<td>48.7</td>
<td>647.8</td>
<td>141.3</td>
</tr>
<tr>
<td>heptanal</td>
<td>5</td>
<td>0.6</td>
<td>4.2</td>
<td>1.2</td>
</tr>
<tr>
<td>pentanal</td>
<td>1147.2</td>
<td>109.8</td>
<td>212.8</td>
<td>48.5</td>
</tr>
</tbody>
</table>

Figure 1. Effect of added oxygen on hexanal levels in anaerobically packed, fresh-pack pickles (480 mL containers) 4 and 28 days after packing.

Figure 2. Effect of added oxygen on heptanal levels in anaerobically packed, fresh-pack pickles (480 mL containers) 4 and 28 days after packing.
than at 4 days of storage. The concentrations of these aldehydes increased linearly with the amount of oxygen initially added to the jars. In contrast, the pentanal concentration declined severalfold between 4 and 28 days of storage in all treatments (Figure 3). Furthermore, after 28 days, the pentanal concentrations were not significantly different among treatments, showing no correlation with the amount of oxygen that had initially been injected (Figure 3). In these treatments, \((E)\)-2-hexenal was detected, but not quantified.

Four days after packing, the dissolved oxygen in the cover solution was positively correlated with the amount of oxygen that had initially been injected (Figure 4). However, oxygen concentrations after 28 days were not significantly different among treatments, regardless of the amount of oxygen initially added. The mean concentration of dissolved oxygen over all treatments after 28 days was 0.63 \((0.06 \text{ mg/L})\).

The effectiveness of turmeric as an antioxidant in fresh-pack dill pickles was evaluated by the addition of turmeric, along with a typical commercial dill spice formulation, to pasteurized pickles. Two replications of this experiment were performed using two different lots of cucumbers. There were no significant differences between the two replications. Treatments were prepared with multiple levels of turmeric oleoresin. The pasteurized pickles were put under oxidative stress by injection of oxygen into the containers to simulate the amount of oxygen that might migrate into a plastic jar during 1 year of storage. Formation of hexanal, pentanal, \((E)\)-2-hexenal, and heptanal decreased logarithmically as the turmeric concentration increased. Trends were the same for all aldehydes. Hexanal results are shown in Figure 5. This relationship between aldehyde concentration and turmeric concentration was maintained during the 10 week storage period. At the highest turmeric concentration, aldehyde levels did not increase during storage. Dissolved oxygen levels showed a trend opposite that of aldehyde concentrations, in that oxygen remaining in jars increased logarithmically as turmeric concentration increased (Figure 6). However, dissolved oxygen levels decreased during the 10 week storage period.

Comparisons of hexanal levels in the four additional turmeric treatments, along with the 0 and 250 mg/L turmeric treatments described above, are shown in Figure 7. Again, all aldehydes measured displayed the same trend. Because there were no significant differences over time within each treatment, the mean aldehyde concentrations of all jars analyzed during 10 weeks of storage are shown. The treatments that had both oxygen injected and turmeric present had aldehyde levels similar to those treatments which did not have injected oxygen, regardless of whether spices were present in the oxygen treatments. Treatments that had oxygen injected but did not contain turmeric had significantly higher aldehyde levels than treatments that contained turmeric or did not receive injected oxygen.

In these six treatments, similar to the treatments with various turmeric levels, dissolved oxygen levels showed trends opposite that of the aldehyde levels (Figure 8). Treatments that contained turmeric tended to have higher dissolved oxygen levels. Treatments that did not have oxygen injected had the lowest dissolved oxygen levels, followed by those which had oxygen injected but no turmeric present.

**DISCUSSION**

Commercial dill pickles are packed into glass containers, hermetically sealed, and pasteurized. Hexanal, heptanal, and pentanal appear to be normal components of commercially
produced fresh-pack dill pickles. These aldehydes were present in all samples analyzed early and late in their commercial shelf life and produced by different manufacturers in different areas of North America. Although hexanal has been found in fresh cucumber slurries (13), the other aldehydes were not found. Therefore, all or at least most of these aldehydes were likely formed in the jar by lipid oxidation utilizing the initial oxygen present in the fresh cucumbers (14), the cover solution, and the headspace of the jars. The heating that occurs in the pasteurization process is sufficient to inactivate cucumber lipoxygenase.

This means that these aldehydes were either formed enzymatically before or during heating or produced by nonenzymatic reactions early in the storage period.

The odor detection threshold for hexanal in water is between 4.5 and 30 µg/L, whereas the thresholds for heptanal and pentanal are 3 and 12 µg/L, respectively (15). In the short-term storage samples, the mean hexanal (146 µg/L) and pentanal (149 µg/L) concentrations were well above their threshold levels (Table 2). Because the short-term storage samples were at the beginning of their commercial shelf life when product quality would be at its highest, these aldehydes appear to be a part of the normal flavor volatiles of commercially produced fresh-pack dill pickles. Therefore, they cannot be considered off-flavor compounds. If they were to increase to substantially higher levels, these aldehydes presumably would be perceived as oxidized off-flavors.

In all of the experimental pickle samples analyzed, hexanal, pentanal, (E)-2-hexenal, and heptanal were found. These were different from the aldehydes that have been found in fermented cucumber slurries exposed to high oxygen levels (7). Pentanal and heptanal, found in the fresh-pack pickles, were not found in the fermented cucumber slurries. The unsaturated aldehydes 2-pentenal, 2-heptenal, and 2-octenal were found in the oxidized fermented cucumber slurries, but were not found in these fresh-pack pickles.

The four aldehydes quantified in this work, along with other aldehydes, have been identified and used as indicators of lipid oxidation in other foods. Pentanal and hexanal, along with 1-octen-3-ol, nonanal, and octanal, have been used to monitor lipid oxidation and warmed-over flavor in pork (16). Pentanal and hexanal have been used to test for lipid oxidation in milk and peanuts (17, 18). Hexanal, 2-hexenal, and 2-octenal were used to monitor lipid oxidation in dry fermented sausages (19). Heptanal and hexanal have been used as indicators of lipid oxidation in oil emulsions, salami, and whey protein concentrate (20–22).

Given the association of the aldehydes detected in acidified cucumbers with lipid oxidation, their occurrence in jars of pickles...
cucumber slices that were filled and stored anaerobically was unexpected. Especially surprising was that >700 µg/L pentanal was formed within 4 days of packing (Figure 3). Between 4 and 28 days of anaerobic storage, hexanal and heptanal concentrations did not change, but pentanal declined to <200 µg/L (Figures 1–3). Lipid oxidation as a result of residual oxygen in the acidified cucumbers was not considered to be possible because any oxygen that might have remained in the cucumber slices after the three vacuum exchanges with oxygen-free gases before they were transferred into the anaerobic hood would have been utilized by the respiration of the cucumber tissue within minutes (23). The present results do not exclude the possibility that aldehydes may have been present in the cucumbers prior to the removal of oxygen. However, Zhou and McFeeters (15) found hexanal, but not pentanal, (E)-2-hexenal, or heptanal, in fresh cucumber slurries. Formation of “oxidative aldehydes” anaerobically suggests a previously unrecognized mechanism for their synthesis. There have been no published reports of the changes in volatile components of processed fruit and vegetable products stored in anaerobic conditions. Mattheis et al. (24) analyzed the volatiles of fresh apples stored in 1.5% oxygen compared to 0.05% oxygen. A large amount of acetaldehyde was formed from ethanol that accumulated in the apple tissue. However, production of saturated aldehydes in the apples from hexanal (C6) to decanal (C10) declined to very low concentrations that would be typical of that used for yellow color in pickled cucumber products. Formation of “oxidative aldehydes” changed minimally (Figure 3). The fact that dissolved oxygen in the pickle brines increased with the concentration of added turmeric (Figure 6) suggested that it prevented the reaction of oxygen with the cucumber lipids. However, dissolved oxygen did decrease moderately during the 10 week storage period (Figure 6).

These results suggest that turmeric at reasonable use levels would be effective in preventing the formation of oxidative aldehydes in plastic containers that have substantial oxygen permeability. Because turmeric is an ingredient with a long history of use as a coloring agent in pickled cucumbers, it would be a compatible natural antioxidant for fresh-pack dill pickles.

LITERATURE CITED


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