Role of the Osmoticum in Bloater Formation of Pickling Cucumbers

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ABSTRACT

Changes in internal gas volume and pressure, and mass flow characteristics of fresh compared to brined cucumbers were measured to study the mechanism of bloater formation. Internal gas volume of fresh cucumbers decreased by about 50% and the resistance to mass flow of gases increased following storage in brine. Internal gas pressure increased by 81 mm Hg in cucumbers stored in brine and only 26 mm Hg in fresh cucumbers in brine following carbonation of the brine. Upon carbonation, cucumbers stored in both 2M NaCl and 3M ethylene glycol exhibited bloater damage accompanied by tissue dehydration, whereas fresh and water stored cucumbers did not bloat. Susceptibility of pickling cucumbers to bloater formation was proposed to be associated with a lowered internal tissue resistance to increased gas pressure.

INTRODUCTION

BLOATER FORMATION is a disorder of pickling cucumbers (*Cucumis sativus* L.) that occurs during brine fermentation. Bloated cucumbers are characterized by various forms of hollow regions in the internal tissue of the cucumbers (Etchells et al., 1974). Gas production, primarily CO_2, from the fermentation of brined cucumbers was implicated as the cause of bloater formation more than 40 years ago (Veldhuis and Etchells, 1939) and has since been associated with several microbial sources (Etchells and Bell, 1950; Etchells et al., 1945, 1968; Fleming et al., 1973a, b). Recent research has been directed toward understanding the mechanism of bloater formation (Fleming and Pharr, 1980).

Fleming et al. (1978) categorized susceptibility to bloater damage into three stages. Cucumbers exhibited little bloater damage if carbonated immediately after brining (stage I, low susceptibility), whereas bloater damage was severe if carbonation was started from 1 to 32 days after brining (stage II, high susceptibility). A reversion to low susceptibility (state III) occurred after 49 days of brining. Recently, a model for the mechanism of bloater formation was proposed by Fleming and Pharr (1980). They proposed that a liquid-clogged layer in the intercellular gas spaces of the epidermal and outer mesocarp tissue formed by brine entrance, acts as a differentially permeable barrier to the diffusion of N_2 and CO_2. A fresh fruit contains about 78% N_2 and only 6% CO_2 (Fleming and Pharr, 1980). Upon submergence of the cucumber into brine, microbial evolution of CO_2 causes a partial pressure gradient to develop, resulting in the diffusion of CO_2 from the exterior brine to the interior of the fruit. It was further postulated that there is a greater transport of CO_2 into the fruit than N_2 out of the fruit which causes the total internal gas pressure of the cucumber to exceed 1 atmosphere, resulting in bloater formation. The observation that cucumbers do not bloat upon artificial carbonation of the brine immediately following addition of brine was attributed to the presence of relatively continuous intercellular gas spaces in fresh fruit, allowing a more rapid exchange and equilibration of interior and exterior gases. However, there is no evidence or suggestion in the proposed mechanism, for the possible occurrence of mass flow of gases from cucumbers in brine upon increased internal gas pressure. In addition, the model fails to explain why the extent and severity of bloater damage may decrease with decreasing brine strength (Fleming et al., 1978).

This study was undertaken to gain a further understanding of physical factors associated with the susceptibility of brined cucumbers to bloater formation. Specific objectives were: (1) to develop a method and to measure mass flow of gases through fresh and brine- or water-stored cucumbers, (2) to measure changes in the internal gas pressure in fresh cucumbers, compared to brine- or water-stored cucumbers upon artificial carbonation of the brine, and (3) to test the hypothesis that a liquid-clogged layer of tissue develops during brine storage of cucumbers.

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MATERIALS & METHODS

Cucumbers

North Carolina size grade 3 pickling cucumbers (3.8–5.1 cm diameter), cv. Calypso, were obtained from a nearby grower on the day of harvest for most of the experiments and from hand-pollinations in a greenhouse for remaining experiments. Cucumbers used in experiments involving single fruit measurements were selected for uniform weight (±10% of the mean fruit weight). Bloater damage was evaluated according to Etchells et al. (1974), and bloater indices were calculated according to Fleming et al. (1977).

Gas exchange of cucumbers

Gas exchange of the internal atmospheres of fruit was accomplished by flowing N_2 through glass gas dispersion tubes at 300 ml/min for 1 hr around single fruit in 1500 ml containers before adding solution. Flow of N_2 was maintained at 50 ml/min following addition of solution to exclude O_2 from the system. For experiments involving samples of fruit, 1.7 kg of fruit were packed into 3.8 liter jars 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, graduated reservoir and a glass rod to support the reservoir as previously described by Fleming and Pharr (1980). Carbon dioxide was introduced through the gas dispersion tubes either immediately following addition of solution or following 48 hr of exposure to solution. Carbonation was initiated at a flow rate of 300 ml/min for the first 30 min to achieve saturation rapidly. The flow rate was adjusted to maintain 50 ml/min following the first 30 min.

Composition of solutions

Acidified, aqueous solutions were added to the containers or jars, while gas flow was maintained to exclude air from the system. The brining treatment used in all experiments was 10.6% (w/w) NaCl, 0.32% (v/v) glacial acetic acid, and 0.20% (w/w) sodium benzoate. NaCl was excluded from this mixture in certain experiments. The acidified, aqueous solutions containing sodium benzoate were used to suppress microbial growth during storage (Fleming and Pharr, 1980).

A 16.8% (v/v) ethylene glycol solution was used in one experiment to approximate the osmotic pressure of the 10.6% NaCl

solution. The concentration of ethylene glycol was interpolated from a table of osmosity values (Weast and Astle, 1979).

Expansion volume

Expansion volume of solution to the exterior of cucumbers was determined by the rise in solution level in the graduated reservoir and was expressed as a percentage of the volume of cucumbers (Fleming et al., 1973a).

Internal gas pressure

A cavity was made through the approximate center of the longitudinal axis of cucumbers with a 0.4 cm diameter cork borer. A no. 19 stainless steel, hypodermic needle was placed into the gas cavity, secured to the fruit surface and connected to a Hg manometer as described previously (Corey et al., 1982). Changes in pressure of the gas cavity were then monitored over a 10-hr period at 1-hr intervals. Cucumbers were cut longitudinally and examined for bloater damage following 10-hr carbonation. Fruit packed in glass jars were given the same gas exchange and solution treatments to provide internal controls. The internal controls verified whether a particular lot of fruit was susceptible to bloater damage for a given treatment. In addition, it was not possible to check the experimental set-up of bloater-susceptible fruit for gas leaks because of the possibility of inducing bloater formation by pressurizing the fruit. Thus, if a treatment rendered the cucumbers susceptible to bloating as verified by the internal controls, the results of internal gas pressure changes were used only for those fruit that displayed bloater damage.

Internal gas volume of tissue components

The internal gas volume of exocarp, mesocarp and seed regions from fresh fruit and fruit held for 48 hr in water or brine solutions was determined. A 25–35g sample of each tissue component obtained from a sample of three fruit was immersed in a 2.0M $MgSO_4$ solution adjusted to pH 2.5 with HCl as described by Jorge (1978). A 10-ml graduated pyrex tube was fitted to an inverted funnel with a rubber stopper all of which was placed in a sealed desiccator jar to trap the gases from the tissue. A vacuum pump was connected to the desiccator lid and the system was subjected to a 737 mm Hg (0.97) atmosphere) vacuum for 2 min. Gases exited the cucumber tissue, and were trapped and measured in the graduated tube upon return to atmospheric pressure.

Mass flow

The flow rate of pressurized air from the interior to the exterior of individual fruit was measured using the apparatus shown in Fig. 1. A gas cavity was bored in the fruit and then equipped with a hypodermic needle setup as previously described (Corey et al., 1982). A flow line was connected from the hypodermic needle in the fruit to a pressurized air line. Connections were made on the inflow line to go through a rubber stopper into a sealed desiccator jar. Individual fruit were pressurized internally at increasing increments of pressure as measured by a pressure gauge (Fisher Scientific Company, Raleigh, NC) connected to the inflow line. An outflow line was inserted through the rubber stopper and connected to a flow tube which was read for each static pressure setting. Flow rates were established for fresh fruit in air and fresh fruit submerged in water or brine by reference to a calibration chart for air flow. Fruit were also held for 48 hr in acidified, aqueous solutions with a continuous N_2-purge, following a N_2-exchange of the internal atmosphere and then tested for flow characteristics.

The flow rate for intact fruit was compared to that of fruit with ends removed for both fresh and brined (48 hr) cucumbers to determine if the skin is a major component of resistance to flow. Flow rates for fruit in this experiment were expressed on a per unit of surface area basis.

Since the mass flow apparatus (Fig. 1) was not sufficiently sensitive to measure flow rates less than about 12–15 cm^3/min, low flow rates were measured by gas entrapment. This was achieved by fitting a 10-ml, graduated pyrex tube filled with brine solution to an inverted funnel placed over the fruit in an open container. The fruit were pressurized internally as previously described, and the volume of gas collected in the graduated tube was read every 5 min for 25 min to establish a flow rate.

Percent change in weight of cucumbers

Individual cucumbers were labeled and weighed prior to packing in 3.8 liter jars. The fruit were reweighed following 48 hr exposure to water, brine or ethylene glycol solution, and the % change in weight calculated.

RESULTS

Internal gas pressure

The pressure of gases inside cucumbers stored for 48 hr in brine or water increased rapidly after carbonation was initiated (Fig. 2). After 4 hr carbonation, internal gas pressure increased by 81 mm Hg in fruit exposed to brine for 48 hr, and by 80 mm Hg in fruit exposed to water for the same length of time. In contrast, internal gas pressure increased by only 26 mm Hg in cucumbers carbonated immediately following addition of brine. All cucumbers brined for 48 hr prior to carbonation bloated. On a scale of slight, moderate, and advanced (Etchells et al., 1974), bloating was slight and was of the honeycomb, lens or balloon type. There was no bloater damage in any of the cucumbers carbonated immediately following addition of brine or after 48 hr exposure to water.

Internal gas volume of tissue components

The internal gas volume of both exocarp and seed regions from fresh fruit was lower than in mesocarp tissue (Fig. 3). Exposure of fresh cucumbers to brine or water for 48 hr

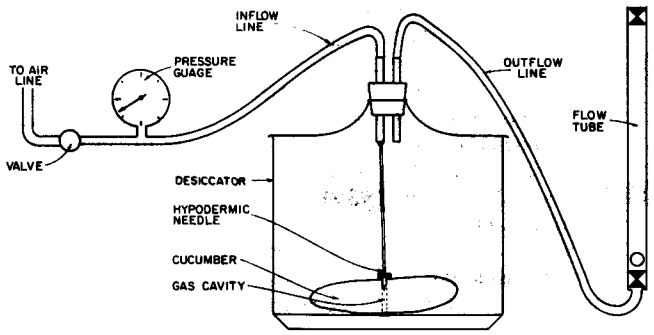

Fig. 1—Diagram of apparatus used for measuring mass flow of pressurized air through cucumber fruit.

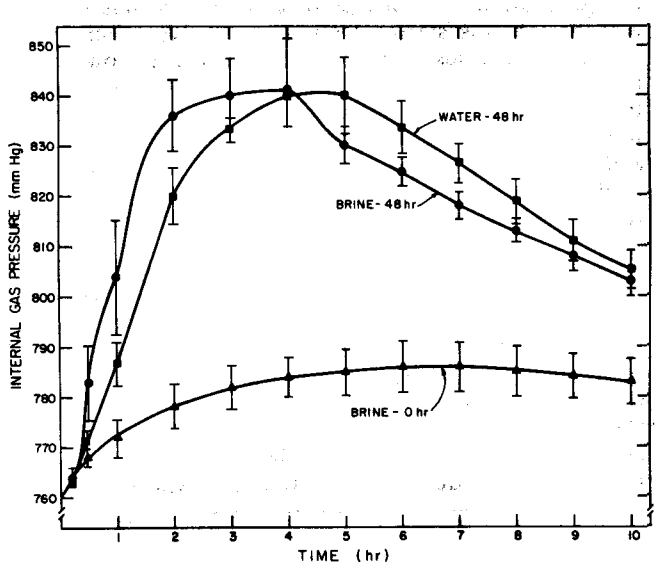

Fig. 2—Internal gas pressure changes in cucumbers held in carbonated brine or water. The cucumbers were N_2-exchanged before addition of liquid. Carbonation was begun either immediately (0 hr) or 48 hr after the addition of liquid. The liquid was continuously purged with N_2 before carbonation when carbonation was begun after 48 hr. Vertical bars represent one standard deviation.

resulted in a significant reduction in the internal gas volume of exocarp, mesocarp and seed region tissue (Fig. 3). Internal gas volume of both exocarp and mesocarp in cucumbers exposed to brine or water decreased by 50–60%. However, the internal gas volume of seed region tissue from fruit exposed to water was reduced by only 29% compared to 51% reduction in fruit exposed to brine.

Mass flow

Air under pressure readily flows through fresh cucumbers in air, indicating the presence of realtively continuous channels from the interior to the exterior of such fruit (Fig. 4). Upon submergence in brine or water, flow was not measurable (apparatus shown in Fig. 1) until an internal pressure of 138 mm Hg was reached in the cucumbers (Fig. 4). However, air bubbles flowing from the surface of the fruit were observed at pressures of 101 mm Hg for cucumbers submerged in water and 84 mm Hg for fruit in brine (Table 1). The flow rate through submerged, fresh fruit at a pressure of 227 mm Hg was only 31.9 $cm^3\ min^{-1}$ · fruit as compared to 97.5 $cm^3\ min^{-1}$ · fruit for fresh fruit in air.

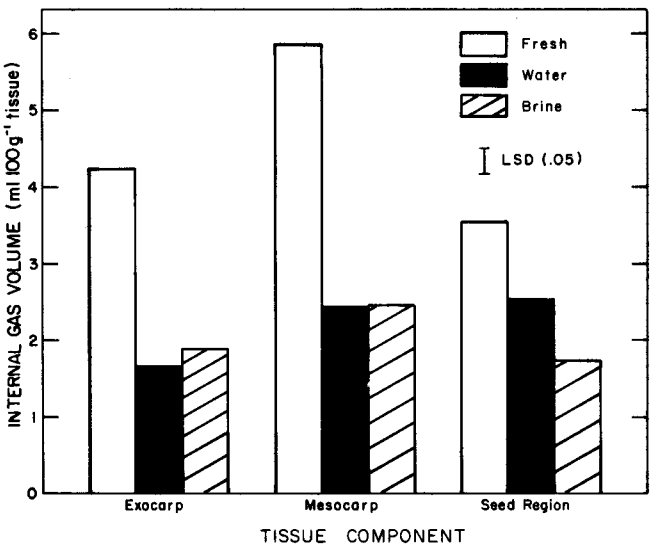

Fig. 3—Internal gas volume of exocarp, mesocarp and seed region tissue of fresh fruit, and of fruit held in brine or water solutions for 48 hr. The fruit were N_2-exchanged before liquid storage. Mean separation is indicated by vertical LSD bar, 5% level.

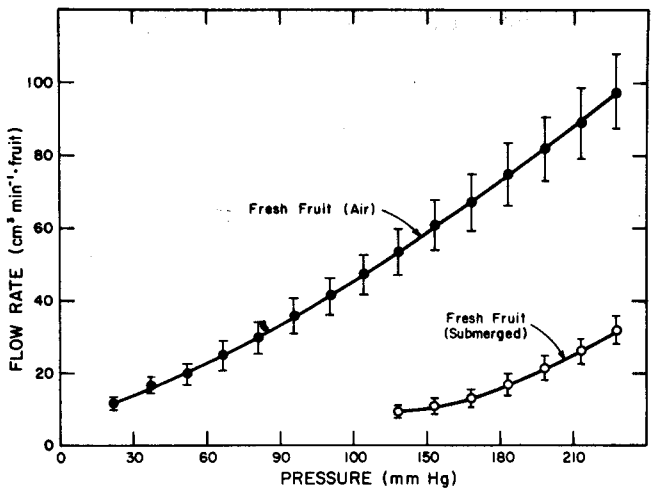

Fig. 4—Flow of pressurized air through fresh cucumbers in air or through cucumbers submerged in water or brine at various pressures. Each point represents the mean of 6–8 replications. Vertical bars represent one stardard deviation.

Gas flowed at a much greater rate in response to internal pressurization from fresh cucumbers with both ends removed than from intact fruit, as measured in air (Fig. 5). There was no gas collected from intact cucumbers pressurized at 70 mm Hg for 5 min following 48 hr brining. However, if both ends were removed from the cucumbers after the 48-hr brining, a flow rate of 4.1 ± 2.5 $cm^3\ min^{-1} \cdot cm^{-2}$ (4 replications) was measured in air at an internal pressure of 70 mm Hg (using the apparatus of Fig. 1).

A measurable flow did not occur through cucumbers exposed previously to water or brine for 48 hr even at pressures as high as 300 mm Hg. However, a slight flow was observed at lower pressures (Table 1) in the form of one to several streams of small air bubbles coming from the surface of each fruit. The pressure was increased to 300 mm Hg inside cucumbers exposed to water and maintained for 2 min. Fruit treated in this manner did not exhibit carpel separation, whereas carpel separation in fruit previously exposed to brine for 48 hr occurred at an average pressure of 202 mm Hg (Table 1). Since the internal gas pressure was increased rapidly and did not simulate closely the natural course of gas pressure development, carpel separation rather than bloater damage was used to describe damage to the

Table 1—Internal pressures for gas flow from cucumbers submerged in water or brine, and for carpel separation

Cover solution[a]	Time held in solution (hr)	Internal gas pressure for: Earliest observable flow (mm Hg)[b]	Carpel separation (mm Hg)
Without NaCl	0	101 ± 17[c]	–[d]
	48	186 ± 66	>288[e]
With NaCl	0	84 ± 7[c]	–[d]
	48	159 ± 20	202 ± 19[f]

[a] Cover solutions contained 0.32% (v/v) glacial acetic acid and 0.20% (w/v) sodium benzoate. NaCl, when added, was 10.6% (w/w).
[b] Values represent means of 4 replications ± 1 standard deviation.
[c] Measurements were made immediately after addition of solutions.
[d] Fruit were later held in solution for 48 hr following flow measurements and were therefore not tested for carpel separation.
[e] Pressure was increased from 186 mm Hg to 288 mm Hg within 30 s and maintained at 288 mm Hg for 2 min.
[f] Severe carpel separation occurred in all fruit.

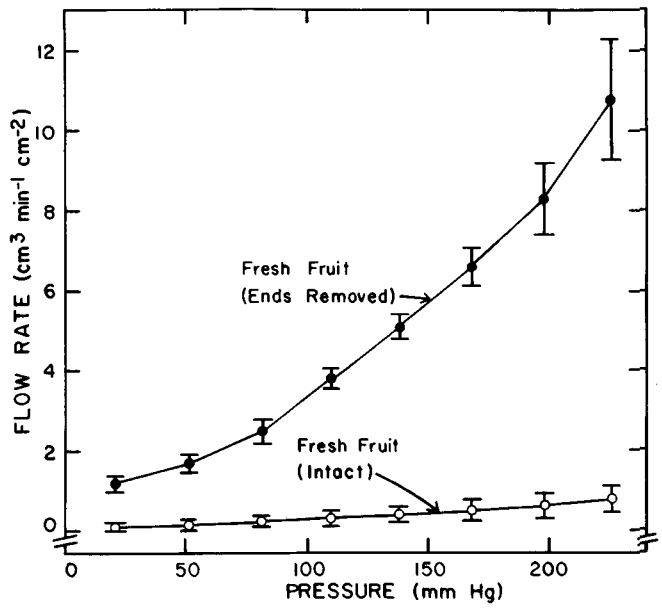

Fig. 5—Flow of pressurized air through intact fresh cucumbers and fresh cucumbers with both ends removed as measured in air. Each point represents the mean of 4 replications. Vertical bars represent one standard deviation.

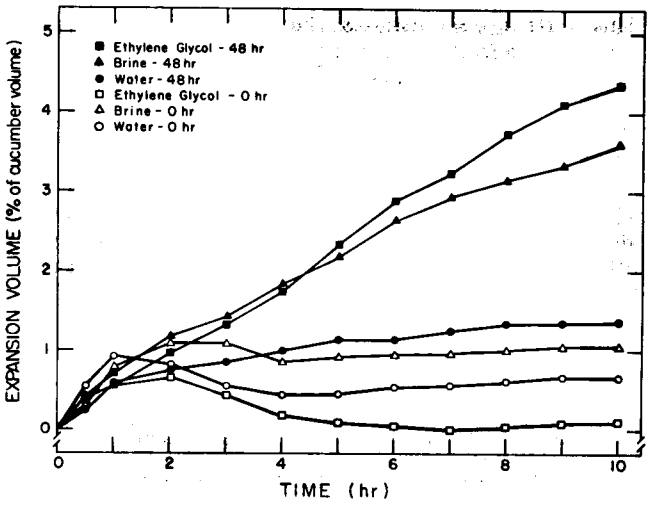

Fig. 6—Expansion volume changes in cucumbers held in carbonated solutions. The cucumbers were N_2-exchanged before addition of solutions. Carbonation was begun either immediately (0 hr) or 48 hr after addition of solution. The cover solutions were water, 16.8% (v/v) ethylene glycol or 10.6% (w/w) NaCl. Each point represents the mean of duplicate 3.8 liter jars, each containing 12—14 fruit.

internal tissue. Carpel separation in fresh cucumbers has been used as a measurement analogous to balloon bloating (Wehner and Saltveit, 1982).

Low flow rates determined by gas entrapment were measured to determine if mass transport of gases can occur through cucumbers in brine. At a pressure of 70 mm Hg, a flow rate of 8.7 ± 4.2 μl min^{-1} · fruit (3 replications) was measured for fresh fruit immediately after submergence in brine. No gas was collected after 25 min at pressures of 70—100 mm Hg from cucumbers exposed to brine for 48 hr.

Effect of osmoticum on bloater formation

Changes in expansion volume after carbonation of N_2-exchanged cucumbers ranged from 0—1.1% in the three external solutions immediately following addition of the solutions (Fig. 6). Bloater damage was absent from these three treatments (Table 2). However, carbonation of fruit after 48 hr exposure to either osmoticum, brine or ethylene glycol, resulted in substantial expansion volume changes (Fig. 6). Bloater damage was significantly greater for cucumbers exposed to NaCl or ethylene glycol solutions than for fruit held only in acidified water for 48 hr prior to carbonation (Table 2). Cucumbers exposed to acidified water for 48 hr increased in weight by 1.65% of their initial weight as compared to losses of 6.15% and 7.43% for cucumbers held in brine and ethylene glycol, respectively (Table 3).

DISCUSSION

A major aspect of the model for the mechanism of bloater formation proposed by Fleming and Pharr (1980) is the formation of a continuous liquid-clogged region of tissue in the outer layer of brine-stored cucumbers. Internal gas pressure in excess of atmospheric pressure upon artificial carbonation of brined fruit was proposed to occur due to the greater inward transport of CO_2 compared to outward transport of N_2 in cucumbers possessing this hydrated layer, thereby causing bloater formation.

Evidence for the clogging of the intercellular passages following brine exposure was obtained in this study. A reduction in the internal gas volume of cucumbers exposed to 48 hr brining was measured throughout the fruit, including the interior seed region tissue (Fig. 3). Therefore blockage of intercellular avenues may occur not solely in the outer layer of tissue as postulated previously by Fleming and Pharr (1980). Further, since cucumbers exposed to brine become dehydrated (Table 3), the mode of clogging is probably not solely by intrusion of brine.

Table 2—Effects of cover solution and the time carbonation was initiated on susceptibility of cucumbers to bloater formation

	Time carbonation was initiated after addition of solution[b]			
	0 hr		48 hr[c]	
Cover solution[a]	Expansion volume after 10 hr (%)	Bloater index	Expansion volume after 10 hr (%)	Bloater index
Without NaCl	0.67	0	0.64	0.87
With NaCl	1.06	0	3.61	9.94
Ethylene glycol	0.12	0	4.35	18.75
LSD (0.05)	0.63	–	0.63	2.34

[a] Cover solutions also contain 0.32% (v/v) glacial acetic acid and 0.20% (w/v) sodium benzoate. NaCl, when added, was 10.6% (w/w). Ethylene glycol, when added, was 16.8% (w/w).
[b] Cucumbers were N_2-exchanged prior to addition of solution.
[c] Purged continuously with N_2 after addition of solution until carbonation was begun at 48 hr.

Table 3—Percent weight change of fresh cucumbers after 48 hr exposure to water, brine or ethylene glycol

External solution[a]	Change in weight from initial fresh weight (%)[b]
Without NaCl	+1.65 ± 0.95
With NaCl	–6.15 ± 1.47
Ethylene glycol	–7.43 ± 1.70
LSD (0.05)	1.01

[a] Cover solutions also contained 0.32% (v/v) glacial acetic acid and 0.20% (w/v) sodium benzoate. NaCl, when added, was 10.6% (w/w). Ethylene glycol, when added, was 16.8% (w/w).
[b] Values represent means of 16 cucumbers ± 1 standard deviation.

The clogging of internal gas spaces in brine-stored cucumbers may also be attributed to both an exosmosis of water and shriveling and collapse of the tissue (Fleming and Thompson, 1982) leading to the reduction in internal gas volume throughout the fruit (Fig. 3). In contrast, the reduction in internal gas volume of cucumbers exposed to water for 48 hr is attributed to the entrance of liquid water into the internal gas spaces, since these cucumbers increase in weight (Table 3).

Although there is a reduction in the internal gas volume of the interior tissue of brined cucumbers, the exocarp is the main component of resistance to the mass flow of gases through the fruit surface in both fresh fruit (Fig. 5) and brined fruit (see Results). Previous work of Fleming et al. (1973a) showed that cucumbers pierced to a depth of 1 inch with a bed of 20-guage needles did not bloat. In addition, bloater damage was also reduced in fruit that were peeled before brining.

The above observations along with mass flow measurements of brined cucumbers with ends removed suggest that mass flow may act to relieve pressure and subsequent bloating of brined cucumbers when resistance to gas transfer is artificially reduced. However, in intact cucumbers there is a reduction in mass flow of gases through brine- or water-exposed cucumbers as measured by an increase in the pressure required to provide observable flow from the cucumber surface compared to fresh fruit (Table 1). This provided additional evidence for the clogging of the intercellular avenues for gas exchange.

Evidence that fresh cucumbers possess relatively continuous intercellular spaces (allowing a more rapid transfer of gases than brine- or water- stored fruit) were obtained from the following observations: (1) lower internal gas

pressure in fresh cucumbers carbonated immediately following brine addition as compared to cucumbers carbonated following exposure to water or brine for 48 hr (Fig. 2), (2) higher internal gas volume of fresh cucumbers compared to brine- or water- stored cucumbers (Fig. 3), and (3) high flow rate of air through fresh cucumbers upon internal pressurization (Fig. 4). Those findings were consistent with the results and ideas of Fleming and Pharr (1980) that gases in fresh cucumbers can exchange rapidly with the ambient gaseous environment.

However, there is a substantial reduction in the mass flow of air through internally pressurized cucumbers immediately following submergence in either water or brine compared to the mass flow rate through the same cucumbers in air prior to submergence. This immediate reduction in the pressure-induced flow of gases through intact fresh cucumbers upon submergence may be explicable on the basis of opposing surface forces of the liquid at the interface of the liquid and openings on the surface of the fruit (Corey, 1982).

Nevertheless, there is the potential for mass flow of gases to occur through fresh cucumbers in brine. The lower internal gas pressure measured in fresh cucumbers carbonated immediately following brine addition compared to brine- or water- stored cucumbers (Fig. 2) may be due to mass flow of gases acting in the release of pressure. The fact that a measurable flow of air was obtained for fresh cucumbers in brine at a gas pressure of 70 mm Hg (i.e. 8.7 μl min^{-1} · fruit) indicated that internal gas pressures of that magnitude would probably not generally be reached in fresh cucumbers carbonated immediately following addition of brine.

The development of internal gas pressure is not a sufficient condition to account for the occurence of bloaters since water-stored cucumbers did not exhibit bloater damage. Further, the acquisition of susceptibility to bloater formation is due to an effect of the NaCl solution. The role of the NaCl in bringing about a bloater-susceptible condition in pickling cucumbers is apparently due to its effect as a strong osmoticum, causing the dehydration of the tissue (Table 3). This idea was supported by the expansion volume (Fig. 6 and Table 2), bloater index (Table 2) and weight loss (Table 3) resulting from 48 hr exposure to ethylene glycol solution having the same osmotic pressure as the NaCl solution. Apparently, there is a decreased tissue resistance to internal gas pressure in cucumbers exposed to the dehydrating effects of a hypertonic solution relative to the tissue resistance of fresh fruit or fruit exposed to a hypotonic solution such as water. Wehner and Saltveit (1982) found that internal air pressures of 0.68–2.0 atmospheres above atmospheric pressure were needed to cause carpel separation in several cultivars of fresh pickling cucumbers. Those measurements are in sharp contrast to the maximum internal gas pressure of about 0.1 atmosphere (80 mm Hg) above atmospheric pressure associated with bloater damage in brine stock as measured in this study. This further demonstrates the large difference in tissue resistance of fresh compared to brined cucumbers.

In summary, findings of this study suggest that the acquisition of susceptibility to bloater formation in pickling cucumbers is associated with the following mutually dependent conditions: (1) a change in the gas exchange properties of the cucumber such that an altered transport of gases occurs leading to the development of internal gas pressure, and (2) a decreased resistance of the tissue to internal gas pressure brought about by exposure to high solute concentration.

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