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Pressure Changes in Oxygen-exchanged, Brined Cucumbers[1]

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Abstract. **A method was developed for determining internal gas pressure changes of pickling cucumbers (*Cucumis sativus* L.) during brine storage. Internal pressure decreased by 55 mm Hg during the first hour after the control fruit had been submerged in brine and then gradually increased over the next 2 hours to about the level of atmospheric pressure that had existed immediately after brining. With cucumbers that were gas-exchanged before brining, the pressure decreased by a maximum of 145 mm Hg when O_2 was the exchange gas, and increased slightly when N_2 was the exchange gas. Pressure changes in O_2-exchanged cucumbers corresponded with changes in the level of brine that surrounded the fruit, suggesting that liquid entered the fruit as a consequence of the partial vacuum. O_2-exchanged, brined cucumbers acquired a translucent, cured appearance, due apparently to filling of the intercellular gas spaces with liquid. Mechanically induced vacuum failed to induce the cured appearance. Respiratory conversion of O_2 to CO_2 in control and in O_2-exchanged fruit, with greater dissolution of the CO_2 than the O_2 which it replaced, is thought to account for the partial vacuum that develops in brined cucumbers.**

Fleming et al. (3) observed that exposure of pickling cucumbers to 100% oxygen prior to brining resulted in apparent advantageous changes in the cucumbers during the early stages of brining. These changes included the attainment of visual cure (translucent appearance of fruit flesh) within a few hours after brine addition, an increase in fruit density, and increased resistance to bloater damage upon artificial carbonation of the brine or upon pure culture fermentation of the brined cucumbers with *Lactobacillus plantarum*. In contrast, N_2-purged cucumbers acquire a cured appearance very slowly, generally taking at least 1 month to attain a fully cured appearance (2). The increased density of cured fruit also minimizes damage to the fruit incurred by the initial high buoyancy pressure of fruit contacting the head boards in brine storage tanks (5).

It was also shown that the internal gas atmosphere of cucumbers exposed to 100% oxygen rapidly equilibrated with the ambient oxygen environment (3). It was postulated that upon addition of brine to the fruit, the internal oxygen was rapidly consumed with accompanying production of CO_2 due to fruit respiration. It was further hypothesized that a partial vacuum developed in the interior of fruit treated in this manner by the dissolution of CO_2 into the fruit tissue, due to the high aqueous solubility of CO_2. The development of a partial vacuum was proposed to draw brine into the intercellular spaces of the fruit tissue, replacing gases, and resulting in the cured appearance and increased density (3) as well as increased resistance to bloater damage (4). Experimental evidence for the interior vacuum was not presented.

This investigation was undertaken to examine aspects of the above hypotheses proposed by Fleming et al. (3) to explain the rapid induction of cure in O_2-exchanged, brined cucumbers. Specifically, the objectives were to: a) develop a method to measure internal gas pressure changes of brined cucumbers; b) determine effects of N_2- and O_2-exchange on internal gas pressure and gas volume changes of brined cucumbers; c) measure compositional changes in the internal gases of gas-exchanged, brined cucumbers; and d) determine the effects of artificially applied partial vacuum on the development of cured appearance.

Materials and Methods

Cucumbers. Size No. 3 'Calypso' pickling cucumbers (3.8–5.1 cm diameter) were harvested from field plots grown at University Research Unit 4 at Raleigh. Fruit were selected based on the absence of obvious physical damage, disease, and shape deformities. Cucumbers used in experiments involving single fruit measurements were selected for uniform weight (± 10%). Following harvest, cucumbers were humidified and held in 13.0 ± 1.0°C storage for not longer than 7 days. Fruit were equilibrated at 22.0 ± 1.0° in humidified containers prior to experimental use.

Gas exchange of fruit prior to brining. Gas exchange of the internal atmospheres of fruit was accomplished by flowing O_2 or N_2 through glass gas dispersion tubes at 300 ml/min for 1 hr around single fruit in 1500-ml containers prior to brine addition. This combination of flow rate and time was found previously to achieve a near complete exchange (3). Nonexchanged fruit (air) served as the control. For experiments involving composite samples of fruit, 1.7 kg of fruit was packed into a 3.8-liter jar to give a 45:55 (w/v) pack-out ratio of fruit to brine. Each jar lid was equipped with a glass gas dispersion tube, expansion reservoir, and a glass rod to support the reservoir as described previously by Fleming and Pharr (4).

Brining. Brine, held at 22.0 ± 1.0°C, was added to the containers or jars of gas-exchanged cucumbers, while gas flow was maintained to exclude air from the system. Flow of O_2 was stopped following brine addition, but N_2 flow was continued at 50 ml/min throughout the experiments in order to maintain an O_2-free environment. The brine composition used in all exper-

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iments was 10.6% (w/w) NaCl, 0.32% (v/v) glacial acetic acid, and 0.20% (w/v) sodium benzoate. This brine solution composition, as used previously by Fleming and Pharr (4), precluded microbial growth. Contraction volume, previously expressed as "brine uptake" (3), was determined by monitoring the drop in brine level in the graduated reservoir from an initial 150-ml mark on the reservoir. Contraction volume was computed as the percent of the total cucumber volume, assuming a cucumber density of 1.0 g/cm^3.

Internal gas pressure measurements. Measurement of internal gas pressure was made using the apparatus shown in Fig. 1. A cavity was made through the approximate center of each cucumber with a 0.4-cm diameter cork-borer. The cavity was then blotted to remove liquid expressed from the cut cells. A 1.6-cm-diameter patch of vinyl plastic electrical tape (No. 4472; Plymouth Rubber Co., Inc., Canton, Mass.) was glued to the fruit surface over each end of the cavity. A 0.5-cm-diameter hole was made in one of the patches for insertion into the cavity of a flanged aluminum needle guide equipped with a hubless No. 19 stainless steel hypodermic needle. The needle guide was fitted between two 1.0-cm-diameter, 0.3-cm-thick rubber septa (No. 69-000010-02; Varian Inst., Walnut Creek, Calif.) and strapped securely with a plastic cable strap (PLT31-CP; Panduit Corp., Tinley Park, Ill.) to the fruit surface. A septum was also placed over the patch on the other end of the cavity to cushion the strap pressure on the fruit surface. The assembly was sealed with Duro Super Glue adhesive (Woodhill Permatix, Cleveland, Ohio) to provide gas-tight seals. Before gas-exchange treatment of fruit, the needle in the fruit cavity was connected to a U-tube Hg manometer using tygon and polyethylene tubing connections. Changes in pressure of the gas cavity were then monitored over a 4-hr time period at 5-min intervals.

Internal gas volume. Cucumbers were gas-exchanged and brined as described previously in 3.8-liter glass jars. A sample of 3 fruit was removed from each jar at 1, 2, 3, and 4 hr following brine addition. Tissue plugs were removed from the fruit in each sample using a 2.0-cm-diameter cork-borer to obtain a tissue weight of 30.0 to 40.0 g. The tissue plugs were immersed in a 2.0 M $MgSO_4$ solution adjusted to pH 2.5 with HCl and were contained by an inverted glass funnel in a desiccator jar as in Jorge (8). A 10-ml graduated Pyrex tube filled with the $MgSO_4$ solution was fitted to the inverted funnel with a rubber stopper to trap the gases from the tissue plugs. A vacuum pump was then connected to the desiccator lid and the system was subjected to a 737 mm Hg (0.97 atmospheres) vacuum for 2 min. Gases exited the tissue and were trapped and measured in the graduated Pyrex tube upon return to atmospheric pressure. Volume of the tissue plugs was determined by liquid displacement in a graduated cylinder prior to gas extraction. The data were expressed as ml gas/100 cm^3 of tissue. The same method was used to determine the internal gas volume of exocarp, mesocarp, and seed region tissue of fresh cucumbers. A sample of 3 fruit was peeled and cut in order to separate the 3 major tissue components. Gas volume was expressed on a per unit weight of tissue basis.

Composition of gas cavity. Cucumbers with a 0.5-cm artificial gas cavity, sealed with tape patches and a septum sampling port, were subjected to the 3 gas-exchange treatments previously described. A 1.5- to 2.0-ml sample was taken from the gas cavities with a 5-ml plastic syringe immediately prior to brine addition and another was taken 1 hr following brine addition. Since the sampled gases were not at atmospheric pressure, the syringes were held under the brine solution immediately following sampling to allow brine to enter the syringe and bring the sampled gas to atmospheric pressure. A 0.25-cm^3 sample of the gas was then analyzed for composition with a Hamilton-Fisher Gas Partitioner (Model no. 29; Fisher Scientific Co., Raleigh, N.C.).

Cl^- concentration. An experiment was conducted to determine the Cl^- concentration of cavity liquid 4 hr following O_2-exchange and brine addition. A control was set up by adding brine solution to the cavity of unbrined cucumbers. After a 4-hr equilibration period, a 2- to 3-ml sample of the cavity liquid was obtained from a 3-fruit composite sample (3 replications) with Pasteur pipettes 4 hr after brine addition. The Cl^- concentration of the cavity liquid was determined by titration with 0.17 N $AgNO_3$, using 2 drops 10% dichlorofluorescein in 70% ethanol as the indicator. A 0.5-ml sample was titrated to a pink end point.

Application of artificial partial vacuum. An experiment was conducted to induce fruit to cure artificially by using a prepro-

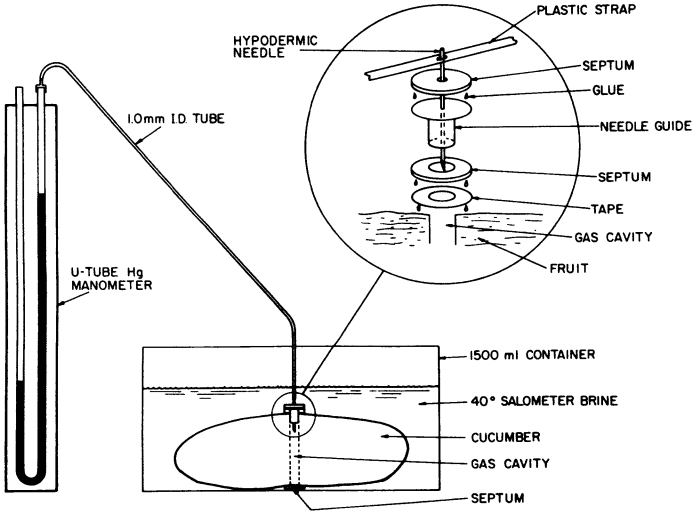

Fig. 1. Diagram of apparatus used for monitoring pressure changes of the interior gases of brined cucumbers.

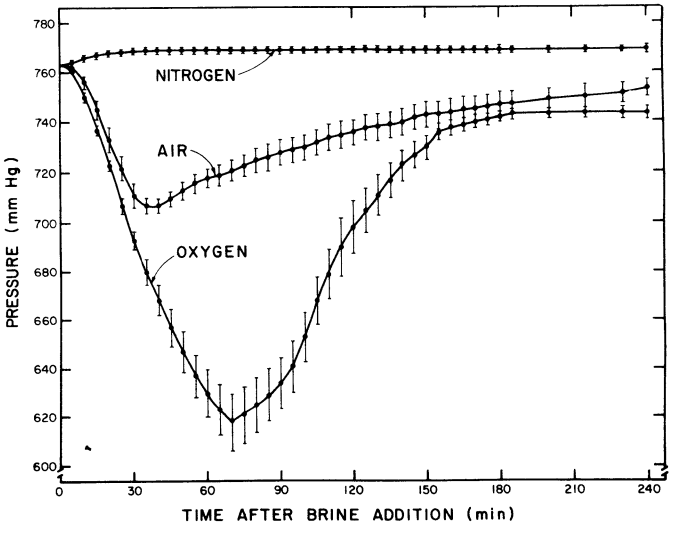

Fig. 2. Gas pressure changes in N_2-exchanged and O_2-exchanged cucumbers or in nonexchanged (air) cucumbers following addition of brine. Each point represents the mean of 4 replications. Vertical bars represent 1 SD.

grammed sequence of partial vacuum values obtained from the experimentally determined values. Single fruit were N_2-exchanged prior to brine addition to exclude O_2, thereby preventing the natural development of partial vacuum. Artificial vacuum was applied to the gas cavity with a 10-ml syringe. Incremental increases in partial vacuum were made and held for 5-min intervals for 70 min. The syringe line was clamped off and the pressure in the fruit was allowed to increase to atmospheric pressure. Fruit were cut longitudinally after 4 hr in brine and examined for visual appearance. In addition, fruit with 2 cavities (3 cm apart) were set up for monitoring the partial vacuum. The partial vacuum was applied in the first cavity and measured in the second.

Results

Internal pressure measurements. Gas pressure in the cavity of O_2-exchanged and brined cucumbers decreased sharply from atmospheric pressure following addition of brine to cover the fruit (Fig. 2). A decrease in gas pressure of 145 mm Hg was measured in the fruit after 70 min in brine. The pressure following attainment of the maximum partial vacuum then increased, presumably due to brine intrusion. It was obvious that brine did enter the O_2-exchanged fruit because brine appeared in the polyethylene tubing connected to the fruit between 2 and 3 hr following brine addition. The gas pressure in control (air) fruit decreased by only 56 mm Hg following brine addition. Pressure increased slightly (6 mm Hg) above atmospheric in fruit pre-exchanged with N_2. After 4 hr in brine, the internal flesh of O_2-exchanged cucumbers appeared fully cured (Fig. 3). A major proportion of this visual cure was evident after 2 hr of exposure to brine, as verified by subjective ratings of 67 to 75% cure present in fruit cut at 90 min and 75 to 85% cure present after 120 min. The internal raw, opaque appearance of N_2-exchanged and control (air) fruit was in sharp contrast to the cured appearance of O_2-exchanged fruit.

Contraction volume. Decrease in brine volume in the graduated reservoir was measured for each of the 3 gas-exchange treatments and was expressed as contraction volume. O_2-exchanged cucumbers were characterized by a sharp decrease in brine volume following brine addition (Fig. 4). The visual appearance of cure (90–120 min) occurred at a time when the major contraction had taken place (Fig. 4). There was little change in brine volume after 2 hr. The maximum contraction volume of about 5.2% for O_2-exchanged fruit closely approximates the value of total intercellular gas space in cucumber fruits determined previously by Jorge (8). There was a slight drop in brine volume for both the N_2-exchanged and control (air) cucumbers.

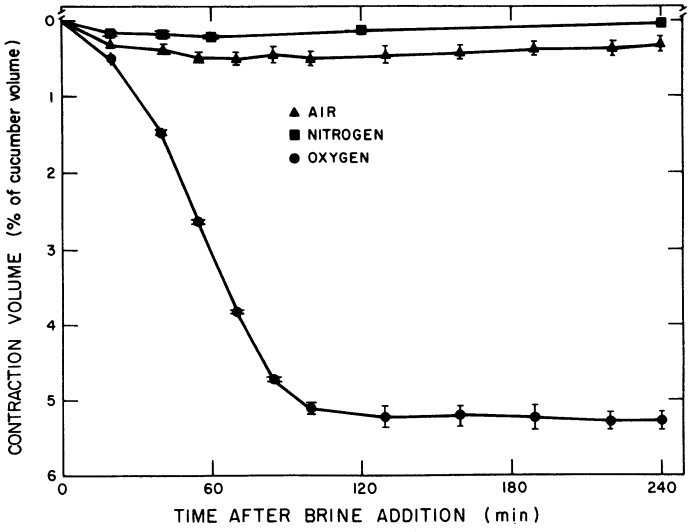

Fig. 4. Contraction volume in N_2- and O_2-exchanged cucumbers or nonexchanged (air) cucumbers following addition of brine. Vertical bars represent the range of duplicate 3.8-liter jars; each containing 12–14 cucumbers.

Internal gas volume. Changes in internal gas volume of tissue plugs removed from intact fruit of the O_2-exchanged, brined cucumbers accompanied changes in brine volume (Fig. 5). Total gas volume inside the fruit declined rapidly in the first 2 hr of exposure to brine from the initial value of 6%, with less than

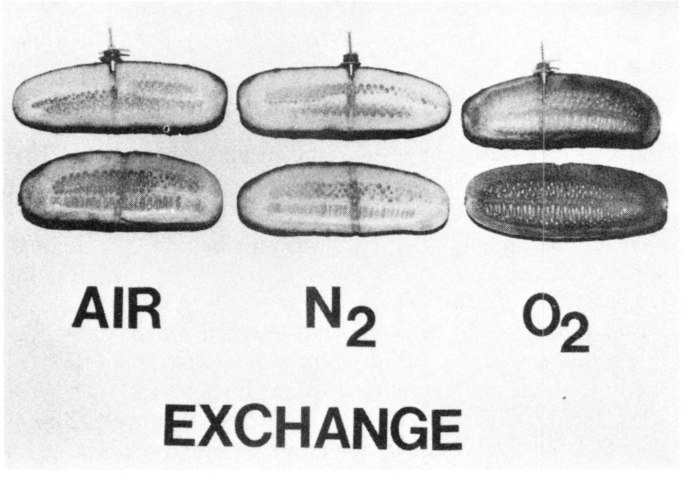

Fig. 3. Translucent, cured appearance of O_2-exchanged cucumbers as compared to the raw, opaque appearance of N_2-exchanged and nonexchanged (air) cucumbers after 4 hr in brine solutions. A portion of the pressure monitoring equipment is pictured in the longitudinally cut fruit.

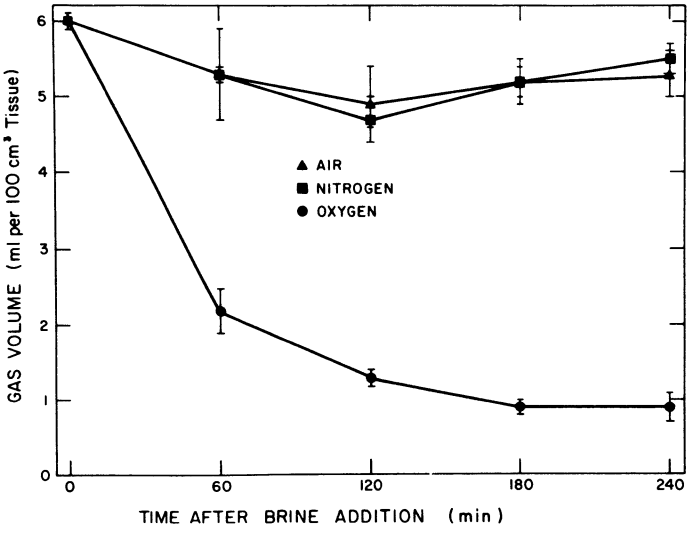

Fig. 5. Internal gas volume of tissue segments taken at hourly intervals from N_2- and O_2-exchanged, brined cucumbers or from nonexchanged (air), brined cucumbers. Each point represents the mean of 3 replications. Vertical bars represent 1 SD.

Table 1. Gas volume of the major tissue components of fresh pickling cucumbers.

Tissue component	Gas volume[z] (cm^3/100 g tissue)
Exocarp[y]	2.90 ± 0.18
Mesocarp	8.17 ± 0.67
Seed region	3.63 ± 0.40
LSD 5%	0.92

[z]Values are means of 3 replications ± SD.
[y]Included small portion of subtending mesocarp tissue.

1% remaining after 4 hr. A small decrease in gas volume was measured for both the N_2-exchanged and control cucumbers following brine addition, which was in agreement with the contraction volume measurements. These small volume changes may be due to shrinkage of the fruit placed in a solution (brine) of high osmotic pressure.

Gas volume of tissue components. Exocarp tissue contained the smallest gas volume of the 3 tissue components analyzed in fresh cucumbers (Table 1). Gas volume of mesocarp tissue was significantly higher than the gas volume of both exocarp and seed region tissue.

Composition of gas cavity. Analyses of gases in the gas cavities of O_2-exchanged (97.1% O_2) and N_2-exchanged (96.3% N_2) cucumbers revealed that a near complete exchange of the internal atmospheres of the fruit with the respective gases was achieved (Table 2). Small residual concentrations of CO_2 and N_2 remained in O_2-exchanged cucumbers and small residual concentrations of O_2 and CO_2 were measured in N_2-exchanged cucumbers. The percentage of O_2 decreased and the percentage of increased in both the O_2-exchanged and control cucumbers 60 min after brine addition. There was also an increase in the percentage of CO_2 in N_2-exchanged cucumbers, but little change in the percentage of N_2 since a N_2 purge was maintained throughout the time of exposure of fruit to brine.

Cl^- concentration. The concentration of Cl^- in the cavity liquid of control fruit (2.62 ± 0.28% w/v) and the exterior brine of O_2-exchanged fruit (11.68 ± 0.19% w/v) were significantly higher (P = 5%) than the Cl^- concentration of cavity liquid from O_2-exchanged fruit (0.60 ± 0.09% w/v). The low Cl^- concentration measured in the cavity liquid of O_2-exchanged fruit confirmed the idea that brine had entered through the fruit surface and not through the patch and seals of the gas cavity. If the latter had occurred, the Cl^- concentration should have been equal to or higher than the Cl^- concentration measured in the cavity liquid of the control.

Artificial partial vacuum. Attempts to induce the artificial curing of fruit by the application of partial vacuum using the experimentally determined values were made. Fruit treated in this manner did not develop the cured appearance. In addition, 2 artificial cavities were made in individual fruit to monitor the response of a cavity located 3 cm from the cavity where a partial vacuum was applied. Following brine addition, the remote cavity responded initially to applied vacuum with a time lag of seconds, reaching the pressure value applied; however, when a partial vacuum was applied after 1 hr of brine exposure, the remote cavity responded with a longer time lag (min) and attained only about 67% of the vacuum applied.

Table 2. Gas composition of artificial gas cavity in gas-exchanged cucumbers immediately following exchange period and after 1 hr in brine.

Treatment	Time (min)	Gas composition (%)[z] O_2	N_2	CO_2
O_2-exchange	0	97.1 ± 1.1	1.3 ± 0.8	1.6 ± 0.3
	60	82.0 ± 6.0	10.7 ± 4.3	7.2 ± 1.7
N_2-exchange	0	1.8 ± 1.8	96.3 ± 1.9	1.9 ± 0.1
	60	trace	95.2 ± 0.3	4.8 ± 0.3
Control (air)	0	29.7 ± 0.4	77.2 ± 0.3	1.9 ± 0.4
	60	2.4 ± 0.2	91.8 ± 0.2	5.8 ± 0.1

[z]Each value represents mean of 3 replications ± SD.

Discussion

Changes preceding and accompanying the curing process. Experimental evidence for the development of a partial vacuum inside O_2-exchanged cucumbers following brine addition was obtained, confirming the hypothesis of Fleming et al. (3). The partial vacuum inside O_2-exchanged cucumbers resulted in a total pressure gradient from the exterior to the interior of the fruit, which caused entrance of brine into the fruit. The Cl^- concentration of the cavity liquid of O_2-exchanged fruit after 4 hr of brine exposure confirmed that the pathway of brine entrance was through the intercellular passages of the fruit rather than through the experimental setup. Entrance of brine was indicated by both the presence of brine in the gas cavity after about 2 hr of brine exposure and by contraction of the exterior brine volume. A decline in the total internal gas volume correlated with the percentage of contraction volume in both magnitude and time. The translucent appearance of the flesh of O_2-exchanged fruit resulted from liquid displacement of the internal free gas volume. In contrast, the large gas volume that remained in N_2-exchanged and control (air) fruit during brining resulted in the raw, opaque appearance of the flesh of these fruit. Although a maximum partial vacuum of 56 mm Hg developed in control (air) fruit, the magnitude of contraction volume and the decline in internal gas volume did not proceed in proportion to the magnitude of partial vacuum, in part explaining the lack of visual cure in these fruit.

Origin of partial vacuum. Analysis of gas composition inside the gas cavity after 1 hr of brine exposure revealed decreased O_2 and increased CO_2 composition, which is indicative of respiratory depletion of O_2. The relatively small changes in O_2 and CO_2 measured in O_2-exchanged cucumbers after 1 hr of brine exposure (Table 2) is attributed to the concomitant decrease in internal gas volume (Fig. 5). The gas remaining in O_2-exchanged cucumbers after 1 hr was still largely O_2, although a substantial depletion of O_2 had already occurred by this time. If an aqueous system is assumed (note: the solubility of CO_2 in tissue fluids would be somewhat lowered by the presence of other solutes) a 100-g fruit could hold about 83 ml of CO_2 in the tissue fluids at saturation. A typical respiratory rate of 0.35 $\mu l\ CO_2\ g^{-1}\ min^{-1}$ for size No. 3 pickling cucumbers (8) would result in only 2.1 ml CO_2 evolution in 1 hr for a 100-g fruit. Hence, CO_2 evolved from respiration would be expected to rapidly partition into the cellular fluids (6) with the result being a lowered gas pressure (partial vacuum) in the fruit.

Nature of partial vacuum. The experimentally determined values of partial vacuum probably would not reflect accurately the absolute pressure in all regions of the fruit and may be lower than what occurred in local regions of the fruit. The fact that

artificial application of partial vacuum using the experimental values did not result in the cured appearance is evidence for the occurrence of pressure gradients inside the fruit. This suggests the presence of localized regions of partial vacuum higher than measured. In addition, the failure of the remote cavity in the 2-cavity experiment to respond completely to applied partial vacuum after 1 hr of brine exposure suggests that discontinuities in the intercellular spaces may develop following brine entrance. The presence of discontinuities within the fruit would result in isolation of different regions of tissue. This may also lead to the development of pressure differences within the fruit.

The existence of localized regions of high partial vacuum appears to be a necessary condition for brine entrance, particularly soon after brine addition. Since both contraction volume and a decline in internal gas volume were found to occur shortly after brine addition, a partial vacuum sufficient to cause brine movement from the exterior into the surface tissue of the fruit probably develops very early during brine exposure. An analysis of the internal gas volume of exocarp, mesocarp, and seed region tissue (Table 1) revealed a significantly lower gas volume in exocarp than in mesocarp tissue. Thus, the rate and magnitude of partial vacuum development in the intercellular spaces of the surface cells may be greater than the internal tissue because of the compact arrangement of the relatively small exocarp cells (7, 9, 10). Once brine entrance is initiated at the surface of the fruit, continued partial vacuum development may promote capillary movement of brine into the tissue, with additional movement of brine occurring because of the capillary nature of the intercellular passages. This would result in the replacement of gas with liquid and the resultant cured appearance.

The likely pathway of brine entrance is believed to be primarily through the stomata (1), but entry may also occur through breaks and discontinuities in the cuticular layer. The postulated regions of high partial vacuum perhaps develop in stomatal and substomatal chambers as O_2 is consumed in the surface cells, thus causing brine entrance.

The results of this investigation support the hypotheses of Fleming et al. (3) regarding the occurrence and origin of the partial vacuum that develops in O_2-exchanged cucumbers following addition of brine. A description of the O_2-exchange process has been extended with regard to the nature of the partial vacuum.

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