

Acidification of Brined Cherry Peppers

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ABSTRACT

Rates and routes of acetic acid penetration into nonheated cherry peppers (*Capsicum annuum*) were determined by monitoring pH over time at ten interior areas. The pH was determined for fresh red and green peppers, brined peppers, and brined peppers that had been exposed to oxygen prior to brining. The pH measurements, scanning electron microscopy, and dye penetration observations indicated that the primary avenue for acid penetration was through the stem into the placenta. The last area to become acidified (pH < 4.6) was the interior of the fruit wall. Peppers that had been exposed to oxygen were more rapidly acidified (24 hr) as compared to those not exposed to oxygen (150 hr).

INTRODUCTION

SECTION 12 CFR 114.80 of the Good Manufacturing Practice (GMP, FDA, 1979) regulations stipulates that "A manufacturer must manufacture, process and pack acidified foods so that a finished equilibrium pH value of 4.6 or below is achieved and maintained in all finished foods." Furthermore, "A manufacturer must thermally process acidified foods to an extent necessary to destroy vegetative cells of microorganisms of public health significance and those of nonhealth significance capable of reproducing in the food under the conditions in which the food is stored, distributed, retailed, and held by the user unless permitted preservatives are used to inhibit the reproduction of these microorganisms." Some pepper products are preserved by addition of sufficient acetic acid and salt to prevent microbial spoilage without the need for heat processing. This process is known as "cold packing" and is particularly useful for the preservation of pepper rings (sliced peppers) which are subject to severe deterioration of textural properties if heated. Manufacturers are reluctant to use the cold pack process for whole peppers because of uncertainties related to the rate of acid penetration into the peppers. The cold-pack process is dependent upon the diffusion of acidified brine into the pepper for preservation of texture and prevention of microbial growth. Since diffusion is a time-dependent event, the question arises as to the time required for all parts of a pepper to achieve and maintain an equilibrium pH of 4.6 or below when submerged in an acidified brine. The objectives of this study were: (1) to compare the rates of pH lowering of different anatomical areas of brined cherry peppers; (2) to determine the routes of brine penetration through cherry peppers; and (3) to determine the effect of changing the internal atmosphere of cherry peppers on the rate of pH lowering when brined.

MATERIALS & METHODS

Peppers

Hand-harvested, disease-free, undamaged cherry peppers (*cv. Capsicum annuum*) grown at the N. C. State University Method Road

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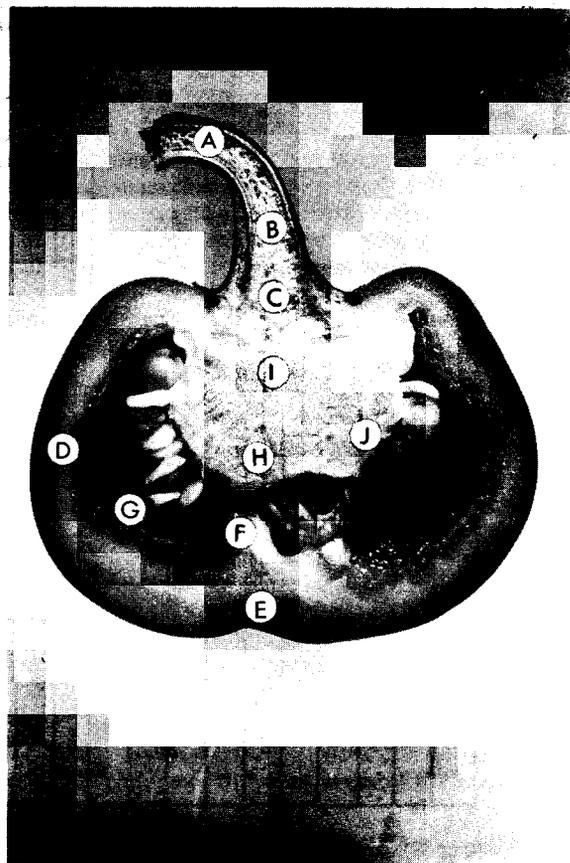


Fig. 1—Areas of cherry pepper from which pH values were measured.

Experimental Farm were used exclusively. Green fruit were used in all experiments except where indicated.

Brining

Unless otherwise noted, 9 peppers of equal number, weight (ca. 14 g/pepper), size, and condition were packed into 12-oz (355 mL) jars to give a pack-out ratio by weight of 35% peppers to 65% brine. Brine consisted of an aqueous solution of 5% NaCl, w/v, and 2.5%, w/v, glacial acetic acid.

In some experiments, dye was added (0.5% w/v Safranin O, Allied Chemical Co.) to the brine. To test the effect of pepper position on brine penetration, peppers were placed individually into 50 mL beakers and weighed. Brine was added at a rate of twice the weight of the pepper. Each pepper was upright with the stem perpendicular to the beaker bottom. Each pepper was buoyant to the point where none of the calyx or stem was submerged in the brine. The beakers were placed in a plastic box and loosely fitted with a cover to prevent brine evaporation and allow gas exchange. The same procedure was used for peppers submerged with their stems down. A group of peppers was completely submerged and kept under brine by means of plastic mesh. In other experiments, peppers were oxygen-exchanged prior to brining. These peppers were packed into 355 mL jars which were fitted with a lid in which was mounted a brine reservoir and a gas sarger, as described previously (Daeschel and Fleming, 1983). Oxy-

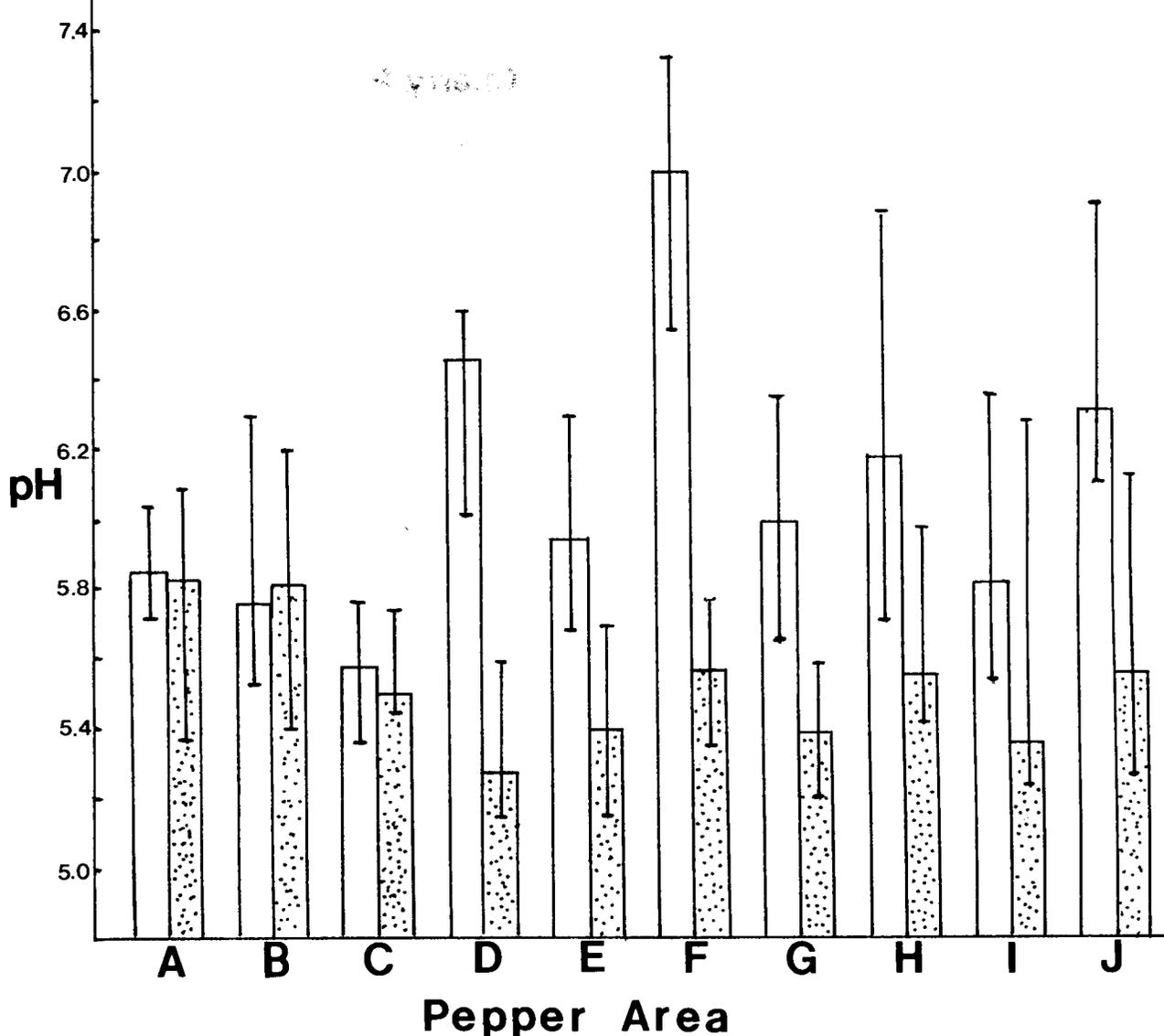


Fig. 2—Median and range limits of pH values of fresh green cherry peppers (open bars) and fresh red cherry peppers (stippled bars) from ten anatomical areas and ten replicate samples.

gen was allowed to flow through the jar at a metered rate of 300 mL/min for 1 hr prior to brine addition.

Chemical assays

The pH was measured with an M-710 micro-combination probe (Microelectrodes, Inc., Londonderry, NH). Individual peppers were removed from jars and sliced as indicated in Fig. 1, and the electrode tip (1.5 mm in diameter) was inserted into the pepper tissue at areas indicated and rotated gently. The pH readings were recorded 30 sec after insertion from the visual read-out from an Orion 701 digital ionanalyzer (Orion Research Inc., Cambridge, MA). Each pepper was discarded after measurement of its internal pH. Subsequent measurements were made on other peppers in the jar. Citric and malic acids were measured in fresh peppers by means of high performance liquid chromatography (McFeeters et al., 1984).

Measurement of oxygen-exchange rate of whole peppers

Preliminary experiments were conducted to determine the length of time needed to exchange the internal atmosphere of fresh peppers with

that of pure oxygen. Peppers were placed in a gas-tight glovebox (Germfree Laboratories, Miami, FL) which had been repeatedly flushed with pure oxygen until a concentration of nearly 100% oxygen was reached. At various time intervals, a 1 mL sample of gas was removed from the interior of a single fruit with a 2 mL syringe fitted with a hypodermic needle. The needle was inserted through a gas-tight rubber septum (13 mm diameter) which was mounted in a hole drilled through the plexiglass wall of the glovebox. The needle was then removed and replaced with a tightly fitting serum cap, while expelling a positive flow from the syringe. After collection of all samples, 0.5 mL gas samples were removed from the syringes through the serum cap and injected into a Fisher-Hamilton gas chromatograph (Fleming et al., 1980). Oxygen concentration was computed by reference to peak heights of gas mixtures of known oxygen concentration.

Scanning electron microscopy

Tissue sections of fresh pepper were fixed in 3% glutaraldehyde in 0.1M sodium acetate buffer, pH 5.5. Sections were dehydrated in ethanol and passed through a graded series of amyl acetate. Liquid carbon dioxide was used as an intermediate fluid for critical point drying in a Ladd 28000 instrument (Ladd Research Industries, Inc.,

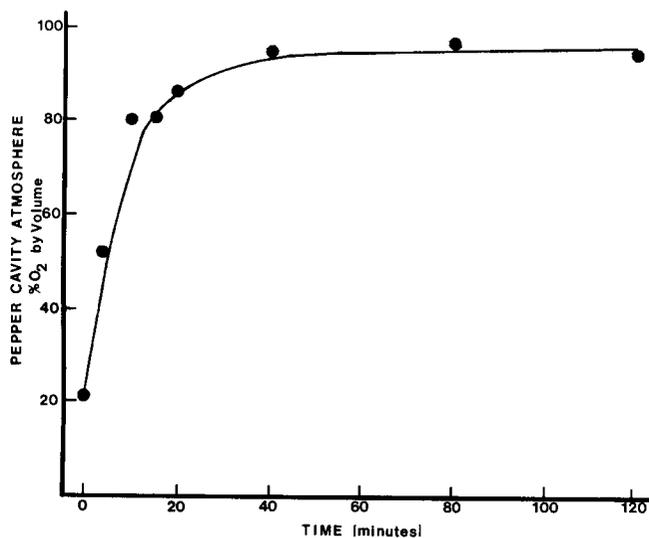


Fig. 3—Internal % O₂ composition of fresh cherry peppers exposed to O₂.

Table 1—Effect of O₂-exchange on the weight of peppers when brined^a

Gas treatment	Initial pepper wt (g)	4 hr after brining	(% Change)	24 hr after brining	(% Change)
O ₂ exchanged	527.8	598.2	(+11.7)	—	—
	534.3	—	—	680.2	(+21.4)
Nonexchanged	517.1	543.5	(+4.8)	—	—
	534.4	—	—	528.65	(-1.0)

^a For this experiment, 26 peppers of approximate equal weight were placed in a 3785 mL jar, oxygen-exchanged, and held submerged in a solution of 5% NaCl and 2.5% glacial acetic acid. The pack-out ratio by weight was 35% peppers and 65% brine.

Table 2—Effect of pepper position on pH of different areas of the pepper fruit during brining^a

Pepper area ^b	Pepper position		
	Stems up	Stems down	Completely submerged
A	5.9 (4.8-6.5)	3.2 (3.0-3.5)	3.4 (3.0-3.7)
B	6.3 (5.3-6.8)	3.5 (3.4-3.7)	3.7 (3.6-4.1)
C	6.0 (5.1-6.7)	3.8 (3.6-3.9)	3.9 (3.5-4.2)
D	5.9 (5.7-6.8)	6.2 (5.9-6.6)	6.1 (5.8-6.5)
E	4.3 (4.0-4.6)	6.3 (5.1-6.9)	4.8 (4.7-5.0)
F	6.1 (5.4-6.4)	6.0 (5.2-6.2)	5.6 (5.3-6.0)
G	5.7 (5.2-6.6)	5.7 (5.4-6.3)	5.8 (5.6-6.0)
H	5.9 (5.7-6.6)	4.8 (4.6-5.8)	5.0 (4.8-5.2)
I	6.1 (5.5-6.8)	4.3 (4.0-4.6)	4.8 (4.5-5.0)
J	6.3 (5.9-6.8)	5.1 (4.5-5.9)	5.0 (4.8-5.1)

^a Time of exposure was for 24 hr in 2.5% acetic acid-5% NaCl brine. The pH values are expressed as the median and range from ten pepper areas and nine replicate samples. The pH values of fresh (not brined) peppers are given in Fig. 2.

^b See Fig. 1 for anatomical location of pepper areas.

Burlington, VT). Tissue was gold-coated in a Polaron E5000 diode sputtering system (Polaron Instruments, Inc., Doylestown, PA) and observed at 20 KeV with an ETEC autoscan microscope (ETEC Corp., Hayward, CA). Linear and gamma operating modes were used.

RESULTS

pH and acid content of fresh peppers

The pH values for ten distinct areas (Fig. 1) of the pepper fruit were determined for both green and red fruit (Fig. 2). Differences in pH among areas in green fruit were as large as 1.5 units, whereas with red fruit the largest pH difference among areas was about 0.5 units. In all sample areas, the pH was lower with red fruit. The largest differences in pH between red and green fruit were observed in area D, which is the interior of the fruit wall and area F which is the interlocular

septum that is contiguous with the fruit wall. Organic acid analysis of homogenous slurries of entire red and green fruit indicated concentrations of malic and citric acids of 2.2 and 5.2 mM for red and 2.6 and 2.4 mM for green fruit. Acetic acid (1.6 mM) was detected in red, but not in green fruit.

Oxygen-exchange in fresh peppers

The internal gas composition of fresh peppers was greater than 90% oxygen (Fig. 3) when exposed to 100% oxygen for 1 hr. Similar results have been obtained for fresh cucumbers (Fleming et al., 1980).

Brine uptake of oxygen-exchanged peppers

Brine uptake was measured by observing changes in pepper weight over time (Table 1). Oxygen-exchanged peppers were heavier than nonexchanged peppers at 4 and 24 hr after brine addition. The nonexchanged peppers exhibited a slight weight loss after 24 hr in brine. This loss may be due to shrinkage of pepper flesh due to water loss in the brine. This effect apparently was masked in the oxygen-exchanged peppers because of the large influx of brine. The oxygen-exchanged peppers, when cut open after being immersed in brine for 24 hr, contained approximately 5-6 mL of brine. The nonexchanged fruit did not contain appreciable amounts of brine (< 1 mL).

Effect of pepper position on rate of acidification

The effect of pepper position during brine storage on the rate of acidification was determined. The data summarized in Table 2 indicated that, when the stems were out of the brine, acidification was not as rapid as when the stems were submerged or when the entire peppers were submerged. This suggests that the route of brine entrance was primarily through the stem. However, the blossom end scar area (E) also appeared to allow brine entry, but to a lesser extent than the stem area.

Rate of pH change of different areas of the brined cherry pepper

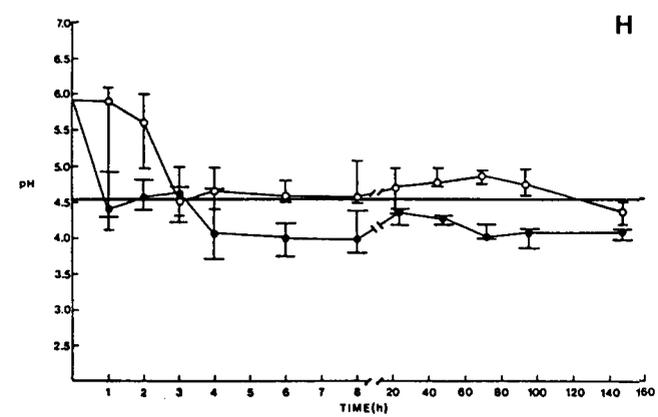
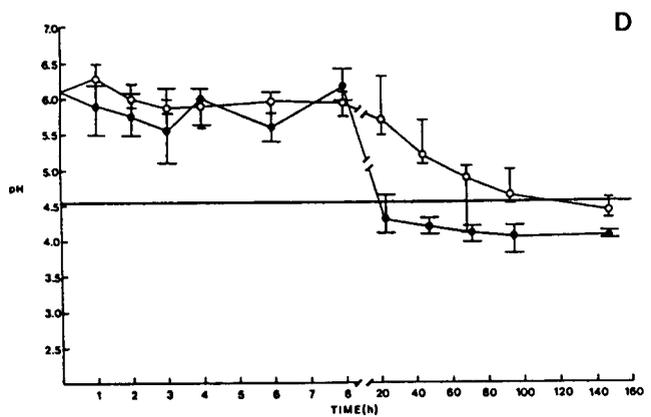
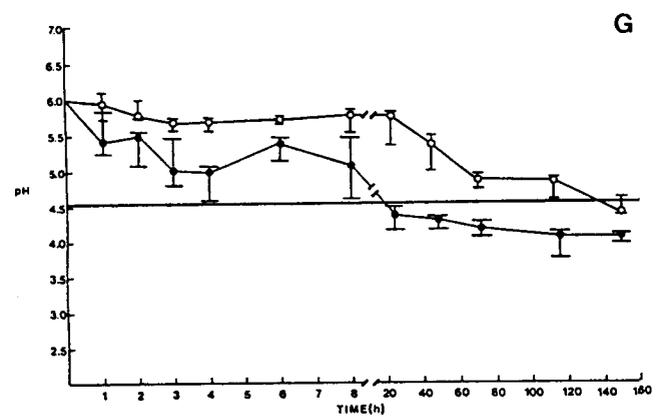
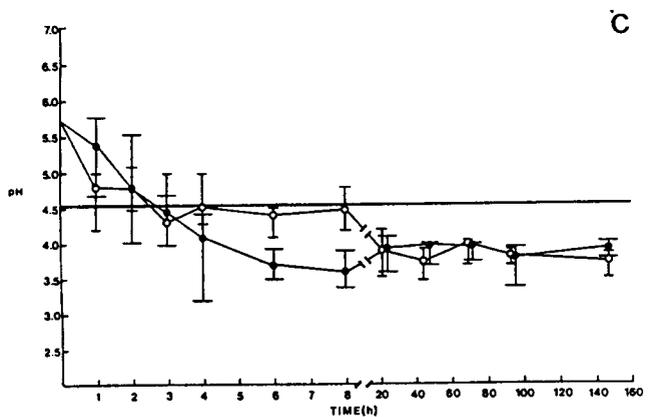
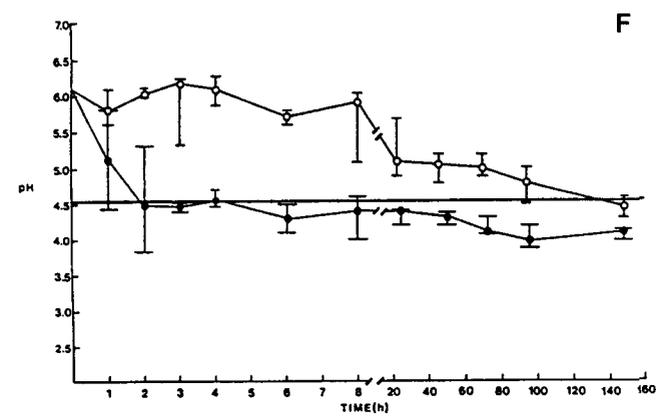
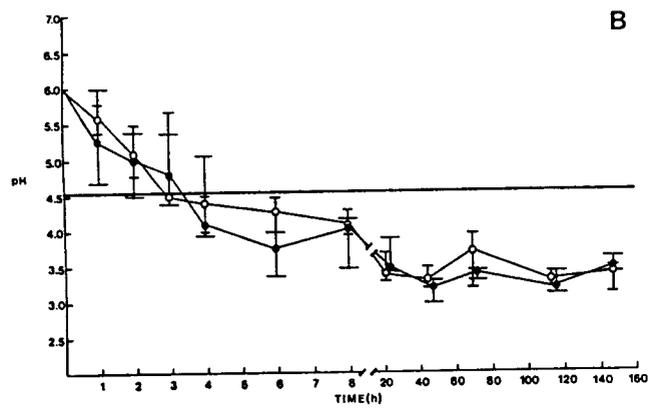
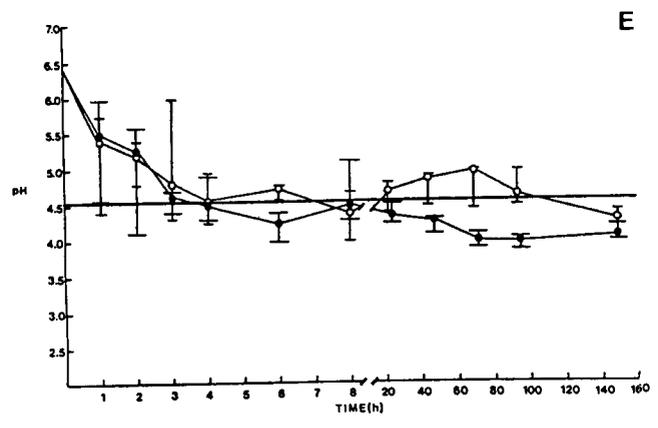
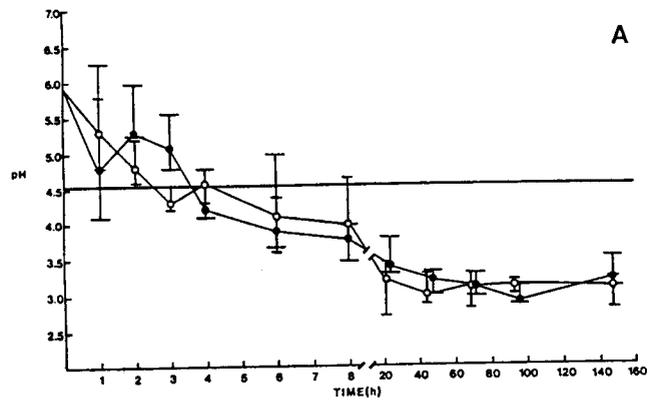
The pH was measured from ten areas of peppers (Fig. 4) which were exposed to oxygen prior to brining. Measurements were also taken from peppers not exposed to oxygen. Areas A, B, and C were rapidly acidified to pH < 4.6, regardless of pre-brining gas exposure, indicating an initial rapid movement of acid through the stem. Again, area E, the site of the blossom end scar, was acidified more rapidly than some areas, but not as rapidly as the stem areas. Areas D, F, and G were the areas most resistant to acidification. Without exposure to oxygen, some peppers could not be acidified below pH 4.6 in these areas after 6 days of submersion in brine. Oxygen-exposed peppers were acidified in all ten areas within 1 day.

Dye Movement into oxygen and nonexchanged peppers

An indication of the directional movement of brine into peppers was obtained by including a water-soluble dye in the brine solution. Peppers exposed to oxygen prior to brining and submerged in dye-brine for 24 hr displayed a homogenous distribution of dye throughout the interior of the pepper fruit (Fig. 5). Peppers not exposed to oxygen exhibited a spotty distribution of dye, primarily being concentrated in the stem area, indicating the primary avenue for liquid penetration is through the stem.

Scanning electron microscopy of cherry peppers

Scanning electron microscopy was employed to determine if anatomical features of the peppers may influence the rates



and routes of acidification. A cross-section of the stem (Fig. 6A) revealed areas of noncontiguous cells known as pith which

could afford routes for brine entrance. The surface of the stem and calyx is undulating and is characterized by the presence

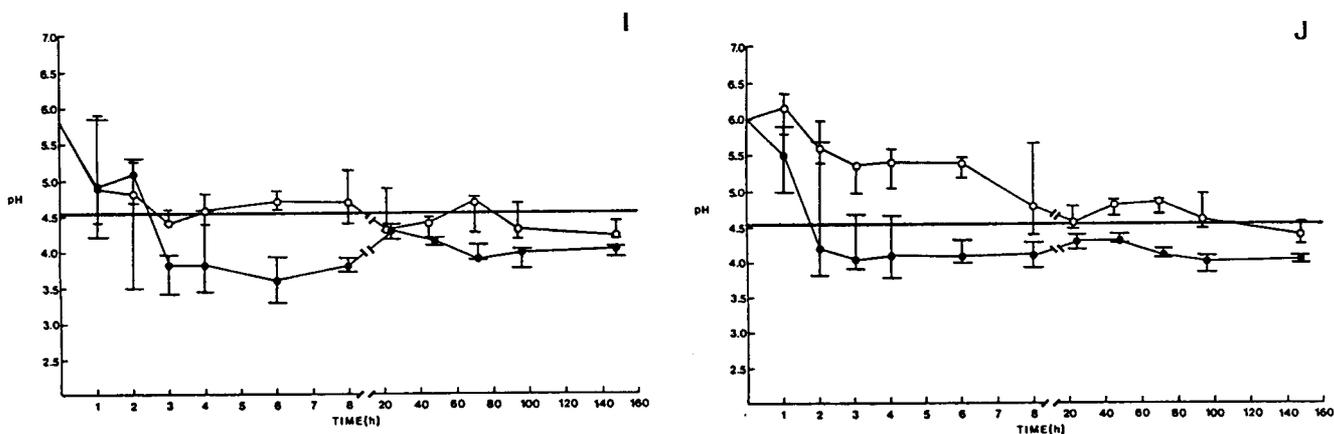


Fig. 4—Rate of acidification of ten different pepper areas in O_2 -exchanged (●) and nonexchanged (○) brined cherry peppers. Values are the median and range limits from replicate samples.

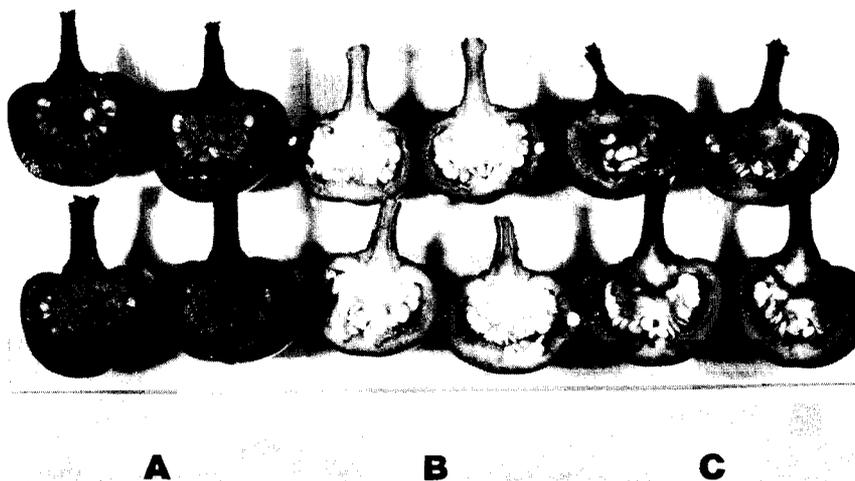


Fig. 5—Effect of O_2 -exchange on brine-dye uptake into brined cherry peppers. O_2 -exchanged brined peppers (A), fresh (not brined) peppers (B), and nonexchanged brined peppers (C).

of stomata (Fig. 6B) which may serve as conduits for brine entry into the fruit. The fruit wall has a smooth, featureless surface devoid of any openings or breaks (Fig. 6C). A waxy or cutin-like material appears to extend three to four cell layers into the cuticle.

DISCUSSION

PREVIOUS STUDIES on pH reduction of peppers by acidification (Sane et al., 1950; Flora et al., 1978; Flora and Heaton, 1979; Sapers et al., 1980) did not address the variability of pH among different anatomical areas within fresh or acidified pepper fruit. Reported pH values were from either samples of blended peppers or the acidified cover liquor. pH values of blended pepper samples may be misleading in that a reported value of below 4.6 may not be representative of all areas of the pepper before it was blended. The data reported in this study have shown this to be true. In a recent study (Stroup et al., 1985), pH values were determined for the centers of cherry peppers which were blanched prior to acidification and then pasteurized. A value of pH 4.4 was reported for peppers acidified with 2% acetic acid for 1 day. Blanching and pasteurization, which are not used in cold packing, probably enhance acidification by disrupting the structure of the pepper. Many factors can be involved in the rate of acid equilibration between

the pepper fruit and the acidified brine liquor. The primary barrier for acid penetration into the fruit is thought to be the waxy component of the cuticle, since waxes generally are considered to offer the primary resistance of plants to transpiration losses (Martin and Juniper, 1970). The hydrophobic nature of the cuticle is apparent since water will easily bead on the surface. Surfaces of the pepper other than the fruit wall include the stem and the calyx. During harvest, the integrity of the stem is breached, which might provide an avenue for the entrance of brine. On the surface of the stem and calyx are large numbers of stomata. These portals would provide avenues of entry for brine and possibly microorganisms. Daeschel and Fleming, (1983) found that liquid enters cucumber fruit through epidermal regions of greatest stomatal density. The experiments in the present study indicate that the stem and calyx are the main areas of liquid penetration into cherry peppers. Peppers brined with the stem and calyx above the brine surface were acidified at a much slower rate than when these areas were submerged. When peppers were completely submerged, the interiors of the stem and calyx quickly became acidified, as compared to the interior areas. Other factors which may influence the rate of acidification are the type and concentration of acidulant, the initial pH, and buffering capacity of the pepper. Evidence obtained with pickling cucumbers (Corey et al., 1983) indicated that a partial vacuum is formed within the

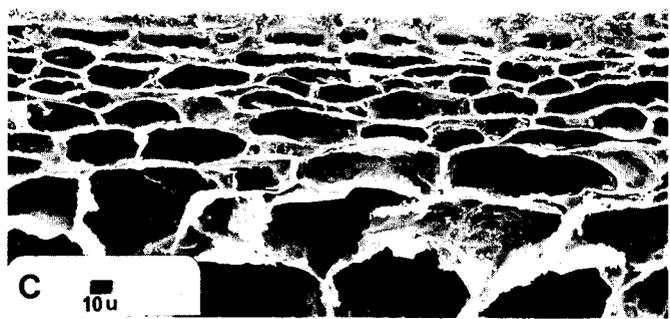
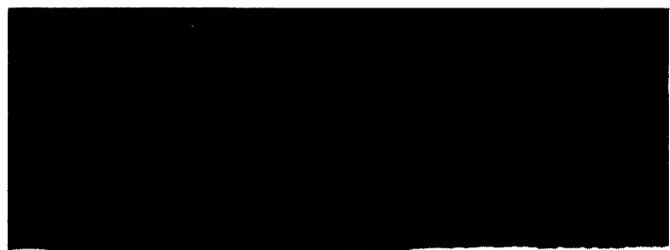
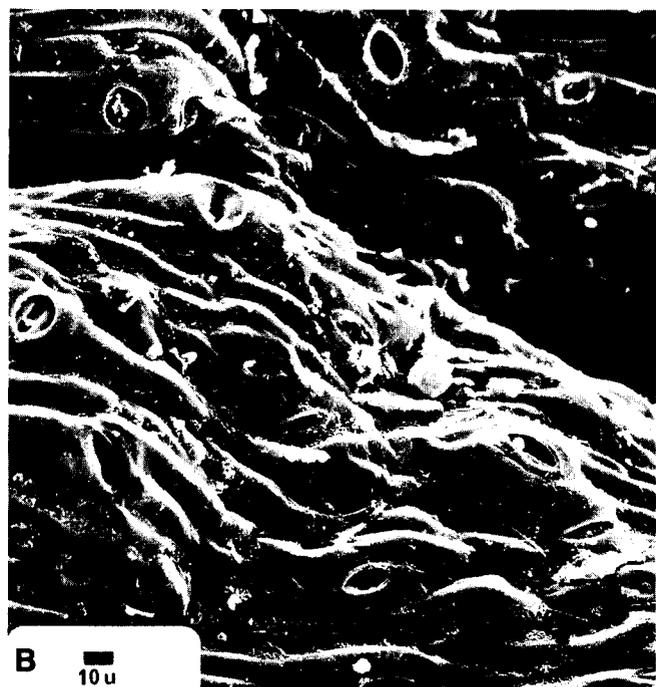
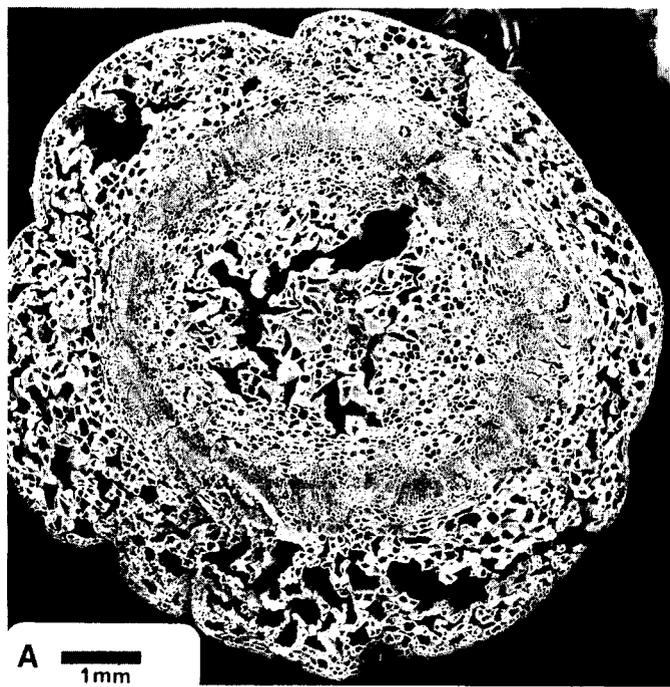


Fig. 6—Scanning electron microscopy of various surfaces of a cherry pepper. Cross-section of the stem (A), outside surface of the stem (B), cross-section of the pepper fruit wall showing the fruit wall surface and adjacent cells (C), and cross-section of the pepper fruit wall showing cutin extending down into the intercellular air spaces of adjacent cells (D).

fruit when brined and is relieved by the uptake of brine. Perhaps a similar phenomenon occurs with peppers.

Fleming et al. (1980) observed that exchange of the internal gas atmosphere of whole, fresh pickling cucumbers with oxygen greatly influenced brining properties of the cucumbers and that the cucumbers rapidly acquired a cured, translucent appearance within a day instead of the usual several weeks required for salt-stock cucumbers. They hypothesized that a partial vacuum was created within oxygen-exchanged and brined cucumbers that caused brine to be drawn into the fruit (by mass flow) and thereby to fill the intercellular air spaces (which amounted to 4 to 6% of the volume of fresh cucumbers), with the resultant cured appearance. They suggested that the oxygen

was metabolized with resultant formation of CO_2 when the fruit were brined. The CO_2 , being about 80 times more soluble than oxygen, was thought to have dissolved in the tissue fluids to a greater extent than the oxygen that it replaced, resulting in a partial vacuum. Occurrence of a partial vacuum in oxygen-exchanged and nonexchanged cucumbers has been experimentally confirmed (Corey et al., 1983).

Peppers exposed to oxygen before brining appear to behave in a fashion similar to cucumbers. The weight of brined peppers was considerably greater when exposed to oxygen prior to brining (Table 1). When the peppers were cut open and examined, it was found that their cavities were more than half full with brine. Smaller amounts of liquid were found in the

nonexchanged peppers. Oxygen-exchanged peppers brined in liquid containing dye were much more heavily stained than nonexchanged peppers (Fig. 5), which is consistent with a greater degree of brine penetration, as indicated above. Although relative rates of dye and acid penetration were not determined, it is likely that both penetrated similarly if mass flow of liquid into the oxygen-exchanged peppers occurred as discussed above. The rate of acidification of oxygen-exchanged was much greater than that of nonexchanged peppers. All areas of the oxygen-exchanged peppers were acidified to pH 4.6 or below within 24 hr as compared to greater than 6 days for nonexchanged peppers. The interior of the fruit wall (area D) was the most resistant to acidification, indicating that acid penetration was most rapid through the stem and into the interior surface of the fruit wall rather than through the outer fruit wall. The blossom scar was also shown to be an avenue for brine entry.

This study has demonstrated that the acidification rate of whole pepper fruit is not homogeneous across the various anatomical structures of the fruit. A pH value of 4.6 has been established as the upper limit for preventing toxin formation by *Clostridium botulinum*. The interiors of fruits, vegetables and seeds are not sterile (Samish et al., 1963; Mundt and Hinkle, 1976; Daeschel et al., 1985), and hence it is conceivable that clostridia may be within or be transported into the fruit interior during brining. Daeschel and Fleming (1981) demonstrated that bacteria can enter cucumbers via stomatal pores during brining. These factors must be considered in assessing the safety of cold-pack processes for peppers. Oxygen-exchange technology may be a feasible approach to provide a more rapid acidification rate to preclude the possibility of growth of *C. botulinum* in cold-packed cherry peppers.

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