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Rapid Softening of Acidified Peppers: Effect of Oxygen and Sulfite

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Evidence was found for two previously unreported, nonenzymatic reactions that affected texture retention in acidified red bell peppers. First, oxygen was found to cause rapid softening of the pepper tissue such that it lost at least 40% of the initial tissue firmness within 2 weeks after acidification. Second, sulfite added to the acidified peppers prevented the softening caused by oxygen. Combined addition of sulfite and calcium chloride resulted in better retention of tissue firmness during extended storage than the addition of either component by itself.

KEYWORDS: **Pepper; *Capsicum annum*; texture; firmness; benzoate**

INTRODUCTION

The factors that result in longer term firmness retention in acidified red bell peppers preserved without added NaCl have recently been investigated (*1*). In that study, sulfite, added as sodium metabisulfite, was used as a preservative to ensure the microbial stability of the stored peppers (*2*). Subsequently, efforts were made to replace sulfite with benzoate to prevent growth of microorganisms in the peppers. When this was done, it was observed that peppers preserved with benzoate softened rapidly compared to the peppers with added sulfite. This suggested either that benzoate accelerated pepper tissue softening or that sulfite inhibited softening. However, there is little in the literature to indicate textural effects on fruit or vegetable tissues by either compound. No published information related to textural effects by benzoate was found. Chassery and Gormley (*3*) found better firmness retention in potatoes treated with sulfite, but the effect was not attributed to the sulfite. Softening has been observed in strawberries stored in a sulfite-containing brine, but softening was attributed to pectolytic enzymes, not to the sulfite (*4*). Paterson et al. (*5*) found that sulfite could accelerate depolymerization of polysaccharides. However, polysaccharide depolymerization would be expected to accelerate rather that inhibit tissue softening. Therefore, the objective of this research was to determine if either preservative affected red pepper firmness during acidified storage and, if a textural effect occurred, to determine how it might occur.

MATERIALS AND METHODS

Pepper Preparation. Red bell peppers were purchased wholesale from a local produce supplier. Peppers were cut into pieces ∼2 cm square. The pieces were blanched for 3 min at 75 °C and then cooled in tap water for 3 min (*1*). In one experiment, pepper pieces were not blanched and blanched at 85 and 95 °C, in addition to the 75 °C standard blanch treatment. Red bell peppers were packed in 236 mL jars with 118 g of pepper pieces and 118 g of cover solution. The cover solution contained acetic acid from food grade vinegar (20% acetic acid) such that the equilibrated concentration was 150 mM. Additions of sodium benzoate (12 mM equilibrated), sodium metabisulfite (various amounts), and calcium chloride (10 mM equilibrated) were added as indicated. The jars were closed manually after the caps had been heated for at least 30 s at ≥90 °C. A slurry of equal weights of blanched red pepper and cover solution was titrated to determine the amount of 3 N hydrochloric acid required to adjust the pH to 3.5 in each jar. Peppers were stored at 30 °C either in an incubator or in an anaerobic hood with a heating unit to maintain that temperature.

Texture Measurement. The firmness of the pepper pieces was determined using a Stable Micro Systems TA-XT2 texture analyzer (Texture Technologies Corp., Hamilton, MA). The maximum force required for a flat, 3 mm diameter, stainless steel punch to punch a hole in the surface of a piece of pepper tissue placed skin down on a steel plate was measured. The crosshead speed was 48 mm/min. Tissue pieces were equilibrated to ambient temperature (22−24 °C) before texture measurements were done. Firmness values reported are the average of punch tests done on 15 tissue pieces from each container. Duplicate jars of each treatment were analyzed at each sampling time.

Oxygen Exclusion. To store peppers under strict anaerobic conditions (<1 ppm of O_2), 118 g amounts of blanched and cooled pepper pieces were weighed into 236-mL open jars. In the antechamber of the anaerobic hood, the air was removed and replaced with nitrogen six times to minimize oxygen that was in the jar or trapped in the pepper tissue prior to the jars being placed inside the hood. The jars of pepper pieces were then held in the hood (oxygen ≤1 ppm) for 1−2 h before the addition of cover solution. Cover solution was deaerated by sonication under vacuum, and then Erlenmeyer flasks containing 118 mL of the solution were placed in the anaerobic hood for 3−4 h before the solution was added to the peppers. Cover solution was added to the jars of peppers in the hood and closed. For jars inside the anaerobic hood, the lids were not heated. These jars were stored in the anaerobic hood.

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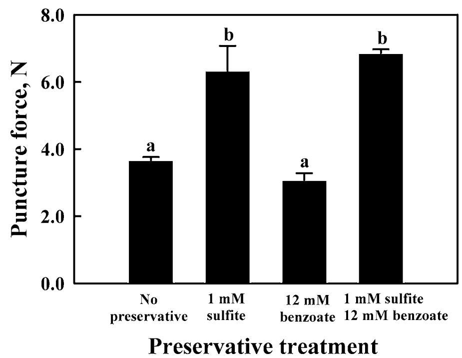

Figure 1. Firmness of red bell peppers stored for 1 week in 150 mM acetic acid, pH 3.5, with benzoate or sulfite added as indicated. Error bars show the standard deviation of the firmness for duplicate jars. Treatments with the same letter were not significantly different in firmness ($p \leq 0.05$).

Oxygen Addition. Jars of red pepper pieces with the oxygen excluded were prepared as described above. However, a hole was cut into each lid with a leather punch. A self-sealing rubber septum was inserted in the hole. Caulk was put around each septum to minimize the possibility that gas could enter or leave the jar. A 10-mL gas-lock syringe was inserted through the septum into the headspace of the jar. An amount of headspace anaerobic gas equal to the volume of oxygen to be added was withdrawn. A second gas-lock syringe was filled with the intended volume of oxygen and the oxygen injected into the jar. Caulk was put on the sealed syringe holes.

Statistical Analysis. Statistical analyses were done using the GLM and ANOVA procedures of SAS (SAS Inc., Cary, NC). Comparisons of treatment means were done by a one-way analysis of variance and the use of the Duncan multiple-means comparison. Texture changes as a function of multiple treatment levels or time were fitted with the linear regression or hyperbolic regression functions of SigmaPlot 8.0 (SPSS Science, Chicago, IL).

RESULTS AND DISCUSSION

Addition of either sulfite or benzoate was required for extended microbial stability of the red pepper pieces at pH 3.5. Microbial stability was assessed visually by the absence of turbidity in the cover solutions of the stored jars and by the absence of gas pressure on the jar lids. Blanched pepper pieces, however, showed no evidence of microbial growth at pH 3.5 for ∼10−14 days when neither preservative was added. This made it possible to carry out storage experiments for 1 week with a control that contained neither benzoate nor sulfite. **Figure 1** shows that blanched pieces of red pepper stored at pH 3.5 in 150 mM acetic acid for only 1 week had softened from 7 N for the fresh peppers to <4 N. If 12 mM benzoate, which was sufficient preservative to prevent microbial growth for extended periods of time, was added to the peppers, a similar loss of firmness was observed. Addition of 1 mM sulfite to the peppers resulted in retention of texture close to that of the fresh peppers. This experiment showed that benzoate had no effect on the firmness of the pepper pieces relative to a control without added benzoate. In contrast, addition of only 1 mM sulfite prevented firmness loss during a 1-week storage relative to peppers without added sulfite ($p \leq 0.05$) at 30 °C.

To determine if the prevention of softening by sulfite addition was concentration-dependent, sulfite was added to blanched red bell pepper pieces at concentrations up to 3 mM (**Figure 2**). Firmness measurements made after 1 week and 1 month of storage at 30 °C showed that firmness retention improved hyperbolically as the sulfite concentration added to the peppers increased ($p \leq 0.0001$).

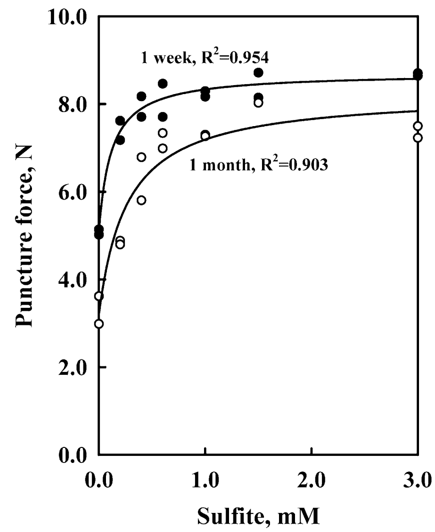

Figure 2. Inhibition of red bell pepper softening by sulfite. Peppers were stored in 150 mM acetic acid and 12 mM sodium benzoate, pH 3.5.

Table 1. Effect of Blanch Temperature on Firmness Loss in Red Peppers without Added Sulfite Compared to the Addition of 2.3 mM Sulfite[a]

	firmness (N)				
blanch temp (°C)	2.3 mM sulfite added	SD[b]	no sulfite added	SD	firmness loss[c] (%)
no blanch	6.83**	0.83	4.42	0.24	35.3
75	7.05**	0.02	3.39	0.09	51.9
85	6.87**	0.18	3.73	0.13	45.7
95	5.39*	0.38	2.71	0.24	49.6

[a] Peppers were stored for 2 weeks at 30 °C. One asterisk (*) indicates that the peppers with added sulfite were firmer than peppers without added sulfite at that blanch temperature at the $p \leq 0.05$ level. Two asterisks (**) indicate that the peppers with added sulfite were firmer than peppers without added sulfite at that blanch temperature at the $p \leq 0.01$ level. [b] Standard deviation. [c] Firmness loss is the firmness of peppers without sulfite divided by the pepper firmness with sulfite added × 100.

The softening of the pepper tissue and the inhibition of softening by sulfite occurring in blanched tissue suggested that both reactions were nonenzymatic. The data in **Table 1** show that in nonblanched pepper tissue and pepper pieces blanched at 75, 85, and 95 °C, peppers without added sulfite were significantly softer than peppers with sulfite added. The firmness loss relative to peppers with added sulfite was similar for all four treatments. This result was consistent with the hypothesis that softening was caused by a nonenzymatic mechanism and that sulfite inhibited the softening reaction nonenzymatically.

Calcium had previously been found to inhibit softening of red peppers (*1*). Therefore, it was of interest to compare the effect of calcium and sulfite on firmness retention. **Figure 3** shows that both added calcium chloride and sulfite significantly reduced softening of red pepper pieces during 3 months of storage. A combination of added calcium plus sulfite prevented softening to a greater extent than did the addition of either component by itself. This result suggested that calcium and sulfite inhibit softening by different mechanisms that to some degree are additive.

Softening of red peppers in low-pH conditions without sulfite was much more rapid than had been previously observed in cucumbers. The possibility that oxygen might be involved in pepper softening was suggested by an observation that pepper

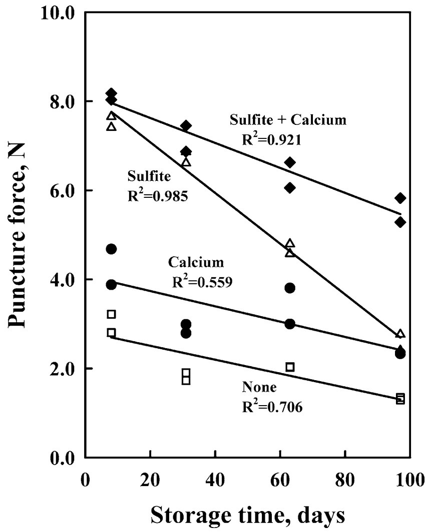

Figure 3. Effect of sulfite (1.0 mM) and calcium (10 mM) on the retention of firmness of red bell peppers stored for 3 months in 150 mM acetic acid and 12 mM sodium benzoate, pH 3.5. All treatments were different from the other treatments at the $p \leq 0.01$ significance level.

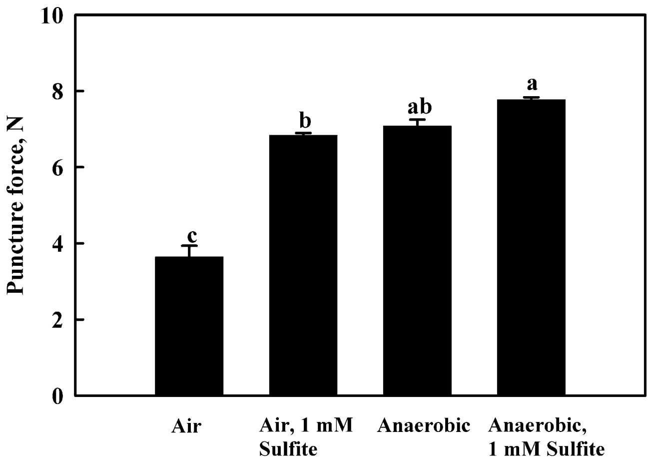

Figure 4. Comparison of aerobic and anaerobic storage on the firmness of red bell peppers stored for 2 weeks. Peppers were stored in 150 mM acetic acid and 12 mM sodium benzoate, pH 3.5. Error bars show the standard deviation of the firmness for duplicate jars. Treatments with the same letter were not significantly different in firmness ($p \leq 0.01$).

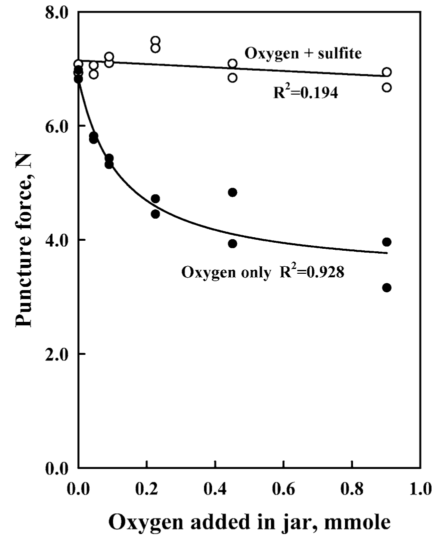

Figure 5. Effect of the addition of oxygen only (●) or oxygen + 2.3 mM sulfite (○) on the firmness stored red bell peppers. Jars of peppers were filled in an anaerobic hood, oxygen and sulfite were injected as shown, and the peppers were stored for 3 weeks in 150 mM acetic acid and 12 mM sodium benzoate, pH 3.5.

pieces near the headspace of jars tended to be softer than pieces near the bottom of a jar. To test the hypothesis that oxygen was responsible for rapid softening, peppers were prepared and stored in an anaerobic hood and their firmness retention was compared to that of peppers prepared and stored in air with or without added sulfite. Firmness retention of peppers prepared anaerobically after 2 weeks of storage was similar to that of peppers prepared in air with 1.0 mM sulfite added (**Figure 4**). Peppers prepared and stored in air were significantly softer that the other treatments ($p \leq 0.001$). The mean firmness of the peppers prepared in air was only 3.45 N compared to 7.09 N for the peppers prepared in anaerobic conditions. Addition of 1.0 mM sulfite to anaerobically stored peppers did not significantly increase firmness. This result indicated very strongly that oxygen was responsible for rapid softening and that in air sulfite inhibited the effect of oxygen on tissue softening. This conclusion was confirmed by the results shown in **Figure 5**. Jars of peppers prepared anaerobically and then injected with both sulfite and increasing amounts of oxygen did not soften significantly, as indicated by the fact that the slope of the curve was not significantly different from zero ($p = 0.49$). Peppers that were packed in an anaerobic hood and stored for 3 weeks with zero addition of oxygen retained the same firmness as peppers with sulfite added. However, firmness declined hyperbolically as increasing amounts of oxygen, but no sulfite, were added to the jars of peppers.

These results demonstrate two new phenomena related to texture changes in acidified plant tissues. First, oxygen has been found to accelerate softening of red bell pepper pieces stored at pH 3.5. Second, a specific effect of sulfite to prevent this softening was observed. Future investigations will be directed toward determining whether the softening observed in red bell peppers occurs in other peppers or other fruits and vegetables, the mechanism by which oxygen accelerates softening of pepper tissue, and, finally, the mechanism by which sulfite inhibits the softening effect of oxygen. Fry and co-workers (*6, 7*) have proposed a nonenzymatic mechanism for cell wall loosening in growing plants and softening in ripening fruits. They suggest that copper ions bound by cell wall components can catalyze the generation of hydroxyl radicals from hydrogen peroxide. The hydroxyl radicals would cause degradation of cell wall polysaccharides. The hydrogen peroxide would be formed when ascorbic acid was oxidized to dehydroascorbic acid by molecular oxygen. Because red peppers contain high levels of ascorbic acid, this could be a plausible mechanism for the oxygen-dependent softening. Sulfite could inhibit such a mechanism by acting as an oxygen scavenger as it is oxidized to sulfate by hydrogen peroxide (*2*).

Conclusion. This work has revealed a previously unrecognized role for oxygen in causing rapid softening of red bell peppers during acidified storage. Sulfite, which is used to prevent darkening and as a microbial inhibitor in acidified vegetables, was found to prevent this rapid softening reaction. In combination with calcium chloride, sulfite was useful in maintaining the firmness of red bell peppers during extended storage.

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