Trends in Food Science & Technology 81 (2018) 185–192

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Review

Viability of commercial cucumber fermentation without nitrogen or air purging☆

Yawen Zhai[a], Ilenys M. Pérez-Díaz[b,*], Joscelin T. Diaz[a]

[a] North Carolina State University, Department of Food, Bioprocessing, and Nutrition Sciences, 400 Dan Allen Drive, Raleigh, NC, 27695, USA
[b] USDA-Agriculture Research Service, SEA, Food Science Research Unit, 322 Schaub Hall, Box 7624, North Carolina State University, Raleigh, NC, 27695, USA

ARTICLE INFO

Keywords:
Cucumber fermentation
Bloater
Microbiota
Carbon dioxide
Purging
Pickling industry

ABSTRACT

Background: Bloated cucumber defect, resulting from the accumulation of the biologically produced carbon dioxide (CO_2) in the fruit, reduces yield and economic gains for the pickling industry worldwide. It was the aim of this review to identify commonalities among effective strategies to reduce bloater defect and determine the theoretical viability of commercial cucumber fermentations without bloater defect and/or purging.
Scope and approach: This article summarizes the known causes of fermented cucumber bloating defect, including sources of CO_2, and the strategies developed to mitigate the production of the carbonic gas such as controlled fermentation, inoculation of selected starter cultures, cover brine acidification and reformulation and the application of air or nitrogen purging.
Key findings and conclusions: It was understood that microbial activity during fermentation, cucumber tissue respiration, as well as the pressure in the fruits and fermentation tanks, ambient temperature and cover brine composition, impact the levels of dissolved CO_2 in the system. Although the biological conversion of oxygen to CO_2 reduces the cucumbers internal gas pressure, the dissipation of the gas from the tissue is reduced by brining. Once the gas accumulates in the cucumber tissue in concentrations high enough to displace it, the irreversible formation of hollow cavities or bloaters occurs. Residual CO_2 is produced by acid-preserved cucumbers, presumably by tissue respiration, which results in the absence of bloating. Thus, microbial activity seems to contribute most of the CO_2 needed for cucumbers to bloat. It is speculated that colonization of the internal cucumber tissue by indigenous microbes, in particular aerobic gram-negative bacteria, results in the localized production of CO_2 causing bloating defect early in the fermentation. It is concluded that effective manipulation of the microbiota, reduction of dissolved oxygen levels and the use of adequately selected starter cultures may enable cucumber fermentations of acceptable quality without purging.

1. Introduction

The formation of gas pockets in fermented cucumbers mesocarp and endocarp or seed cavity results in bloater defect, which causes relevant yield and economic losses for the pickling industry worldwide (Fleming, 1979; Fleming, Thompson, Etchells, Kelling, & Bell, 1973b; Jones, Etchells, Veerhoff, & Veldhuis, 1941). It even prevented the implementation of a promising technology to ferment cucumbers without sodium chloride (NaCl), incorporating the use of calcium acetate as a buffer, in 1995 (Fleming, McDonald, McFeeters, Thompson, & Humphries, 1995). Economic losses are mainly associated with the inadequacy of bloated fermented cucumbers for the manufacture of chips, which are characterized by undesirable holes and appearance, occasionally resembling a half moon.

Fig. 1 describes the acuteness of the injuries (slight, medium and severe) and type of tissue disruption (honeycomb, lens or balloon) that may be observed in bloated fermented cucumbers (Wehner & Fleming, 1984). All possible combinations of injury acuteness and type of tissue disruption have been documented. The extent of cucumber tissue damage caused by bloating is measured by the bloater index. The bloater index for batches of fermented cucumbers is calculated from the numerical percentage of bloaters adjusted by the degree of severity and tissue disruption type as defined by the weighted damage values proposed by Fleming and colleagues (Fleming, Thompson, Bell, & Monroe,

☆ Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

* Corresponding author.
E-mail address: Ilenys.Pérez-Díaz@ars.usda.gov (I.M. Pérez-Díaz).

https://doi.org/10.1016/j.tifs.2018.05.017
Received 26 June 2017; Received in revised form 4 May 2018; Accepted 19 May 2018
Available online 21 May 2018

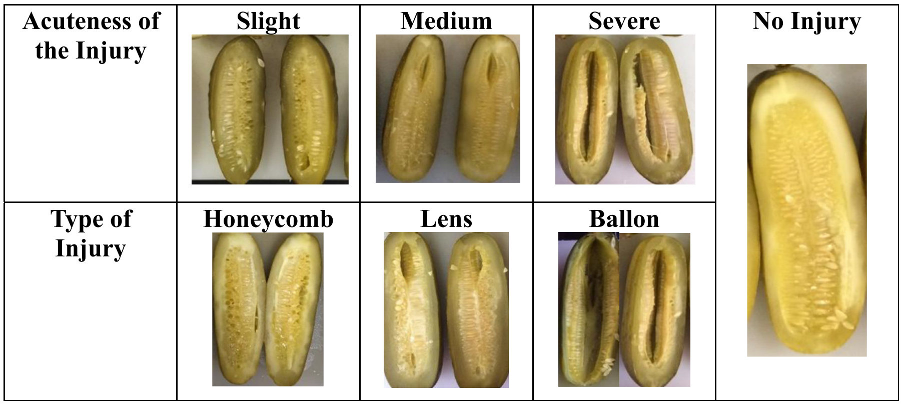

Fig. 1. Description of types of bloater defect injuries for quantification and evaluation purposes. All combinations of acuteness and injury type have been documented.

1977; Fleming, Thompson, & Monroe, 1978b). In general, larger size cucumbers, such as 3A (3.8–5.1 cm diam.) and 3B (5.1–5.7 cm diam.) are more vulnerable to bloater defect as compared to smaller sizes (less than 3.8 cm diam.) (Fleming, Thompson, Etchells, Kelling, & Bell, 1973a).

Although the formation of hollow cavities in fermented cucumbers is associated with an elevated concentration of CO_2 accumulated internally, the level of dissolved carbon dioxide (dCO_2) in fermentation cover brine is not linearly correlated with bloater index. Corey, Pharr, and Fleming (1983a) determined that cucumbers brined with 2 M (11.7%) NaCl, representative of the typical un-equilibrated fermentation cover brine, creates a vacuum in the fruits mesocarp and endocarp increasing the resistance to gases mass flow. Although conversion of O_2 to CO_2 enables the flow of liquid into cucumbers, the dissipation of CO_2 is limited (Corey, Pharr, & Fleming, 1983b). Thus, measurement of CO_2 levels within brined cucumbers likely has more predictive value as compared to dCO_2 in fermentation cover brines.

This article documents cucumber bloater defect to gain insights regarding the viability of purging-free commercial cucumber fermentations. It includes the causes of bloater defect, sources of CO_2 conducive to bloating, strategies to mitigate the production of such gas in fermentations and the application of air or nitrogen (N_2) purging to reduce bloater index in commercial operations. Application of N_2 purging in commercial cucumber fermentations increases production cost. Although air purging is a more economical solution to minimize bloating defect, it incorporates oxygen in the system triggering chemical oxidation, growth of undesired aerobic microbes, cucumber tissue softening and other side effects. A solution to effectively manage fermented cucumber bloater defect is still needed.

2. Sources of CO_2 in cucumber fermentation that influence bloater index

CO_2 is produced by cucumber tissue respiration or the microbiota metabolic activity, which includes that from yeasts, molds and bacteria. Production of CO_2 in cucumber fermentation is demonstrated to be the culprit in the development of bloater defect especially that derived from microbial activity (Etchells & Jones, 1941). The gas contained in cucumbers hollow cavities, either CO_2 or a mixture of CO_2 and hydrogen (H_2) (Jones et al., 1941; Veldhuis & Etchells, 1939), matches the composition of the gas dissolved in fermentation cover brines. The production of such gases corresponds to the presence *Enterobacter* spp. in combination with yeasts and selected lactic acid bacteria (LAB) (Etchells, 1941; Etchells, Borg, & Bell, 1968; Etchells, Fabian, & Jones, 1945; Fleming et al., 1973b). Given that the metabolic activity of yeasts results in the formation of ethanol and CO_2 from sugars (Etchells & Jones, 1941), such microbial population was originally associated with bloating. However, it was demonstrated that the incidence of bloaters in fermented cucumbers remained unchanged in batches where yeast growth was suppressed by supplementing with up to 0.1% potassium sorbate or sorbic acid, a yeast inhibitor (Etchells et al., 1968). Enterobacteriaceae, such as, *Enterobacter aerogenes*, and *Escherichia coli*, and halophilic bacteria can produce H_2 and CO_2, possibly inducing bloater defect (Etchells et al., 1968; Veldhuis & Etchells, 1939). However, such microbes are known to be sensitive to the acidic pH that develops as the result of fermentation, reaching undetectable levels by the third day of the process (Etchells et al., 1968). Microorganisms that have been implicated in the formation of bloaters include heterofermenter, facultative heterofermenter and homofermenter LAB, in particular those able to decarboxylate malic acid (Etchells & Bell, 1950; Etchells et al., 1968; Fleming et al., 1973b, 1973a; McFeeters, Fleming, & Daeschel, 1984; McFeeters, Fleming, & Thompson, 1982). The heterofermenter LAB, *Lactobacillus brevis*, have been isolated from defective commercial batches and found able to produce sufficient CO_2 during fermentation to cause cucumber bloating (Etchells et al., 1968). Additionally, it is estimated that the malic acid decarboxylating activity of selected facultative heterofermenter LAB, such as *L. plantarum*, can generate 84 mg of CO_2 per 100 g of cucumbers in a fermentation, which is sufficient to cause bloaters (Daeschel, McFeeters, & Fleming, 1985).

Given the influence of the microbial activity on the incidence of bloaters, factors affecting the microbial composition and behavior, such as brining conditions, cover brine strengths, supplementation with lactic acid or sugar and temperature (Jones et al., 1941; Samish, Etinger-Tulczynska, & Bick, 1957) also influence bloater incidence. While supplementation of cucumber fermentations with lactic acid, vinegar or sugar induces a significant increase in bloaters, a reduction in salinity (0–30° Salometer) has the opposite effect (Jones et al., 1941). Larger amounts of gases are measured in 40–80% saturated cover brines than in the 20 and 30% counterparts, likely due to lower gas solubility in the high salt solutions (Fleming et al., 1973a; Veldhuis & Etchells, 1939).

Additional sources of CO_2 include the natural water carbonation, respiration of the fresh cucumber tissue and incorporation of atmospheric CO_2 in the fermentation system. Thus, cucumber size, cultivation conditions, and variety have also been identified as factors influencing the extent of bloating, due in part to their role in tissue respiration (Fleming et al., 1973b). Cucumber flesh is rich in channels that represent a transport system for gases including CO_2 and air (Fleming, 1984). The O_2 supplied in the equilibrated atmospheric air becomes a substrate for cucumber tissue respiration. The CO_2 produced by tissue respiration is trapped in bloaters, in particular in the brined fruits. When pasteurized cucumber jars were examined for CO_2 levels, it was found that about 7% of the gas still formed in the absence of microbial activity (Fleming et al., 1973a). This observation suggested that tissue respiration contributes to CO_2 production. The contribution of tissue respiration to the total amount of CO_2 varies depending on the physiological state of the fruit prior to brining and post-harvest storage conditions (Eak & Morris, 1956). Pre-brining conditions and O_2, acid and NaCl levels also influence respiration rate (Fleming et al., 1973a). Differences in resistance to bloating has been observed among cucumber varieties (Wehner & Fleming, 1984).

Other sources of fermented cucumber damage that may exacerbate the quality issues associated with bloater defect include pre-harvest fruits condition, tanking injuries of the fresh fruits and hydrostatic pressure in tanks with more than 6 ft depth (Fleming et al., 1977). Fresh cucumbers tanking injuries result from the deposition of the fruits into the fermentation vessels filled with cover brines of low buoyancy force. Increasing tank depths augments the buoyancy force on cucumbers near the top of the tank, restrained below the free liquid surface (Fleming, 1979; Fleming et al., 1977; Humphries & Fleming, 1986).

The mechanism of bloater formation proposed by Etchells et al. (1968) and modified by Fleming (1984) suggest that the CO_2 produced in the cover brine diffuses into the cucumbers as part of the equilibration process or homeostasis. Once 12.5 mM of CO_2 is produced from sources other than malic acid degradation (McFeeters et al., 1984), the internal tissue pressure increases producing enough force to displace the endocarp and/or seed cavity tissue towards the exocarp or skin, forming hollow cavities or gas pockets within the fruit. Thus, bloater defect initiates when 20–60 mg of CO_2 per 100 mL of cover brine accumulates in the system, depending on brining conditions, such as salt level and temperature, microbiota, initial sugars and malic acid content in the cucumbers and variety of cultivar (Etchells & Jones, 1941; Fleming, 1979, 1984; Fleming et al., 1973b, 1973a; McFeeters et al., 1984). Evaluation of bloater resistance in pickling cucumbers using a brine carbonation method suggest that the selection of cultivars with balloon bloater resistance for tissue displacement may reduce the necessity for purging in commercial tanks (Wehner & Fleming, 1984).

3. Utilization of air or N_2 purging to reduce bloater index in commercial scale operations

Studies have shown that controlled cucumber fermentation and purging of cover brines effectively reduces bloater damage (Fleming, 1979). N_2 or air purging is most commonly used in the pickling industry to reduce the incidence of bloaters. A review by Fleming (1979) is the most comprehensive revision on the application of N_2 purging to reduce the incidence of bloaters. This concept proposes that a source of N_2, an inert gas, at the bottom of a container, using a diffuser or sparger, can generate relatively small bubbles that increase in size as they travel through the fermentation cover brine from the bottom of the tank to the top, trapping CO_2. This was based on the observation that the absorption of CO_2 by N_2 bubbles is faster than the surfacing of the mixed gas bubbles. Fig. 2 describes some of the earliest systems proposed for N_2 purging, in which bubbles of the gas are emitted at the bottom of commercial tanks, through the holes made on plastic concentric or spiral pipes.

It has been proposed that bloating incidence can be reduced if levels of CO_2 are maintained below 30% and 50% saturation at temperatures above and below 75 °F, respectively (Fleming, 1979), in cover brines with 25° salometer (10% NaCl). CO_2 saturation is defined as the division of the measured CO_2 in the cover brine by the maximum level of CO_2 possible at a given temperature and pressure by 100 (Etchells et al., 1968; Fleming, Thompson, Bell, & Hontz, 1978a). Readers are encouraged to review Fig. 1 from Fleming, Etchells, Thompson, and Bell (1975) for more details on the solubility of CO_2 at variable temperatures and salt concentrations.

Continuous purging has been proven to be more cost efficient as compared to intermittent purging, typically applied for periods of 4 or 20 h, followed by a shutdown period of 4 h. Intermittent purging is often needed at a higher flow rate to be as effective as continuous purging (Fleming et al., 1975). Most publications in this area coincide that a minimum N_2 gas flow rate of 40 square cubic feet per hour (SCFH) is needed to minimize the incidence of bloaters in commercial tanks of 40,000 L capacity.

A subsequent proposal for purging to reduce the incidence of bloaters pioneered by Costilow, Bedford, Mingus, and Black (1977) was based on the observations that N_2 sparging from the bottom of the tanks lacked uniformity as the gas bubbles would find paths of less resistance where cucumbers are not in close proximity. It was also considered that the N_2 bubbles may move differently throughout the matrix in terms of time and may coalesce to form larger bubbles that would remain in the system for a longer period of time, making the system less efficient. The system shown in Fig. 3 was proposed by Costilow et al. (1977) to overcome the challenges described above. The objective of both of the systems shown in Fig. 3 is to concomitantly purge and circulate the cover brine for a more efficient removal of gaseous CO_2 from the fermentation tanks. The application of one of such systems adds the ability to mix-in ingredients for the adjustment of salometer, acidity or preservative levels, as well as for the incorporation of rain water throughout the fermentation tanks, preventing its accumulation on top. It was estimated that these systems are more efficient at removing CO_2 with purging at lower flow rates, translating into a lower demand for gases and thus reducing the production cost. Fig. 4 shows the system most commonly used at the commercial scale to date, which essentially combines the most efficient features of the two systems initially proposed by Costilow et al. (1977). The main modification made was a shorter head on the purging system located right at the interface between the surface of the cover brine and the atmospheric air, enabling the delivery of the cover brine from the bottom of the tank to the center top, which induces a more efficient circulation. It also accommodates

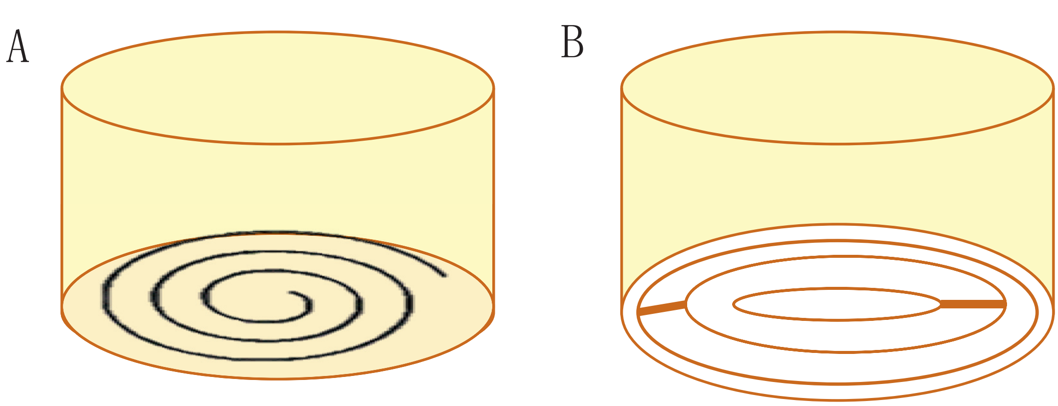

Fig. 2. Schematic of a fermentation tank bottom equipped with N_2 purging systems. Panel A shows a representation of a spiral gas dispensing pipe of 3/4″ diameter flexible plastic drilled with 14, 1/64″ diameter holes made on a 30° angle with respect to the contact point on the surface of the tank and interspaced every 2 ft. The concentric configuration shown on panel B was constructed with the same specifications.

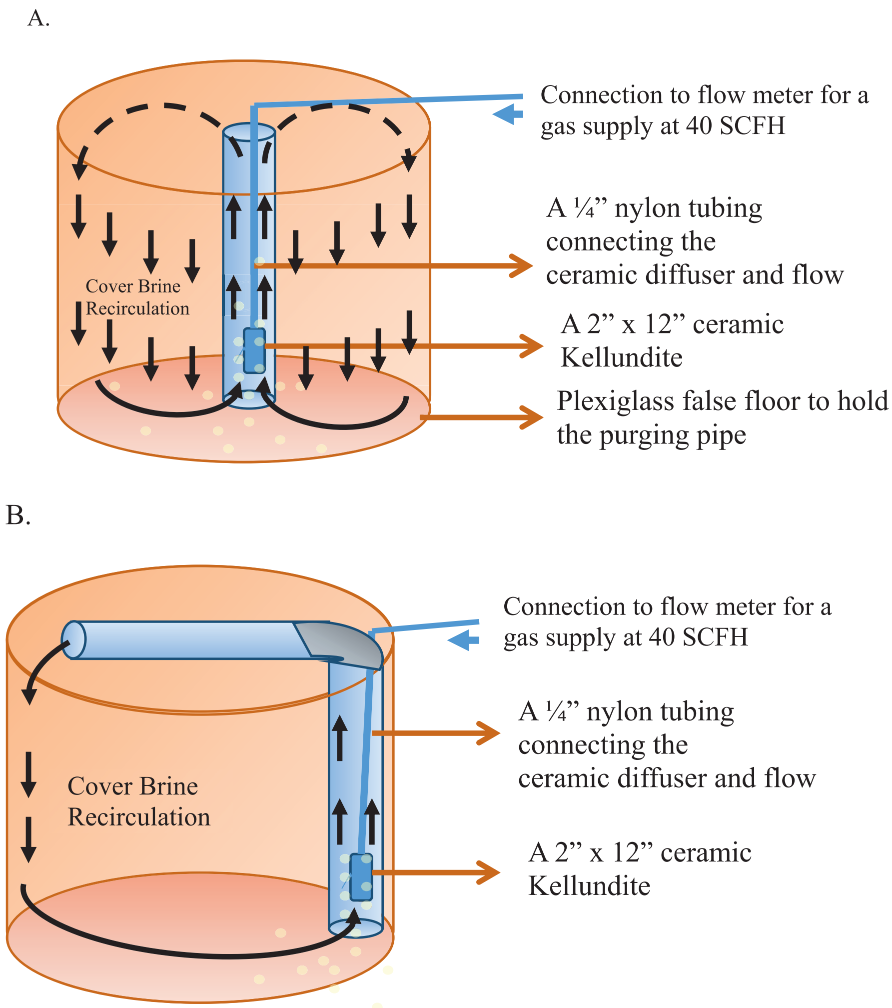

Fig. 3. Purging system designs proposed by Costilow et al. (1977). Panel A shows the incorporation of a false floor of Plexiglas at the bottom of a fermentation tank to hold a centric 4″ diameter pipe that serves as a case for a ceramic N_2 gas diffuser with 10 μm pores. The delivery of N_2 bubbles to the bottom of the tank forces the liquid to move up the pipe. Deposition of the cover brine on the surface results in its inevitable movement to the bottom of the tank propelling circulation. Panel B shows the schematic representation of a similar system with the purging system located to the side of the tank to facilitate its secure placement and removal as needed, if the operator stands on a wooden platform around the tank. Delivery of the cover brine from the bottom of the tank to the opposite side on the surface results in circulation next to the tank wall as opposed to thru the center of the tank.

for placement of the purging system on the side of the tank, eliminating the need for a false bottom.

For the purging to function properly, the CO_2 has to be present in the system in its gaseous form. This is also true for bloater formation. The CO_2 first dissociation constant (pKa1 in a 6% NaCl at 25 °C) is 5.94, meaning that 50% of it exists as carbonic acid and the remaining 50% as gaseous CO_2 (Harned & Bonner, 1945). Therefore, the application of purging in a system with a pH of 5.94 or above would only eliminate

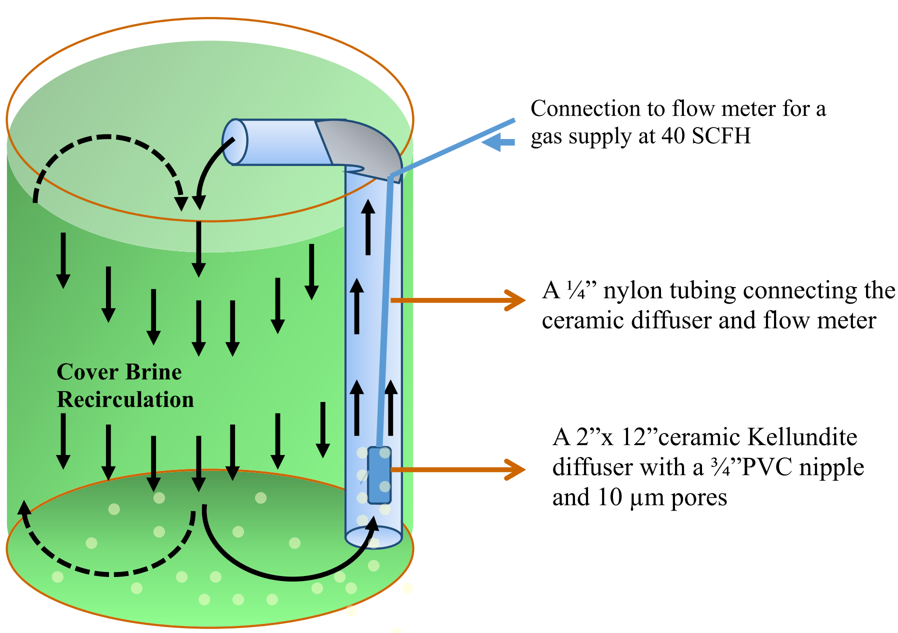

Fig. 4. Schematic representation of the purging system currently most commonly used in the pickling industry in the US. A diffuser is located on the side of the tank, closer to a wooden platform surrounding it, for easiness of removal/insertion. The diffuser is encased in a 4″ PVC pipe, which is perforated at the bottom to deliver N_2 or air gas. The incorporation of the gas in the liquid forces the liquid to move up the pipe. Given that the head of the pipe is elongated to reach the center of the tank, the cover brine moving up the pipe is delivered to the center of the tank and moves down once on the surface, initiating the circulation of the liquid through the center of the system.

50% or less of the total CO_2. Remaining dissolved gas is consequently available for the formation of bloaters. A reduction in pH, meaning natural or artificial acidification, is the main consideration in increasing the levels of gaseous CO_2. A fermentation system pH of 4.94 or 5.0 is thus conducive to the presence of 90% or more of the CO_2 as gas, which can cause bloaters and may also be effectively removed by purging. A delay in the application of purging after filling up the tanks, can thus result in high incidence of bloaters. Accumulation of enough CO_2 in the fermentation system is the only requirement for formation of bloaters and its development is irreversible. Similarly, maintenance of an initial pH of the fermentation at 5.0 or below benefits the conversion of the pre-existing CO_2 in the fermentation system to the gas form and can be effectively removed by purging. The effect of cover brine equilibration with the cucumber water soluble components results in changes in pH, which may cause an increase to values above 5.0, initially.

Costilow, Gates, and Bedford (1981) also proposed the most economical air purging instead of N_2 purging as an effective way to reduce the incidence of bloaters without significantly sacrificing the quality of the fermented stocks. Although this group did not observe significant differences among air and N_2 purged commercial fermentations, air purging at high rates (100 mL air/minute) in 1 gallon jar fermentations were characterized by rapid and extensive cucumber softening (Gates & Costilow, 1981). Cucumbers softening has been observed as the results of aerobic mold growth on the surface of the brined cucumbers in commercial fermentations purged at high air flow rates (Costilow, Gates, & Lacy, 1980). Up to 6 ppm of dissolved oxygen may be present in cucumber fermentations depending on salt concentration and temperature. In continuously aerated cover brines, the dO_2 levels increased at the outset of the fermentations followed by a decrease and the development of an O_2 uptake demand and film yeasts growth (Potts & Fleming, 1979). However, N_2 purged fermentations presented a negligible dissolved oxygen level and low O_2 uptake, conducive to the dominance of the desired LAB (Potts & Fleming, 1979). To date, air purging has become the preferred method used within the pickling industry to reduce the incidence of bloaters, given the low cost of application. However, air purging is not only a relevant source of O_2 for softening-associated molds, but also for the growth of other undesirable aerobic organisms, off-flavors, oxidation and enhanced CO_2 production from aerobic biological processes including tissue respiration (Fleming, 1979; Zhou, McFeeters, & Fleming, 2000). Consequently, the utilization of air purging to reduce the incidence of bloaters necessitates the identification of strategies to minimize its impact on the quality of the fermented cucumbers. The use of N_2 purging for at least the first 2 d of laboratory scale fermentations is known to prevent tissue softening and improve the quality of fermented cucumbers (Gates & Costilow, 1981).

4. Utilization of controlled fermentations to reduce bloater index in commercial scale cucumber fermentations

Controlled lactic acid fermentation of cucumbers was developed by Etchells, Bell, Fleming, and Thompson (1976a) with the aim to reduce the inconsistency on the salt-stock quality resulting from the heterogeneous and complex microbiota present in the fresh cucumbers. To some extent controlled cucumber fermentation is one of the practices commonly used in the pickling industry to minimize bloater defect (Etchells, Bell, Fleming, Kelling, & Thompson, 1973; Wehner & Fleming, 1984). Controlled fermentations are characterized by the steps described in Fig. 5 (Etchells et al., 1976a; Etchells, Fleming, Bell, & Thompson, 1976b). The blanching of cucumbers followed by inoculation with a starter culture prior to fermentation, has also been effective in reducing bloater index (Gates & Costilow, 1981). In essence, the main objective of the steps recommended for controlled fermentation aim at reducing the density of the cucumbers microbiota.

5. Utilization of malic acid decarboxylase deficient (MDC-) LAB as starter cultures for commercial cucumber fermentations

Preferred starter cultures for cucumber fermentations include the facultative heterofermenter lactic acid bacteria (LAB), *L. plantarum* and *L. pentosus* and the homofermenter LAB, *Pediococcus* spp. (Etchells et al., 1976b, 1976a). Such LAB are known to dominate in cucumber fermentations (Pérez-Díaz et al., 2016) and produced relatively low levels of CO_2 as compared to the heterofermenter LAB, *L. brevis*. Although *L. plantarum* and *L. pentosus* prevail in cucumber fermentations producing mostly lactic acid, they are both able to produce CO_2 from a number of decarboxylation pathways such as the conversion of malic acid to lactic acid. Thus, the utilization of a starter LAB deficient in malic acid decarboxylation has been suggested as an additional strategy to reduce the incidence of bloaters in cucumber fermentations (Daeschel, McFeeters, Fleming, Klaenhammer, & Sanozky, 1987). However, cultures naturally deficient in malic acid decarboxylation, such as *L. plantarum* FS965, isolated from cheddar cheese, and *L. plantarum* WSO-M35 and MOP3-M6, derived from the chemical mutagenesis of a cucumber fermentation isolate, are unable to complete the desired conversion of sugars to lactic acid (McDonald, Shieh, Fleming, McFeeters, & Thompson, 1993; McFeeters et al., 1984). Cultures deficient in malic acid decarboxylation also present longer growth lag phases and generation times in cucumber juice (McDonald et al., 1993; McFeeters et al., 1984). Improved performance of cultures deficient in malic acid decarboxylation may be achieved by blanching the fresh cucumbers prior to fermentation, and sanitizing the fermentation vessels, so that the naturally occurring LAB are suppressed (McDonald et al., 1993). Inoculation of the deficient starter cultures to 2 log of CFU/mL higher than the microbiota enables its prevalence (Breidt & Fleming, 1992).

6. Application of acidification of cover brines to reduce bloater index in commercial scale cucumber fermentations

Acidification of the fermentation system to a pH around or less than 4.6 can help with the surfacing of CO_2. The cover brine pH is a factor affecting CO_2 solubility, as it determines the proportion of dissolved CO_2 with respect to the total gas content (Fleming et al., 1973b). More of the HCO_3^- species exists at pH 3.6 or lower (Greenwood & Earnshaw, 1997, p. 310). Two species, H_2CO_3 and HCO_3^-, exist to 50% each at an equilibrated pH of 6.35 (25 °C) (Greenwood & Earnshaw, 1997, p. 310). Thus, adjustment of the initial fermentation pH well below 6.35 should shift the equilibration of the chemical species of CO_2 towards the gaseous form, which is more readily removed from cover brines by air or N_2 purging.

Additionally, cover brine acidification with acetic acid to 0.16% reduces gas production by the Enterobacteriaceae and molds naturally present in the fresh cucumbers, as high hydrogen ion concentration in solution inhibits microbial growth (McDonald, Fleming, & Daeschel, 1991). Molds are associated with cucumber tissue softening, characterized by soft spots and skin blisters on cucumbers (Gates & Costilow, 1981; Potts & Fleming, 1982). The population of Enterobacteriaceae is known to reach numbers as high as 8 log of CFU/g of cucumber tissue and 5 log of CFU/mL of cover brine 3-day post-brining in non-acidified fermentations (McDonald et al., 1991). Such population densities decline by at least 2 log of CFU/mL by day 5 after brining. Additionally, selected members of the Enterobacteriaceae family such as *Erwinia* spp., are known to colonize the internal cucumber tissue and produce CO_2 from fermentative metabolism in the presence of O_2 (Samish, Etinger-Tulczynska, & Bick, 1963). Upon acidification of cover brines with 0.16% acetic acid, the numbers of Enterobacteriaceae steadily decline to below detectable levels 5 days after brining (McDonald et al., 1991).

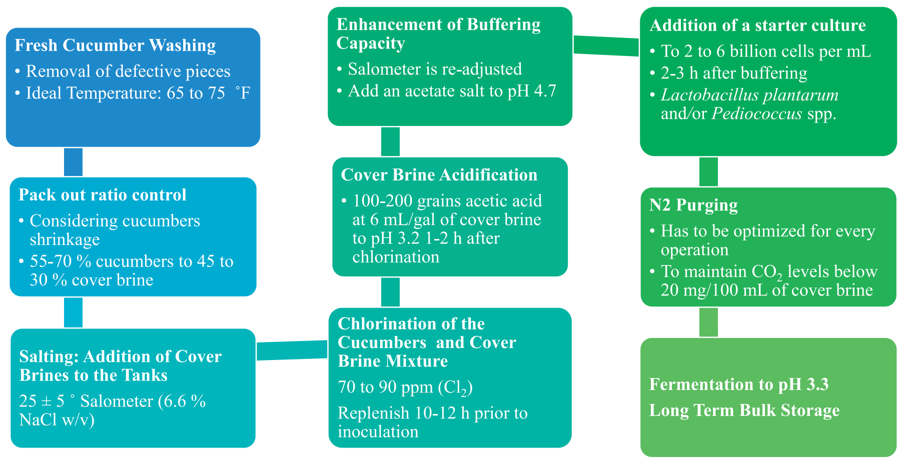

Fig. 5. Description of controlled cucumber fermentation processed proposed by Etchells et al. (1976a, 1976b) and Fleming et al. (1978a).

7. Bloater defect in low salt cucumber fermentations

The typical cucumber fermentation process uses a minimum of 6% NaCl (as equilibrated concentration) resulting in the generation of waste waters from commercial operations with a significant content of chlorides and organic matter (McFeeters & Pérez-Díaz, 2010; Pérez-Díaz et al., 2015). Treatment of such waters and disposal of sludge from water treatment ponds increases the cost of production and can result in environmental pollution, if not managed adequately (Fleming et al., 2002). Low salt and NaCl-free cucumber fermentations has been evaluated by several researchers as of today with the aim of ameliorating the environmental impact from commercial production (Etchells et al., 1976b, 1976a; Fleming et al., 1995; Guillou, Floros, & Cousin, 1992; Pérez-Díaz et al., 2015). The NaCl-free preservation of cucumbers by acidification yields fruits of unacceptable quality due to excessive bloater defect and the development of rising pH spoilage (Etchells et al., 1976b, 1976a; Guillou et al., 1992). Laboratory scale NaCl-free cucumber fermentations resulted in pickles of acceptable quality when the fresh cucumbers were blanched, brined with a calcium acetate buffer and *L. plantarum* was used as a starter culture (Fleming et al., 1995). Although cucumber bloating was prevented by the blanching step in such laboratory scale fermentations, pilot scale commercial fermentations without NaCl resulted in severely bloated fruits with significantly compromised texture (Fleming et al., 1995). A fluctuating bloater incidence, from undetectable to severe, was observed in cucumber fermentations brined with reduced NaCl (2.7%) under anaerobic conditions, which was localized at the top 3 ft of the tanks. This was presumably due to a localized higher buoyancy pressure and lower hydrostatic pressure (Fleming, McFeeters, Daeschel, Humphries, & Thompson, 1988). Bloater damage was minimal in cucumber fermentations brined with 4% NaCl using the Bag-in-Box fermentation technology proposed by Fleming et al. (2002). NaCl free cucumber fermentations brined with calcium chloride ($CaCl_2$) and potassium sorbate in open top tanks subjected to air purging are reported to support higher levels of CO_2 production within the first 3 days and more bloaters as compared to fermentations brined with 6% NaCl (Fig. 6; McMurtrie, 2016; personal communication with processors). Potential causative factors for an increase incidence of bloaters in cucumber fermentations brined with $CaCl_2$ include the application of air purging on a time limited scheduled at a lower flow rate (McMurtrie, 2016) and the reduction of the starter culture lag phase and generation time (Pérez-Díaz et al., 2015). Faster microbial growth in cucumber fermentations brined with 0% NaCl and 1.1% $CaCl_2$ results in the accumulation of higher levels of CO_2 in the vessel early in the process (Fig. 6). This situation contrasts with the slow production and gradual release of CO_2 and less bloater incidence that typically results from fermentations brined with NaCl (Fig. 6).

8. Future trends and conclusions

Are commercial cucumber fermentations without air or N_2 purging viable?

Common to all strategies proposed to reduce bloater index in commercial cucumber fermentations is the goal of reducing the density of the microbiota, which is expected to result in a limited production of CO_2. Washing of the fresh fruits, chlorination of the fermentation mass, cover brine acidification, blanching and N_2 purging are all hurdles for microbial viability. Although reducing the microbiota, represents an attractive and viable option in the task of reducing CO_2 production and, consequently, the incidence of bloaters, it hinders opportunities to use the microbial diversity to impart unique characteristics to finished products. Application of the aforementioned strategies, in particular purging, represent extreme and costly approaches to control bloater index in cucumber fermentations.

It is factual that *Enterobacteriaceae* and molds colonize the cucumber tissue and influence the quality of the fermented food. Molds can induce tissue softening. *Enterobacteriaceae* produce CO_2 in the brine, which is the primary cause of bloater formation. Together these observations suggest a more targeted approach may be developed to minimize the incidence of the defect. Expanding the knowledge of how specific microbes may be involved in cucumber tissue colonization, internal CO_2 production and formation of hollow cavities can set the stage for the development of control strategies targeting the inhibition of selected causative agents. With the current technological advances, it should be possible to develop cost effective inhibitory systems, targeting the causative agents of bloater.

A proactive approach to the reduction of bloater index in commercial fermentations is, thus, envisioned as the ability to identify the presence of causative agents in fresh cucumbers and at production sites, using practical and rapid methods for microbial identification. Results from the tests can provide the basis for a fresh cucumber fermentation fitness standard, which would benefit growers and processors. Fresh cucumber lots containing high loads of bloater-causing microbes can be designated as prospects for fresh-pack products or relish fermentations as opposed to processing by fermentation of the whole fruits. An improved understanding of the microbes directly involved in cucumber bloater defect may also be conducive to enhanced guidelines for the cultivation conditions of such cucumbers in ways that contamination with the undesired microbes is prevented.

Although, it has been also observed that the majority of the dCO_2 in cover brines comes from microbial activity and not tissue respiration (Fleming et al., 1973a; Zhai & Pérez-Díaz, 2018); the exclusion of O_2 as

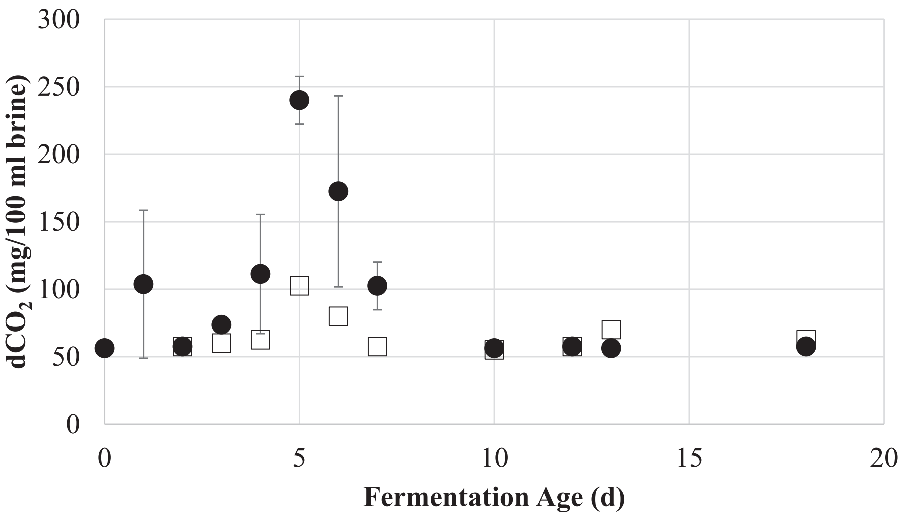

Fig. 6. Production of carbon dioxide during cucumber fermentations brined with 100 mM calcium chloride and 6 mM potassium sorbate instead of 1.03 M sodium chloride. Data represents the means for three and two commercial cucumber fermentations brined with $CaCl_2$ and potassium sorbate (●) and sodium chloride (□), respectively, and processed as described by Pérez-Díaz et al. (2015). All vessels were filled with one lot of cucumbers and exposed to the same environmental temperatures (18.4 and 25.4 °C). Dissolved CO_2 was measured as described by Fleming et al. (1973a) using a benchtop Map-Pak Combi Gas Analyzer (AGC Instruments, Co., Clare, Ireland).

the precursor for CO_2 and bloater formation seems to be an additional effective approach to reduce the incidence of bloaters. The various strategies discussed above suggest that elimination of O_2 from the fermentation system by N_2 purging, results in low bloater index. Cucumber fermentations in closed jars, with limited oxygen content, brined with an acid, a buffer and 4% NaCl, results in a marginal bloater index (Zhai & Pérez-Díaz, 2018). Additionally, it has been reported that cucumbers exposed to a pure oxygen atmosphere in a close system (O_2-exchanged cucumbers) prior to brining are less prone to bloating, given that the gas is converted to CO_2, which has a lower internal pressure (Corey et al., 1983b, 1983a; Fleming, 1984; Fleming, Humphries, Brock, & Pharr, 1983; Fleming & Pharr, 1980; Fleming, Pharr, & Thompson, 1980). Together these observations suggest decarboxylation reactions by microbes in the absence of O_2 may be insufficient to cause severe bloating (Zhai & Pérez-Díaz, 2018) and that O_2 availability for biological activity is a critical factor in the incidence of bloater defect. For instance, conversion of molecular O_2 to CO_2 early in the process would be instrumental in preventing the proliferation of aerobic microbes, tissue respiration and in reducing internal cucumber tissue pressure. Although, utilization of chemical reduction to remove oxygen from a fermentation system represents a theoretically viable alternative to purging, such an approach may be cost inefficient and would generate reactive oxygen species that are inhibitory to lactic acid bacteria and damaging for the cucumber tissue (Brooker, 2011; Hayyan, Hashim, & Al Nashef, 2016). While it seems logical to consider that N_2-exchanged fresh cucumbers may serve the purpose of removing both oxygen and carbon dioxide from the fresh fruit, such type of pretreatment prevents the internalization of a significant number of lactic acid bacteria into the tissue upon brining (Daeschel & Fleming, 1981).

In enhancing the reduction of bloater index in cucumber fermentations, it must be also considered that most bacteria, in particular LAB, utilize a number of decarboxylating reactions to produce metabolic energy (Poolman et al., 1991). Thus, the elimination of O_2 from a fermentation system may result in an opportunity for LAB to conduct decarboxylation reactions at a higher frequency, which would still cause some cucumber bloating. Although, the use of malic acid decarboxylase deficient starter cultures has been tested, its impact may be overshadowed by the ability of LAB to decarboxylate phenolic compounds and amino acids (Matthews et al., 2004; Pessione, 2012; Wolken, Lucas, Lonvaud-Funel, & Lolkema, 2006). An exhaustive selection of starter cultures for cucumber fermentations to identify candidates with a low decarboxylating potential and possibly enhanced peroxidase activity may result in the availability of an additional and more cost-effective approach to reduce the incidence of bloaters. Robust starter cultures with a reduced decarboxylating potential and enhanced peroxidase activity would need to effectively outcompete the natural microbiota.

Funding sources

Ms. Yawen (Karen) Zhai was partially funded by Mount Olive Pickle Company, Mt. Olive, NC.

Acknowledgements

The authors thank Ms. Sandra Parker for excellent administrative assistance.

References

Breidt, F., & Fleming, H. P. (1992). Competitive growth of genetically marked malolactic-deficient *Lactobacillus plantarum* in cucumber fermentations. *Applied and Environmental Microbiology, 58*(12), 3845–3849.

Brooker, R. J. (2011). *Genetics: Analysis and principles* (4th ed.). New York: McGraw-Hill Science 978-07-352528-0.

Corey, K. A., Pharr, D. M., & Fleming, H. P. (1983a). Role of the osmoticum in bloater formation of pickling cucumbers. *Journal of Food Science, 48*(1), 197–201.

Corey, K. A., Pharr, D. M., & Fleming, H. P. (1983b). Pressure changes in oxygen-exchanged, brined cucumbers. *Journal of the American Society for Horticultural Science, 108*(1), 61–65.

Costilow, R. N., Bedford, C. L., Mingus, D., & Black, D. (1977). Purging of natural salt-stock pickle fermentations to reduce bloater damage. *Journal of Food Science, 42*(1), 234–240.

Costilow, R. N., Gates, K., & Bedford, C. L. (1981). Air purging of commercial salt-stock pickle fermentations. *Journal of Food Science, 46*(1), 278–282.

Costilow, R. N., Gates, K., & Lacy, M. L. (1980). Molds in brined cucumbers: Cause of softening during air purging of fermentations. *Applied and Environmental Microbiology, 40*(2), 417–422.

Daeschel, M. A., & Fleming, H. P. (1981). Entrance and growth of lactic acid bacteria in gas-exchanged, brined cucumbers. *Applied and Environmental Microbiology, 42*(6), 1111–1118.

Daeschel, M. A., McFeeters, R. F., & Fleming, H. P. (1985). *Modification of lactic acid bacteria for cucumber fermentations: Elimination of carbon dioxide production from malate. Developments in industrial microbiology, Vol. 26*, Arlington, VA: Society for Industrial Microbiology339–346.

Daeschel, M. A., McFeeters, R. F., Fleming, H. P., Klaenhammer, T. R., & Sanozky, R. B. (1987). *Lactic acid bacteria which do not decarboxylate malic acid and fermentation therewith. 1987 May 19.* U. S. No. 4,666,849.

Eak, I. L., & Morris, L. L. (1956). Respiration of cucumber fruits associated with physiological injury at chilling temperatures. *Plant Physiology, 31*, 308.

Etchells, J. L. (1941). Incidence of yeasts in cucumber fermentations. *Food Research, 6*(1), 95–104.

Etchells, J. L., & Bell, T. A. (1950). Classification of yeasts from the fermentation of commercially brined cucumbers. *Farlowia, 4*(1), 87–112.

Etchells, J. L., Bell, T. A., Fleming, H. P., Kelling, R. E., & Thompson, R. L. (1973). Suggested procedures for the controlled fermentation of commercially brined pickling cucumbers-the use of starter cultures and reduction of carbon dioxide accumulation. *Pickle Package Science, 3*, 4–14.

Etchells, J. L., Bell, T. A., Fleming, H. P., & Thompson, R. L. (1976a). *Controlled bulk vegetable fermentation. 1976 January 13.* U. S. No. 3,932,674.

Etchells, J. L., Borg, A. F., & Bell, T. A. (1968). Bloater formation by gas-forming lactic

acid bacteria in cucumber fermentations. *Applied Microbiology, 16*(7), 1029–1035.
Etchells, J. L., Fabian, F. W., & Jones, I. D. (1945). *The Aerobacter fermentation of cucumbers during salting. 200*, Michigan Agricultural Experiment Station Technical Bulletin56.
Etchells, J. L., Fleming, H. P., Bell, T. A., & Thompson, R. L. (1976b). *The controlled fermentation process compared with a salt-free method for preservation and storage of pickling cucumbers.* St. Charles, IL: Pickle Packers International, Inc Advisory statement published and distributed.
Etchells, J. L., & Jones, I. D. (1941). An occurrence of bloaters during the finishing of sweet pickles. *Fruit Products Journal, 20*(12), 370–381.
Fleming, H. P. (1979). Purging carbon dioxide from cucumber brines to prevent bloater damage–A review. *Pickle Pak Science, 6*(1), 8–22.
Fleming, H. P. (1984). Developments in cucumber fermentation. *Journal of Chemical Technology and Biotechnology, 34B*, 241–252.
Fleming, H. P., Etchells, J. L., Thompson, R. L., & Bell, T. A. (1975). Purging of CO_2 from cucumber brines to reduce bloater damage. *Journal of Food Science, 40*(6), 1304–1310.
Fleming, H. P., Humphries, E. G., Brock, A. D., & Pharr, D. M. (1983). Brining of oxygen-exchanged cucumbers in pilot tanks: A status report. *Pickle Pakage Science, VII*(1), 16–23.
Fleming, H. P., Humphries, E. G., Fasina, O. O., McFeeters, R. F., Thompson, R. L., & Breidt, F., Jr. (2002). BAG-IN-BOX TECHNOLOGY: Pilot system for process-ready, fermented cucumbers. *Pickle Pak Science, VIII*(1), 1–8.
Fleming, H. P., McDonald, L. C., McFeeters, R. F., Thompson, R. L., & Humphries, E. G. (1995). Fermentation of cucumbers without sodium chloride. *Journal of Food Science, 60*(2), 312–315 319.
Fleming, H. P., McFeeters, R. F., Daeschel, M. A., Humphries, E. G., & Thompson, R. L. (1988). Fermentation of cucumbers in anaerobic tanks. *Journal of Food Science, 53*(1), 127–133.
Fleming, H. P., & Pharr, D. M. (1980). Mechanism for bloater formation in brined cucumbers. *Journal of Food Science, 45*(6), 1595–1600.
Fleming, H. P., Pharr, D. M., & Thompson, R. L. (1980). Brining properties of cucumbers exposed to pure oxygen before brining. *Journal of Food Science, 45*(6), 1578–1582.
Fleming, H. P., Thompson, R. L., Bell, T. A., & Hontz, L. H. (1978a). Controlled fermentation of sliced cucumbers. *Journal of Food Science, 43*(3), 888–891.
Fleming, H. P., Thompson, R. L., Bell, T. A., & Monroe, R. J. (1977). Effect of brine depth on physical properties of brine-stock cucumbers. *Journal of Food Science, 42*(6), 1464–1470.
Fleming, H. P., Thompson, R. L., Etchells, J. L., Kelling, R. E., & Bell, T. A. (1973a). Bloater formation in brined cucumbers fermented by *Lactobacillus plantarum*. *Journal of Food Science, 38*(3), 499–503.
Fleming, H. P., Thompson, R. L., Etchells, J. L., Kelling, R. E., & Bell, T. A. (1973b). Carbon dioxide production in the fermentation of brined cucumbers. *Journal of Food Science, 38*(3), 504–506.
Fleming, H. P., Thompson, R. L., & Monroe, R. J. (1978b). Susceptibility of pickling cucumbers to bloater damage by carbonation. *Journal of Food Science, 43*(3), 892–896.
Gates, K., & Costilow, R. N. (1981). Factors influencing softening of salt-stock pickles in air-purged fermentations. *Journal of Food Science, 46*(1), 274–282.
Greenwood, N. N., & Earnshaw, A. (1997). *Chemistry of the elements* (2nd ed.). Woburn, MA: Butterworth-Heinemann 0-08-037941-9.
Guillou, A. A., Floros, J. D., & Cousin, M. A. (1992). Calcium chloride and potassium sorbate reduce sodium chloride used during natural cucumber fermentation and storage. *Journal of Food Science, 57*(6), 1364–1368.
Harned, H. S., & Bonner, F. T. (1945). The first ionization of carbonic acid in aqueous solutions of sodium chloride. *Journal of the American Chemical Society, 67*, 1026–1031.
Hayyan, M., Hashim, M. A., & AlNashef, I. M. (2016). Superoxide ion: Generation and chemical implications. *Chemical Reviews, 116*(5), 3029–3085.
Humphries, E. G., & Fleming, H. P. (1986). Closed-top fermentation tanks for cucumbers. *Transactions of the American Society of Agricultural Engineers, 2*(1), 45–48.
Jones, I. D., Etchells, J. L., Veerhoff, O., & Veldhuis, M. K. (1941). Observations on bloater formation in cucumber fermentation. *The Fruit Products Journal, 20*(7) 202-206, 219-220.
Matthews, A., Grimaldi, A., Walker, M., Bartowsky, E., Grbin, P., & Jiranek, V. (2004). Lactic acid bacteria as a potential source of enzymes for use in vinification. *Applied and Environmental Microbiology, 70*(10), 5715–5731.
McDonald, L. C., Fleming, H. P., & Daeschel, M. A. (1991). Acidification effects on microbial populations during initiation of cucumber fermentation. *Journal of Food Science, 56*(5), 1353–1356 1359.
McDonald, L. C., Shieh, D.-H., Fleming, H. P., McFeeters, R. F., & Thompson, R. L. (1993). Evaluation of malolactic-deficient strains of *Lactobacillus plantarum* for use in cucumber fermentations. *Food Microbiology, 10*(6), 489–499.
McFeeters, R. F., Fleming, H. P., & Daeschel, M. A. (1984). Malic acid degradation and brined cucumber bloating. *Journal of Food Science, 49*(4), 999–1002.
McFeeters, R. F., Fleming, H. P., & Thompson, R. L. (1982). Malic acid as a source of carbon dioxide in cucumber fermentations. *Journal of Food Science, 47*(6), 1862–1865.
McFeeters, R. F., & Pérez-Díaz, I. M. (2010). Fermentation of cucumbers brined with calcium chloride instead of sodium chloride. *Journal of Food Science, 75*(3), C291–C296.
McMurtrie, E. K. (2016). *Quality of cucumbers fermented in acidified and non-acidified calcium chloride brines for reduced environmental impact of brining operations*Thesis of Master degree, Department of Food, Bioprocessing and Nutrition Sciences, 2016, under the direction of Dr. Suzanne Johanningsmeier.
Pérez-Díaz, I. M., Hayes, J. S., Medina-Pradas, E., Anekella, K., Daughtry, K. V., Dieck, S., et al. (2016). Reassessment of the succession of lactic acid bacteria in commercial cucumber fermentations and physiological and genomic features associated with their dominance. *Food Microbiology*. https://doi.org/10.1016/j.fm.2016.11.025.
Pérez-Díaz, I. M., McFeeters, R. F., Moeller, L., Johanningsmeier, S. D., Hayes, J. S., Fornea, D., et al. (2015). Commercial scale cucumber fermentations brined with calcium chloride instead of sodium chloride. *Journal of Food Science, 80*(12), M2827–M2836.
Pessione, E. (2012). Lactic acid bacteria contribution to gut microbiota complexity: Lights and shadows. *Frontiers in Cellular and Infection Microbiology, 2*, 86.
Poolman, B., Molenaar, D., Schmid, E. J., Ubbink-Kok, T., Abee, T., Renault, P. P., et al. (1991). Malolactic fermentation: Electrogenic malate uptake and malate/lactate antiport generate metabolic energy. *Journal of Bacteriology, 173*(19), 6030–6037.
Potts, E. A., & Fleming, H. P. (1979). Changes in dissolved oxygen and microflora during fermentation of aerated, brined cucumbers. *Journal of Food Science, 44*(2), 429–434.
Potts, E. A., & Fleming, H. P. (1982). Prevention of mold-induced softening in air-purged, brined cucumbers by acidification. *Journal of Food Science, 47*(5), 1723–1727.
Samish, Z., Etinger-Tulczynska, R., & Bick, M. (1957). The presence of bacteria within cucumber tissue. *Records of the Agricultural Research Station, 8*, 101.
Samish, Z., Etinger-Tulczynska, R., & Bick, M. (1963). The microflora within the tissue of fruits and vegetables. *Journal of Food Science, 28*(3), 259–266.
Veldhuis, M. K., & Etchells, J. L. (1939). Gaseous products of cucumber pickle fermentations. *Food Research, 4*(6), 621–630.
Wehner, T. C., & Fleming, H. P. (1984). Evaluation of bloater resistance in pickling cucumbers using a brine carbonation method. *Journal of the American Society for Horticultural Science, 109*(2), 261–265.
Wolken, W. A. M., Lucas, P. M., Lonvaud-Funel, A., & Lolkema, J. S. (2006). The Mechanism of the tyrosine transporter TyrP supports a proton motive tyrosine decarboxylation pathway in *Lactobacillus brevis*. *Journal of Bacteriology, 188*(6), 2198–2206.
Zhai, Y., & Pérez-Díaz, I. M. (2018). Evaluation of the use of malic acid decarboxylase deficient starter culture in NaCl free cucumber fermentation to reduce bloating incidence. *Journal of Applied Microbiology, 124*(1), 197–208. https://doi.org/10.1111/jam.13625 ([Epub ahead of print]).
Zhou, A., McFeeters, R. F., & Fleming, H. P. (2000). Development of oxidized odor and volatile aldehydes in fermented cucumber tissue exposed to oxygen. *Journal of Agricultural and Food Chemistry, 48*(2), 193–197.