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Cucumber Fermentation

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1. Introduction

Humans have consumed fermented cucumber products since before the dawn of civilization. The earliest record of fermentation dates before 6000 BC in the Fertile Crescent (Demarigny 2012). There is fossil evidence that fruits and vegetables were undergoing lactic acid bacteria (LAB) fermentation before mankind inhabited the earth (Schopf and Packer 1987). Through time, human populations have developed these processes to create different products and prolong the shelf-life of highly perishable vegetables, such as cucumbers, thereby increasing food security. Almost every culture around the globe include specific fermented foods in their dietary customs and traditions. Fermented cucumbers are turned into a product called jiang-gua in Taiwan, khalpi in Nepal and India, paocai in China, oiji in Korea and are referred to as pickles in many parts of the United States, Europe, and Canada (Das and Deka 2012, Di Cagno et al. 2013, Kumar et al. 2013, Tamang 2010, Jung 2012). Most cucumbers are fermented in a salt solution. But to make khalpi, cucumbers are cut into pieces, sun dried for two days, put into bamboo vessels and left to

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ferment at room temperature for three to seven days. While hundreds of different commercial vegetable fermentation processes are practiced across the globe, cucumbers, cabbage and table olives are currently the most economically relevant commodities.

With an increasing world population, lactic acid fermentation is expected to play an increasingly important role in preserving fresh fruits and vegetables. According to Steinkraus (1994), fermentation plays the following five key roles in modern society: 1) preservation of substantial amounts of otherwise perishable products; 2) biological enhancement of the raw materials with protein, vitamins, essential amino acids and fatty acids; 3) enrichment of human diets through improved texture, appearance, flavor and aromas; 4) decreased energy requirements for preservation; and 5) cottage industry opportunities. Cucumbers are fermented as an economical means of storage in a well-preserved state between harvests, making them available for processing into finished products year round. Cucumber plants take only a couple of months to reach maturity and can produce many fruits. The mature fruits, which can be picked in various sizes, are composed of 95 per cent water and contain about 2.5 per cent sugar. The plants can produce high yields on different soils and complement crop rotations. In small plots, the plants can be grown on trellises to increase the yield per area. The production statistics for the top five cucumber-growing nations are given in Table 1. China has produced the largest amount of cucumbers for the past 50 years, nearly 25 times that of Turkey, the second top producer.

Table 1. Cucumber Production Trends 2008-2012

Country	Cucumber Production (millions of metric tons)				
	2008	2009	2010	2011	2012
China	42.2	44.2	45.7	47.3	48.0
Turkey	1.70	1.70	1.80	1.70	1.70
Iran	1.50	1.60	1.70	1.50	1.60
Russian Federation	1.10	1.10	1.20	1.20	1.30
Ukraine	0.80	0.90	0.90	1.00	1.00
United States	0.90	0.90	0.90	0.80	0.90

Adapted from FAO Stat. 2015

High yields of cucumber require a means of bulk storage. Because the plants grow best when there are warm days and nights, its shelf-life is very short, especially if there is no means of cooling the fruits after harvest. For this reason alone, prior to 1940, virtually all commercial cucumber products were fermented, which is still the case in the developing world today. Cucumber fermentations are energy efficient as they require no heat or refrigeration. In many areas of the world, brined cucumbers are stored in earthen pots, barrels or concrete basins where they undergo a

natural lactic acid fermentation. When managed properly, the fermented products can be edible for years and are ready for direct consumption or turned into sauces and condiments to complement meals. Today, the majority of shelf-stable cucumber products in industrialized societies are acidified, and then pasteurized or refrigerated. The fermented cucumber category is now represented primarily by institutional hamburger dill chips and institutional and retail relishes. The sale of fermented cucumbers represents greater than one billion dollars in sales annually in the United States alone. Value is added throughout the pickle manufacturing and distribution system, culminating in finished products that are valued much higher than the unprocessed vegetable commodities. In this way, the fermented cucumber industry supports the livelihood of growers, packers, manufacturers, transporters and a variety of related suppliers and distributors. However, the ultimate beneficiaries remain the consumers whom have access to safe, affordable and high quality food.

This chapter highlights the current knowledge associated with cucumber fermentation. We start with a detailed description of the industrial-scale process, followed by information that explains the scientific basis of the microbial and chemical changes that occur during cucumber fermentation, and finish with trends, current innovations and future directions for cucumber fermentation.

2. Industrial Process Overview

The experience of visiting a pickling tank-yard is quite interesting. As you approach the field of well-organized tanks, the sight of large tanks frothing with foam alerts one to the scale of operations of the modern pickle plant. Hundreds or thousands of tanks are seen, each holding 15 to 20 tons of fermenting cucumbers. The sounds of air blowers and machinery to move pickles to and from tanks is continuous. The strong smell can be quite pleasing or quite disturbing, depending on the quality of fermentation and one's predisposition to pickles. In large tank-yards, teams of dedicated personnel sample, test and adjust fermentation brines and devices in an effort to control the fermentation process guided by science-based written protocols and experiences of local experts. In colder climates, one may see crews chopping ice from frozen caps. On a closer look at the brine and pickles, you can notice different stages of fermentation even within a matter of days. Newly filled tanks of brined cucumbers will have clear and salty-tasting brine devoid of any subtle flavors. After two or three days of the initial stages of fermentation, the brine tends to turn turbid and develop a pleasing aroma, a subtle acidity, and fresh cucumber flavor. At this stage, the light green chlorophyll color still remains and the cucumber itself retains the opaque white interior. A frothy foam begins to form on the tank surface as a result of the active gas purging initiated at brining to control levels of dissolved carbon dioxide (CO_2) that can destroy cucumbers and tanks alike if not properly managed.

As the fermentation progresses in the surrounding brine during the first two weeks, subtle changes take place in the cucumber. Measured levels of acidity, primarily lactic acid, begin to increase, corresponding with decreasing pH and increasing brine turbidity. Rates of CO_2 development remain high. Carbon dioxide can be introduced from carbonates in the water that makes up the brine, residual respiration of the cucumber, and from the fermentation itself, requiring nearly continuous sparging. Levels of salt may need adjustment to compensate for absorption by the cucumbers (now becoming pickles), dilution from rain-water or evaporation and loss of water from the system. The color of the cucumbers begin to change from bright green skin and white opaque flesh, to a duller olive green skin and flesh that begins to move towards translucence in appearance. Ideally, textures remain crisp at this stage and, hopefully, throughout storage. The density and buoyancy of the cucumber also changes from very buoyant to more buoyant neutral allowing the cucumbers to settle, relieving much of the upward pressure on the cover boards that keep cucumbers below the brine surface, which itself is exposed to the environment. Odors and flavors change substantially as the primary lactic acid fermentation reaches completion. The subtle fresh cucumber aromas and flavors are lost or masked at this stage. A mildly acidic and pleasing taste from lactic acid fermentation is now predominant, but is still too salty for consumption without further processing. If the purging of CO_2 gas was successful, the structure of the cucumber remains intact, aside from some minor shriveling and shrinkage due to osmotic action of the salt brine. If dissolved CO_2 is not adequately controlled, hollow pickles can result that will pop when broken, revealing poor quality and an associated loss of value.

After the completion of fermentation (conversion of the fermentable sugars to lactic acid) and a few more weeks for curing (the loss of internal opacity) for a total of about four weeks, the pickle is ready for processing. At this stage, the processor can use the pickle as a finished product or boost salt levels for long-term storage in the same tank for up to one year. Continued monitoring of brines, including assays for softening enzymes and pH, help identify secondary fermentations by opportunistic fungi and bacteria in the earliest stages, prior to significant product losses.

Not all cucumber varieties yield high-quality, firm pickles. In the US, pickling variety cucumbers are bred to yield firm pickles after brining and fermentation. The most common pickling varieties in the US and Europe have approximately a 3:1 length to diameter ratio. In other countries, pickling varieties can be 10:1 or higher. Lebanon is a good example where the local varieties are 10:1 and make excellent quality pickles. In China, most cucumbers are grown for fresh market with very few being pickled by lactic acid fermentation on a commercial scale.

While most cucumber fermentations are done on whole cucumbers, many processors will also ferment cut cucumbers that are by-products

from fresh pack operations, e.g. spears, or they will pre-dice green cucumbers for relish and ferment the dices in closed tanks. These fermentations will occur very rapidly due to the large surface area of the cuts and rapid diffusion of sugars into the surrounding brine. Products treated in this way should be used quickly as storage is more complex than that with whole pickles, which have retained their protective outer skin. In tank-yards which ferment mainly relish, whole nubs and crooks (whole cucumbers, which are broken or misshapen) are often mixed with finely cut cucumber pieces to eliminate the tight wads of relish that form if only cut products are packed at a high pack-out ratio. However, it is important to monitor these fermentation tanks closely as many losses are incurred with this combination due to a higher incidence of spoilage.

What makes industrial cucumber fermentation different than that at home or in the laboratory? While the microenvironment should be very similar, the problem becomes one of scale. Materials, tank design, compressive and buoyant forces, diffusion distances and exposure to the environment — all present problems when scaling up from a few small vessels to many very large tanks. Sanitation and preventing cross contamination are challenging due to the sheer scale. While technology has advanced in other high-value fermentation industries, e.g. dairy and alcohol, cucumber fermentation remains strikingly similar to historical methods in a crock pot with a porous cover held down with a heavy stone. Part of this has to do with the challenges presented by the cucumber fruit shape and internal structure itself, a large cylindrical structure with three internal carpal sections susceptible to damage on account of rough handling, osmotic pressures or high levels of diffused CO_2. Gentle handling methods and avoidance of excessive drops are requirements for good quality pickles in addition to methods of purging CO_2 from the tank and maintaining as close to an anaerobic environment as possible given the tank design. Another reason for slow change is due to the fact that fermented pickles have had an extraordinary safety record throughout the years.

2.1. Fermentation Vats

It is speculated that the first scale-up was most likely from stone or clay crocks (4-10L) to wooden barrels (60-200L). Wooden barrels built by coopers would have made a more efficient fermentation vessel for commercialization and transport by land or by sea. Wooden barrels were still being used as late as the 1970s; however, where barrels are still currently used, plastic has become the material of choice. The majority of the first large pickle tanks were cast offs from closed breweries or those upgrading to larger tanks. Cylindrical wooden tanks (10,000-30,000L) made from beveled staves dado cut and fit to thick plank flooring were held together with thick steel hoops to withstand extreme pressures

encountered during fermentation. Wooden boards laid on the surface of the leveled cucumbers and held secure with 4″ × 4″ crossboards and locked to the tank with 4″ × 6″ planks counteracted the extreme upward buoyant forces, keeping the cucumbers below the brine surface during the fermentation. Wooden tanks had to be kept full with brine or water even when emptied of pickles in order to keep the wood swelled and tight fitting. After a time, the exposed wood above brine level dries out and shrinks, allowing brine to leak through the gaps. As the tanks aged, leaks between the staves had to be stopped by pushing oakum into the gaps. Many similarities are seen between the maintenance of wooden tanks and wooden sailing ships. In the 1970s and 80s, as many of the old wooden tanks came in disrepair, there was a controversy about whether the wood harbored the essential bacteria required for the fermentation, but this myth was quickly dispelled and there seems to be no difference in the quality of fermentation in plastic or wood tanks. Over time, fiberglass reinforced plastic (FRP) with an inner layer made of an approved food contact material dominated over the polypropylene tanks, primarily due to ease of repair and structural stability over wide temperature extremes. FRP can be multi-layered and cross wound for strength and impregnated with UV light barriers to extend the tank life to more than 15 years.

The tank design has not changed much throughout the years and is typically an open top cylindrical tank with flat bottom that can rest on a concrete pad or be partially set into the ground. As the tanks are open top, slightly tapered walls allow nesting of the tanks for economical freight when shipping new tanks from the manufacturer to the factory. In the US, the standard tank diameter is limited to 11.5 ft to allow legal over the road delivery with one driver. Larger diameter tanks would be designated as wide loads and would be more costly to ship. Tank heights are typically equal to or just slightly greater than the tank diameter, providing a reasonable volume capacity to surface area ratio which optimizes FRP material. FRP tanks are often reinforced with a thick band of fiberglass near the top that maintains the structural integrity even when the tank walls are relatively thin. The top of the tank contains some hardware, such as lifting lugs for proper movement of tanks during installation and brackets for securing the 'hold down' cover which is made from wood boards as described earlier or from semicircular FRP covers with holes that allow free movement of brine. The covering system of these open top tanks holds the cucumbers at least 15 cm below the surface of the brine, which is exposed to air and sunlight. While any exposure to air (oxygen) is not ideal, today's technology relies on the strong UV light from the sun to suppress growth of aerobic yeast and molds on the surface. The open top component of the design has been a necessity due to the enormous pressures developed during the fermentation and will be discussed in more detail later. The barriers to a fully-closed tank system are primarily due to CO_2 removal, material handling of the pickles during loading and

unloading and the cost of replacement of open-top tanks throughout the industry. As open-top tanks are exposed to the environment and sources of contamination and security risk, only a concerted effort by the industry could overcome the technical barriers, which include material handling and effective purging, to prepare for the eventual replacement of the open-top tank technology over time. In fact, a number of cucumber fermentation facilities around the world have adopted closed-tank fermentations. Closed tanks eliminate the threat of contamination that the current open-tank system imposes, reduce color and flavor defects associated with photo-oxidation during storage and improve microbial stability during bulk storage.

Because an active fermentation process takes place in these tanks, frequent monitoring of chemical, physical and organoleptic qualities is necessary. Samples may be pulled from the surface and from internal regions by sampling at the discharge of the side arm purger (discussed in detail later) or from sampling wells attached to the side of the tank with access from the surface near a platform or walkway. Testing can be done from tank side in some cases or in a laboratory on the premises.

In the following paragraphs and in Figure 1, the steps in commercial cucumber fermentation are described.

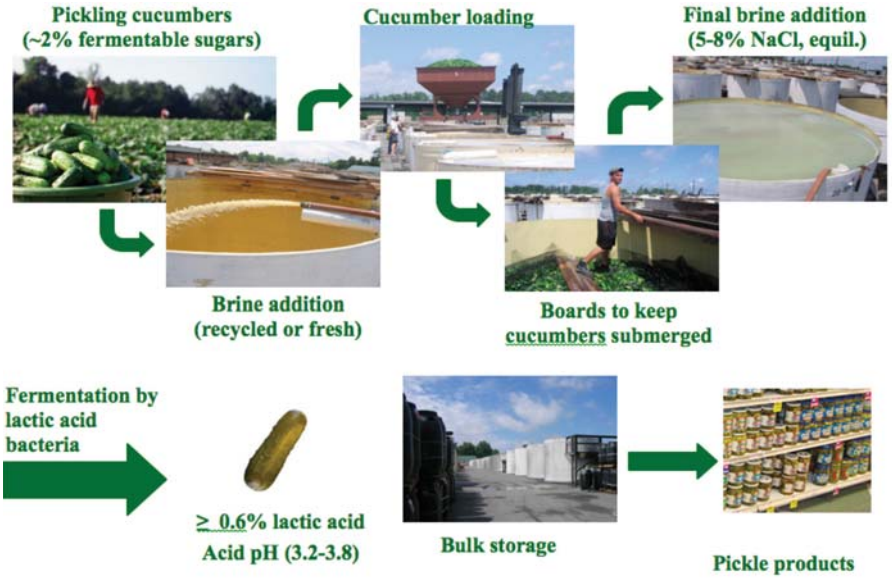

Fig. 1. Process Steps in Cucumber Fermentation. (***Adapted from Johanningsmeier*** (**2011**)).

Prior to the cucumber harvest, open top tanks are cleaned thoroughly. In some cases, a sanitizing solution is used as well as a good potable-water rinse. Accessories are checked for functionality, especially the side-arm purgers and diffusers. An initial salt brine is prepared of proper salinity

and if other chemicals are to be added, like calcium chloride, then it should be done during preparation of the final brine solution. Typically, the salt level of this initial cover brine (and cushion brine) will equilibrate to a salt concentration lower than the target of 6 per cent in order to prevent severe osmotic shriveling of the cucumbers. Dry salt adjustments after two or three days will then get the equilibrated salt levels to the target values.

The cucumbers are typically graded by size, sorted and inspected, culling out any spoiled cucumbers and foreign material. Pre-grading of cucumbers at this stage allows better control of fermentation and makes production planning much simpler when the product is ready to be processed for use in finished products. Management of sizes in the pickle industry is a big challenge and success in the business depends on balancing the supply and demand by size. Each size cucumber will have a different bulk density and impact the pack out ratio in any given tank. The pack out ratio coupled with the size and maturity of the cucumber influences the amount of sugars present and the buffering capacity of the system, affecting fermentation rates and degree of completion as measured by pH and lactic acid production. In some cases, with very large diameter cucumbers, fermentation would stop before all sugars were consumed, allowing for secondary yeast fermentation to take place during the storage phase.

Just prior to filling the tank with cucumbers, a portion of the brine is added as a cushion to the cucumbers that will be dropped into the tank. This cushion brine serves to break the fall of the cucumbers. The second function is to begin the osmotic shrinking process immediately, so that the targeted quantity of cucumbers fit into the tank. A typical target weight is calculated by taking the working volume of the tank as weight of water and multiplying by 0.65 to calculate the weight of the cucumbers within. This will vary slightly by the bulk density which is a function of the cucumber size. The correct pack out ratio in the tank is critical in order to achieve the proper final acidity of the system for preservation.

At the end of filling, the cucumbers are generally heaped in a mound that look impossible to fit; however, within a matter of hours, osmotic shrinking will begin to show a settling of the mound. This action is due to the salt brine cushion that was applied using only half of the calculated brine required. The brine rises in the tank as the cucumbers are added. Manual leveling of the cucumber mounds is typical and the head cover is applied and secured prior to final brining. If the tank is large, a person may walk on the surface of the cucumbers in order to apply and secure the cover. It is critical to avoid contamination and damage from boots or leveling equipment at this stage. Once the hold-down cover is secured, the remaining brine is added about 15 cm over the head cover, and the side-arm purger is started. It is desirable to complete all of these steps within 24 hours after the start of filling; however, in reality it may not always be possible. In some conditions, satisfactory fermentations have been observed

when this process is completed within 48 hours. However, softening and spoilage of cucumbers becomes very likely when filling and brining a tank takes more than 48 hours.

2.2. Controlling the Fermentation Process

Cucumber fermentation relies on the presence of naturally-occurring LAB or in some cases inoculum from a pure starter culture. The brining process creates a competitive advantage for the LAB to flourish while inhibiting the growth of spoilage organisms. Historically, while salt (sodium chloride – NaCl) was used for preservation, there was no established consensus on how much to use. The use of very high salt concentrations hindered a rapid lactic acid fermentation and allowed growth of halophilic yeast, leading to uncertain quality and shelf-life. Too little salt allows spoilage bacteria to proliferate. Many processors today use an equilibrated salt level of about 6–7 per cent (wt/vol) to achieve the desired fermentation based on research done starting in the 1940s. After the active fermentation, it is a common practice to use the pickles immediately or add more salt to protect the product from subsequent spoilage if long-term storage is desired. In colder climates, the extra salt is intended to protect the product from freeze damage. Although the freezing point is lowered, it is generally not sufficient to completely prevent freezing of the brines.

2.3. Purging

Purging is started immediately after capping and brining and continues on an established on-off schedule for at least 14 days, which is the normal period required to complete the fermentation. From the very start of brining and during the entire fermentation process, large quantities of CO_2 (carbon dioxide) are generated due to the presence of carbonates in the water used for brine, continued respiration of the cucumbers after harvest and as a result of malic acid degradation to lactic acid and CO_2 by fermentation microbiota. Practically, purging of CO_2 is only required during the initial five to seven days of fermentation, but, as a precaution, it is usually continued for 14 days or until the sugars are depleted. When the fermentation vessels are small, dissolved CO_2 can be eliminated by various mechanical interventions. Just shaking a crock or kicking a drum can release quantities of CO_2 gas seen visually with the gas bubbles escaping from the surface. In large tanks, the volumes and distances are much larger and CO_2 cannot easily escape, generally staying in the solution until saturation is reached. If unaddressed, CO_2 can create pressures inside the cucumber and destroy the internal tissue causing pickles to become hollow to various degrees. This phenomenon is referred to as bloater damage and is a major source of lost product and inefficiency. Prior to the wide implementation of purging routines, dedicated inspection operations were needed to hand-squeeze every pickle in search of hollow centers. In

some cases, pressures were so great that hold-down covers would blow off, splitting the 4 by 6 inch wooden planks. In the 1980s, all this changed when a purging device called the side-arm purger was invented (Costilow et al. 1977). Almost overnight, bloater defects and the labor-intensive sorting lines disappeared. The solution was low cost and technically simple, bubbling nitrogen gas (to prevent oxygenation) through a small pore-size diffuser in a side-arm gas lift pump which processed brine from the bottom of the tank and lifted it to the tank surface where the CO_2-laden nitrogen bubbles escaped into the atmosphere. The entire volume of brine is processed within a matter of two or three hours, depending on the liquid pumping rate. Later, cost pressures forced processors to look at lower-cost compressed air or blower air as replacement for the nitrogen gas. While effective at eliminating CO_2 and bloating, issues with soft centers and yeast growth developed. Interventions to mitigate these negative consequences, included additions of potassium sorbate, acetic acid and intermittent purging cycles (to allow time each day for the tank to go anaerobic) were used to minimize aerobic yeasts and molds from proliferating. Today, as the salt levels are being further decreased, there is the need to re-evaluate the value of pure nitrogen sparging versus air.

There are a number of factors that make side-arm purging effective. The first is production of many small bubbles that create a very large surface area for a given volume of purging gas. Exposure of the large surface area to the CO_2-laden brine allows a fast transfer of CO_2 into the gas bubble by simple diffusion. Secondly, as the bubbles rise in the purging tube, brine is pumped from the bottom of the tank on to the tank surface where the bubbles pop and are released into the atmosphere, releasing the CO_2 with them. In an efficient system, the treated brine that is spread over the tank surface falls downward in a layered flow pattern, replacing the brine that is pumped up from the bottom. Short circuiting flow patterns are not typical as long as the brine is spread fairly evenly over the surface. Thirdly, the purging gas flow rate must adequately remove dissolved CO_2 at the rate that it is generated. Today, there are purging protocols that work fairly well for standard tank sizes and the conditions experienced by individual processors, e.g. the carbonates present and fermentation rates experienced. Ideally, a purging control system based on CO_2 measurement and feedback control to the purger would optimize the use of purging gas and may be a financial incentive to return to nitrogen as the purging gas of choice.

2.4. Monitoring and Record Keeping

It is critical to monitor the condition of the tanks at a programmed frequency throughout fermentation and storage. Frequency of testing will vary, being very frequent during the 14–21 days of active fermentation and less frequent during storage up to one year. Examples here are typical of many sampling programs but should not be construed as a

recommendation nor as a full quality program for fermented cucumber pickles. Complete records of each tank and daily activities in the tankyard are critical.

The first step in monitoring is to check the condition of the tank and equipment prior to filling and brining. Proper sanitation and cleanliness is critical to avoid any gross contamination that would influence the fermentation. Next is to monitor the tank preparation, filling, capping, brining and purging that initiate the fermentation. A simple status-control board is typical where workers and management can see the progress of each tank at every stage. Entire tanks of cucumbers can be lost without a reliable control system. Salt measurement is the first and most important monitoring and control function as proper equilibrated salt concentration initiates the fermentation by selecting the naturally-occurring lactic acid bacteria and inhibiting spoilage organisms present on the fresh cucumbers. Salt is monitored daily for the first week and adjusted as needed with dry salt on the surface to achieve the processor's desired equilibrated target, typically 6 per cent although many within a range of 5–7 per cent. Monitoring the performance of the side-arm purger is done daily along with a daily visual examination of the tank surface. Observation, by an experienced and trained employee of bubbles along with a good brine flow is generally sufficient to assure proper function. Brine flow can also be measured periodically and should be sufficient to process the entire tank within two to three hours (60 L per min brine flow for a 20-ton tank would be typical). However, as mentioned before, removal of CO_2 is controlled by measuring the dissolved CO_2 levels and adjusting the purging protocols accordingly. In practice this is rarely done. The pH is also monitored daily during this phase. Depending on whether the salt brine was made fresh or from recycled brine, the pH of the brine may start at neutral or acidic values and within a matter of days decrease to levels below 4.6 and continue to decrease throughout the fermentation as lactic acid is produced. Concurrent visual examination of the brine will show increasing turbidity, indicating the growth of sufficient LAB for proper fermentation. In rare cases, when the pH does not decrease and no brine turbidity develops, it means that a stalled fermentation has occurred and an immediate intervention by an expert authority must be initiated.

After the first week, when monitoring indicates that fermentation is underway, tests for titratable acidity (assumed as primarily lactic acid) and fermentable sugars is tracked at a frequency of once or twice per week. Salt and pH testing frequencies can also be reduced at this point. Monitoring the cucumber itself is also desirable if a cucumber sampling port is available. The cucumber will be sliced, looking for internal integrity and tasted for both flavor and texture. After 14 to 21 days, when lactic acid levels are more than 0.6 per cent and reducing sugars are negative, it can be presumed that primary fermentation is complete and the cucumbers are almost ready for processing, depending on the cured appearance standard

of the processor. If the product is to be stored for an extended period of time in the tanks, the processor will monitor the brine chemistry and tank conditions no less than once a month, watching for any signs of off-odor or pH rise, which indicate an undesirable secondary fermentation.

After the completion of fermentation, it is ideal to test every tank for the activity of polygalacturonase (PG) softening enzymes in the brine. Knowledge of the presence of these enzymes is a useful tool for quality-improvement strategies and also to manage inventories. Although the presence of softening enzymes may not indicate poor quality, it is generally a good predictor of soft pickles in the future. Therefore, utilizing and properly processing tanks identified with PG activity early while pickles are still firm can optimize overall yield and quality. Brines intended for recycling are frequently tested prior to reuse for endo-PG activity, using a diffusion plate assay (Buescher and Burgin 1992).

2.5. Material Handling

Material handling involves moving both fresh and fermented cucumbers as well as the fermentation brine. Pickles and brine can be handled separately or sometimes together where the brine acts as the transport medium. The movement of pickles is done in a manner that avoids hard impacts, since the cucumber pickle can be damaged quite easily. Under the right conditions, a cucumber hitting a hard surface on end can develop internal damage in both the tissue and the internal structure (Marshall et al. 1972). This can happen in as little as a 3-ft drop. Damaged cucumber tissue occurring after harvest, but before brining, can trigger internal development of softening enzymes which can affect the texture weeks or months later during storage.

With very small stationary tanks, loading and unloading can be done manually by dumping buckets of cucumbers into the top of the open tank and onto the cushion brine. The fresh cucumbers contain about 5–6 per cent air by volume (Corey et al. 1983a, b), making them less dense than the brine and buoyant. Once fermented and fully cured, the cucumbers are denser than the brine and tend to settle. Unloading small tanks can be achieved manually with long handle nets.

Loading large tanks is fairly straightforward and involves dry conveyance of cucumbers elevated and directed toward the tank center. While many processors just drop them on to the cushion brine, some take precautions to avoid drops by using canvas lowerators which gently provide a cascading fall as the tank gets filled. Unloading large tanks is a bit more complicated. With large open tanks there are two basic methods in use today — one is a conveyor with buckets inserted into the tank. With some manual assistance, it will dig into the tank and convey pickles into a bin placed outside the tank. The buckets have holes to drain the brine as the pickles are being lifted out. Brine needs to be lowered as the tank

becomes empty of pickles but sufficient brine needs to remain to allow the pickles to move freely. Towards the end of the process, a worker will direct the final pickles into the conveyor buckets with a long handle net. This conveyance method would not work in a closed tank. The other popular method today is a pumping system. Special large inlet centrifugal pumps with recessed impellors pump both pickles and brine out of the tank and on to a screen where the brine is separated and returned to the tank. This method is about four times faster than the bucket conveyor method, but requires about ten times the investment in equipment. With the right design and equipment, pickles of all sizes can be pumped and carried to various locations in the processing plant, using brine as the transport medium. Other methods of handling fermented pickles include the use of flumes and air-lift pumps (Demo 2002).

2.6. Processing for Finished Product

Fermented pickles in the completed state are too salty for consumption. The fermented fruits are generally over 7 per cent salt when the fermentation and storage process is complete. In order to make most pickled products, the fruits are 'desalted' by soaking in water. Pickles are typically transported to designated processing tanks, separated from the fermentation brine and flushed with potable water so that sufficient salt is removed. This diffusion process can take place within 24 hours, depending on the amount of salt. Enough salt is removed to meet the finished product specifications with a formulated cover brine. During this process, the natural flavors created by fermentation are also diluted, allowing the finished product to take on the characteristics of the other ingredients added. Sweet items are often flavored with vinegar, sugar, cinnamon and cloves; kosher dills are flavored with vinegar, garlic, onions and herbs.

3. Fermentation Microbiota

3.1. Natural Cucumber Fermentation

Cucumber fermentation is possible due to the presence of a number of microorganisms on the fresh fruits that are responsible for the chemical changes observed with time. Raw cucumbers contain a wide variety of different microorganisms mostly on the surface of the cucumbers, including aerobic bacteria, LAB, yeasts and molds. When properly handled (i.e. not washed excessively or treated with anti-microbials), raw cucumbers will contain LAB as a minor part of their natural microbiota. On average, aerobic plate counts of raw cucumbers account for 4 to 5 log CFU/g while yeast and LAB account for 1.5 and 2.5 log CFU/g, respectively. Raw cucumber also contains species from the *Enterobacteriaceae* family in orders of 1 to 4 log CFU/g. Several enterobacteria may grow at the beginning of fermentation, producing CO_2 and hydrogen, which may influence the

initial development of anaerobic conditions. However, the numbers decrease drastically at the beginning of fermentation due to sensitivity to the acidic environment that develops as the fermentation proceeds (Etchells et al. 1945). Relatively low numbers of LAB naturally present at the beginning of fermentation outcompete the other natural microbiota due to the ability to survive in extreme environments, characterized by high salt and acid (Breidt 2006, Hutkins 2006).

LAB comprise a versatile group of microorganisms that are present at different stages of the fermentation process. Singh and Ramesh (2008) reported that the dominant LAB microbiota observed during cucumber fermentation were comprised of the genera *Lactobacillus*, *Pediococcus*, *Lactococcus* and *Leuconostoc*. Following a PCR approach with and without enrichment, the authors were able to observe *Lactobacillus* as the genus predominant after 72 hours of fermentation. *Leuconostoc* spp. were observed after 12 hours of fermentation in co-existence with *Lactobacillus* spp. After 36 hours of fermentation, the *Leuconostoc* population decreased and was not observed thereafter. At this point, species from the *Pediococcus* genus increased in number and remained in co-existence with *Lactobacillus* spp. until 72 hours of experimentation. *Lb. plantarum* is the predominant LAB species in cucumber fermentations. This homofermentative organism produces primarily lactic acid from glucose and fructose via the Embden-Meyerhoff-Parnas pathway (Breidt et al. 2007). Other LAB present during fermentation, such as *Pd. pentosaceus*, *Lb. brevis and Ln. mesenteroides* (Singh and Ramesh 2008), are in general heterofermentative and use the phosphoketolase pathway to produce lactic acid, CO_2, ethanol and acetic acid (White 2007). The ability of LAB to dominate fermentation depends on the capability to overcome the fermentation environment, rich in salt (6–7 per cent of NaCl) and high in acid. Although a simple NaCl brine has a pH near neutrality when the fermentation starts, it sharply decreases in a short period of time due to lactic acid production achieving values commonly between 3.1–3.5 (Breidt et al. 2013a). Among the LAB reported, *Lb. plantarum* and *Pd. pentosaceus* are more acid tolerant (Daeschel et al. 1987, Sandhu and Shukla 1996, Harris 1998) and therefore can be observed during the late stages of fermentation.

Yeasts, also naturally present on fresh cucumbers, may participate in the fermentation process to varying degrees. Two types of yeasts are commonly observed in cucumber fermentation: film-forming yeasts from the *Debaryomyces*, *Endomycopsis*, *Zygosaccharomyces*, and *Candida* genera that use an oxidizing metabolism to increase biomass (Etchells and Bell 1950a); and subsurface yeasts, such as *Saccharomyces cerevisiae* and *S. rosei*, which primarily carry out ethanol fermentation, converting a portion of glucose to ethanol and CO_2 (Etchells and Bell 1950b, Daeschel et al. 1988). Yeasts have been considered as contributors of flavor and growth factors during lactic acid fermentation in cucumber pickles (Etchells 1941b, Daeschel et al. 1985), and it has been hypothesized that the presence of yeasts helps establish

LAB as the dominant bacterial species (Daeschel et al. 1988). Yeasts may do this by contributing vitamins, nitrogen, amino acids and peptides to the fermentation brine which are important for the metabolic activity of LAB. During the initiation of fermentation, which lasts between two to three days, the number of LAB and yeast increases rapidly while undesirable bacteria and molds are eliminated by competition. LAB populations can reach up to 8 log CFU/mL while yeast may reach counts of ~5 log CFU/mL. This is greatly favored by the fermentation conditions, the ability of LAB and yeasts to tolerate relatively high salt conditions, and the ability of oxidizing yeasts to increase in biomass due to the presence of oxygen from air-purging routines commonly implemented during the first week of fermentation. At the end of the fermentation process, which might last up to three weeks, LAB counts average 6 log CFU/mL while yeast counts decrease to 4 log CFU/mL.

Although lactic acid is the major product of the fermentation process, some other by-products are formed. Carbon dioxide is generated from respiration of cucumbers when submerged in brine (Potts and Fleming 1979) and due to decarboxylation of malic acid by *Lb. plantarum* during fermentation (McFeeters et al. 1982a). Several LAB present in vegetable fermentations have an inducible malolactic enzyme which converts malate to lactate and CO_2 (Johanningsmeier et al. 2004). The presence of coliforms and yeasts also increases the chances of CO_2 production. Excessive CO_2 can lead to bloater pickles, an undesirable quality produced by the formation of gas pockets in the cucumber flesh (Corey et al. 1983b). To remove CO_2, Fleming et al. (1975) recommended nitrogen-purging routines and, if possible, maintaining anaerobic conditions during fermentation and bulk storage. Currently, the pickle industry commonly uses air-purging to prevent bloater damage. The change in the practice has been mainly based on costs since air displaces CO_2 from the fermentation tanks in a fashion similar to the proposed nitrogen-purging. Air-purging is commonly applied during active lactic acid fermentation (seven to 10 days in summer months and up to a month in colder temperatures); however, there are processors that follow a continuous air-purging schedule even during storage of the fermented product (personal communication, unpublished). Potts and Fleming (1979) observed that introduction of air into fermentation might lead to changes in the microbiota present in the fermenting cucumbers. Oxygen availability induces the growth and establishment of aerobic microbiota, including oxidizing yeast and undesirable aerobic spoilage bacteria, which might alter the characteristics of the fermented product. To limit the growth of aerobic microorganisms, particularly molds and yeasts, 0.1 per cent sorbic acid (0.13 per cent as potassium sorbate) or 0.9 per cent acetic acid can be used (Bell and Etchells 1952, Bell et al. 1959, Etchells et al. 1961, Binsted et al. 1962). Excessive growth of aerobic microorganisms which can cause spoilage problems is also controlled by stopping the purging for several hours each day (Breidt et al. 2007).

3.2. Ecology of Bacteriophage

Cucumber fermentations are driven by a variety of LAB naturally present on cucumbers. The metabolic activities of LAB determine the quality and safety of the final fermentation product (Pederson and Albury 1969). Many factors influence the metabolic activities of LAB. Bacteriophages (phages) are one such important factor because phages are natural killers of bacteria. The presence of phages against LAB in the fermentations can potentially lead to significant mortality of LAB, thereby influencing the bacterial ecology, the dynamics of the fermentation and subsequently the quality of the fermented products (Lu et al. 2012).

Phages are ubiquitous in nature. Many phages have been isolated from food environments. Phages in dairy fermentation have been extensively studied for decades. In contrast, phages in cucumber fermentation have only recently been studied. A few reports on phages from cucumber fermentation are found in the literature and all these reports focus on the phages infecting LAB. The two well-studied phages isolated from cucumber fermentations are phages ΦJL-1 and ϕps05. ΦJL-1 infects the starter culture *Lb. plantarum* MU45 while ϕps05 infects another starter culture *Pd. acidilactici* LA0281 (Lu et al. 2003a, Yoon et al. 2007). A recent pioneer study explored the phage ecology in a commercial cucumber fermentation and provided a glimpse into the diversity of LAB phages (Lu et al. 2012). The study obtained 576 LAB isolates from fermentation. Using these LAB isolates as potential hosts, 57 phage isolates were obtained from the same fermentation, indicating that about 10 per cent of LAB isolates were sensitive to phage attacks. The phage hosts included a variety of LAB such as *Lb. brevis*, *Lb. plantarum*, *Weissella paramesenteroides*, *W. cibaria* and *Pd. ethanolidurans*. However, most of the phages infected the two predominant LAB in fermentation — *Lb. brevis* and *Lb. plantarum*. The study showed that all the phages were isolated during the active period of fermentation, 3 to 30 days, and no phage active against LAB was isolated on Day One or after Day 30 in fermentation. The number of phages isolated on each sampling day correlated well with LAB counts (Fig. 2). It is known that LAB on fresh vegetables account for a very small portion (< 1 per cent) of the bacterial population (Mundt et al. 1967, Mundt and Hammer 1968, Mundt 1970) and this small LAB population is dominated by *Ln. mesenteroides*, one of the least salt-tolerant (Konisky 1989, Lund et al. 2000) and least acid-resistant (Pederson and Albury 1969, McDonald et al. 1990) LAB involved in vegetable fermentation. The high salt concentration (6 per cent NaCl, much higher than 2 per cent NaCl used in sauerkraut fermentation) and the low pH (4.0–4.4) of recycled or acidified brine used in the beginning of cucumber fermentation could greatly inhibit *Ln. mesenteroides*. Therefore, very few LAB hosts were present on Day One for phage replication, and phage activity was under the detection limit. After the fermentation started, the concentration of LAB increased rapidly, which provided more

hosts for phage replication. As a result, the number of phages being isolated increased (Fig. 2). As the fermentation continued, the massive production of acids and the resulting low pH became increasingly inhibitory to both LAB and their phages. On Day 30, only one phage against an acid-resistant LAB host was isolated because the brine pH was low (3.4), which inhibited most other phages and their hosts.

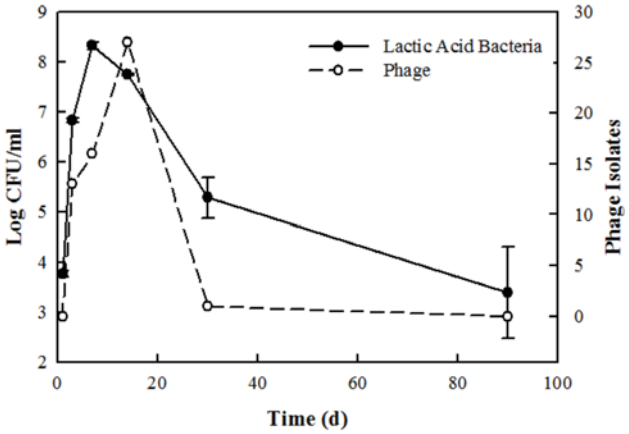

Fig. 2. Lactic Acid Bacteria (LAB) Counts and the Numbers of LAB Phage Isolates in Commercial Cucumber Fermentation (*Adapted from Lu et al. 2012*)

The phage ecology study showed that LAB phages in cucumber fermentation are highly diverse. Morphologically, most of the phages isolated from cucumber fermentation are tailed phages with icosahedral heads, but they vary in head and tail structures, belonging to different phage families, *Myoviridae* or *Siphoviridae* (Fig. 3). These phages also differ in host ranges. Some phages are species- or strain-specific, while other phages are capable of infecting multiple species. Although rare, two phages were found to be able to infect *W. cibaria*, *Lb. plantarum* and *Lb. brevis* (Lu et al. 2012). In contrast, most phages isolated from sauerkraut fermentations are species-specific and no phages were found to be able to infect LAB from different genera (Lu et al. 2003b). A variety of molecules on LAB cells can serve as receptors for phage infection, such as polysaccharides, (lipo) teichoic acids and membrane proteins (McGrath and van Sinderen 2007). Phages with a broad host range may be able to use more than one type of receptors present on different hosts, or the same receptors present on different hosts, allowing those phages to attack a wider variety of host LAB species in one genus or in different genera. These phages can also play important roles in phage ecology and genetic transfer through transduction among different LAB hosts, thereby promoting genetic diversity in microbial communities.

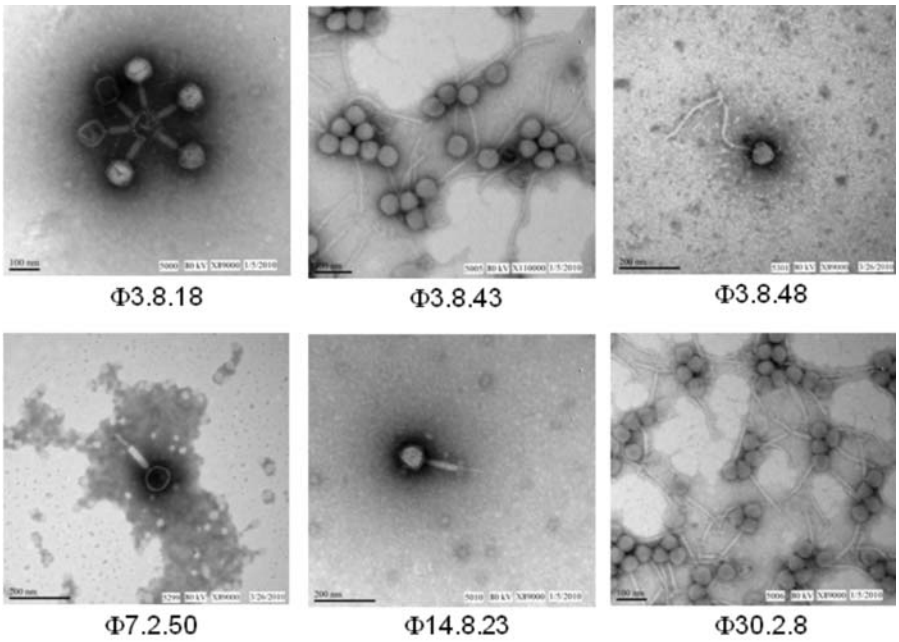

Fig. 3. Transmission Electron Micrographs of Six Phages Isolated from a Commercial Cucumber Fermentation (*adapted from Lu et al. 2012*).

The currently available data from SDS-PAGE analysis and restriction endonuclease digestion revealed a variety of structural protein profiles and restriction-banding patterns of the phages isolated from cucumber fermentations (Lu et al. 2003a, Yoon et al. 2007, Lu et al. 2012). However, the details and extent of the genetic diversity of phages in cucumber fermentation are largely unknown due to the scanty genome sequence database. So far, only one phage, ΦJL-1, from cucumber fermentations has been sequenced (Lu et al. 2005). Sequence analysis showed that the genome of ΦJL-1 is made of a linear double-stranded DNA (36,674 bp) containing 46 possible open reading frames (ORFs). Proven or putative functions were assigned to 17 ORFs, including five structural protein genes. Similar to several other LAB phage genomes, ΦJL-1 genome had a modular organization with functionally-related genes clustered together (Lu et al. 2005). The genome did not contain a lysogeny module, indicating that ΦJL-1 is not a temperate phage. To better understand the genetic diversity of phages in cucumber fermentation, a larger phage sequence database is needed for comparative sequence analysis.

The currently available data provide only a glimpse into the abundance and diversity of the phages active against LAB in cucumber fermentation that could cause significant mortality to LAB populations. As the disposal of high-salt waste from cucumber fermentation causes increasing concern,

technologies for low-salt cucumber fermentation are under development. These technologies may require the use of LAB starter cultures to ensure normal fermentation. The naturally-present phages could lead to starter culture failure. Therefore, phage-control strategies may be essential in those types of fermentation. Further study is greatly needed in order to get the whole picture of phage ecology in cucumber fermentations. This may include, but is not limited to: 1) studying phage ecology in cucumber fermentation in various geographic locations; 2) evaluating the impact of phages on these fermentations; and 3) sequencing more phages from cucumber fermentations. In addition, the potential of starter culture failure caused by phage infection should also be investigated if the fermentations require the use of starter cultures.

4. Secondary Fermentation and Spoilage

Over the years, there have been sporadic reports of spoilage in commercial tanks of brined cucumbers after apparently normal fermentation. The spoilage is characterized by a gradual increase in pH and decrease in titratable acidity followed by a very rapid increase in pH above 4.6, gas and odor production, and the potential for germination and outgrowth of clostridium spores. More detailed information on how this spoilage proceeds and the organisms involved has been limited in part to the sporadic occurrence of the event and the inability to predict the conditions that lead to secondary fermentation. The diversity in microbiota that is present during and after the lactic acid fermentation makes the isolation and identification of potential causative agents very challenging. The first documented post-fermentation cucumber spoilage was reported by Fleming et al. (1989). As part of experiments to reduce salt levels during fermentation, cucumbers that fermented normally in a closed 1000 litres tank with low NaCl concentration (2.3 per cent) spoiled several months after completion of the fermentation. The spoilage was characterized by complete depletion of lactic acid and production of butyric and propionic acids. Butyric acid production was attributed to *Clostridium tertium*, which was isolated from the spoiled brine. However, the authors concluded that this bacterium was not able to initiate the spoilage process since it was only able to convert lactic acid into butyric acid at pH 5 and above.

Later studies demonstrated that pH and NaCl content are important factors to modulate the spoilage process under anaerobic conditions (Kim and Breidt 2007, Johanningsmeier et al. 2012). Spoilage has been reproduced at pH 3.8 and above and NaCl concentrations of 4 per cent and below under anaerobic conditions. Organisms isolated from early laboratory-spoilage experiments were identified as *Propionibacterium*, *Clostridium* and *Lactobacillus* spp., but these microorganisms were not responsible for the initiation of secondary fermentation at the conditions prevailing once the primary fermentation is completed. More recently, *Lb. buchneri*, an aciduric

heterofermentative LAB, was isolated from both laboratory-reproduced spoilage (Johanningsmeier et al. 2012) and commercial fermentations (Franco and Pérez-Díaz 2012a) and shown capable of initiating spoilage by metabolizing lactic acid into acetic acid and 1,2-propanediol in fermented cucumber media under both aerobic and anaerobic conditions (Johanningsmeier and McFeeters 2013).

The natural microbiota present on and in cucumber fruits includes LAB, yeasts, enterobacteria and *Clostridium* spp. If oxygen availability is considered, it is reasonable to postulate that organisms other than those reported under anaerobic conditions may have a role in the post-fermentation spoilage of fermented cucumber pickles. For instance, under aerobic conditions, *Lb. plantarum* is able to grow faster and reach higher cell densities as compared to anaerobic conditions (Bobillo and Marshall 1991). This bacterium produces mainly lactate when glucose is present. However, once the sugar is exhausted, an oxygen-dependent pathway promotes the formation of acetic acid at the expense of lactic acid (Murphy and Condon 1984, Murphy et al. 1985, Bobillo and Marshall 1991). Other microorganisms with similar 'lactate oxidizing' systems are *Pd. pentosaceus*, *Lb. casei*, *Lb. sakei*, *Streptococcus faecium* and *Str. faecalis* (Thomas et al. 1985, Malleret et al. 1998).

During bulk storage in open tanks, yeasts may grow on the surface of the brine and oxidize the organic acids produced during primary fermentation (Etchells and Bell 1950a, b, Bell and Etchells 1952). It has been observed in the laboratory that common pickling-spoilage yeasts, such as *Zygosaccharomyces globiformis* (Belland Etchells 1952) may grow even in the presence of extremely low concentrations of oxygen (personal communication/unpublished). Additionally, various yeasts have been related to spoilage problems in the table olive industry (Vaughan et al. 1969, Durán Quintana et al. 1979). Under aerobic conditions, species from the genera *Candida*, *Pichia* and *Saccharomyces* are capable of utilizing lactic and/or acetic acids (Dakin and Day 1958, Ruiz-Cruz and Gonzalez-Cancho 1969).

Other microorganisms of interest belong to the *Enterobacteriaceae* family. These bacteria, commonly present in fresh produce, are usually inhibited by the acidic conditions and low pH that develop as the primary fermentation proceeds (Etchells et al. 1945). However, a recent study reported that *Enterobacter cloacae* might be a vector of contamination in fermented green olives (Bevilacqua et al. 2009). *Enterobacter* sp. were also identified as the causative agents in an unusual gaseous spoilage in high-salt fermentation that resulted in the production of hydrogen gas (Etchells 1941a, Etchells et al. 1945). A number of anaerobic organisms have been isolated that relate to fermented cucumber spoilage (Fleming et al. 1989, Kim and Breidt 2007). Among those, *Clostridia* spp. are of interest due to their ability to sporulate under stress conditions and germinate once environmental conditions are favorable.

Investigations into the development of secondary cucumber fermentations have been limited by the inability to predict the event on the commercial scale and by its sporadic occurrence in a small number of fermentation tanks. In the 2010-cucumber-brining season, a considerable number of commercial tanks spoiled due to secondary fermentation, which resulted in the loss of lactic acid and increased pH to an extent that led to discarding of fermented cucumbers (Franco et al. 2012). Microbiological analysis of the brines showed a diverse microbiota composed of yeasts and LAB (Fig. 4). Oxidative yeasts, identified as *Pichia manshurica* and *Issatchenkia occidentalis*, were observed only in spoiled samples, while *Candida etchelsii* was observed in stable commercial samples. The study of this outbreak also confirmed that selected LAB, different from those that carry out primary fermentation, are capable of proliferating during secondary fermentation. These spoilage LAB occurred simultaneously with the spoilage yeasts. Among these bacteria, the LAB *Lb. buchneri* and *Pd. ethanolidurans* were frequently observed in spoiled samples. Figure 4 shows the different colony morphologies of these spoilage microorganisms. Although *Pd. ethanolidurans* persists in these spoilage conditions, it is not capable of metabolizing lactic acid or other spoilage intermediates (Johanningsmeier et al. 2012, Johanningsmeier and McFeeters 2013). Under aerobic conditions, *P. manshurica* and *I. occidentalis* were able to initiate lactic acid utilization at pH values as low as 3.5, resulting in an increase in pH and decrease in redox potential. These changes favored the establishment of bacteria, such as *Lb. buchneri*, *Pd. ethanolidurans*, *Enterobacter* and *Clostridium* spp. whose metabolic activity resulted in acetic, propionic and butyric acid production (Franco and Pérez-Díaz, 2012 a, b, 2013).

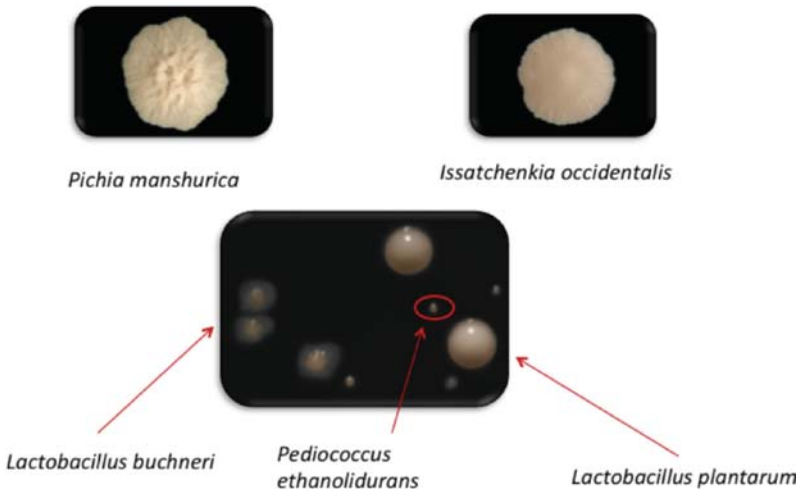

Fig. 4. Yeast and Bacterial Colonies Observed in Commercial and Laboratory Reproduced Spoiled Fermented Cucumber. (*Adapted from Franco and Pérez-Díaz* (*2012a*)

Although, lower NaCl concentrations were considered as important for the development of spoilage in fermented cucumber pickles under anaerobic conditions (Kim and Breidt 2007, Johanningsmeier et al. 2012), data gathered from these commercial spoilage samples indicated that fermentations carried out at 0–6 per cent NaCl and 0.2–1.1 per cent $CaCl_2$ are susceptible to spoilage with the typical air purging routines used in commercial production. Certain spoilage yeasts and LAB are known for being halotolerant (Deák 2008), which, when combined with the ability to utilize organic acids as a source of carbon, gives these organisms a unique competitive advantage in an environment characterized by high NaCl and lactic acid concentrations.

A number of LAB species have been isolated from commercial and laboratory reproduced spoilage samples (Johanningsmeier et al. 2012). Among these bacteria, *Lb. buchneri* and *Lb. parafarraginis* were able to utilize lactic acid in fermented cucumber while converting it to acetic acid and 1,2-propanediol. Subsequent to such conversion, 1,2 propanediol can be converted into propionic acid and propanol by organisms like *Lb. rapi*, also isolated from spoiled fermented cucumbers (Johanningsmeier et al. 2012, Johanningsmeier and McFeeters 2013). A secondary fermentation initiated by *Lb. buchneri* in the absence of the oxidative spoilage yeasts would be preferable on the commercial scale over spoilage induced by oxidative yeasts. This is due to the possibility of having slower lactic acid utilization and the conversion of lactic acid into acetic and propionic acids, which would maintain a more acidic pH with time. The presence of oxidative yeasts and aerobic conditions accelerates the removal of lactic acid, leading to a rapid rise in pH and gas formation that could cause bloating of whole cucumbers. Additionally, the presence of oxidative yeasts accelerates the spoilage associated with the lactic acid bacterium *Lb. buchneri* (Franco et al. 2012). Recently, Breidt et al. (2013b) reported that gram-positive *Firmicutes* dominated the early stages of spoilage fermentation. As pH values approach 5, gram-negative anaerobes mostly representative of the *Bacteriodetes*/*Chlorobi* group dominated, along with the presence of butyric acid in the spoiled brine samples. Metabolic activity of *Propionibacterium* and *Pectinatus* species isolated from more advanced spoilage samples was demonstrated in brine or media with a pH above 4.9, resulting in lactic acid conversion into propionic acid with a further increase in pH.

Based on current scientific evidence, it is logical to conclude that the spoilage process is a complex microbiological process in which biochemical changes are often related to the establishment of specific and diverse microorganisms in a successive fashion. During long-term storage, environmental conditions could favor the establishment of oxidative yeasts, such as *P. manshurica* and *I. occidentalis* when sufficient oxygen is available (Franco and Pérez-Díaz 2012a, b, Franco et al. 2012). While under anaerobic conditions, fermented cucumbers are susceptible to spoilage by *Lb. buchneri* and *Lb. parafarraginis* (Johanningsmeier and McFeeters 2013) unless the pH of 3.2 and 6 per cent NaCl are maintained (Johanningsmeier et al. 2012).

These microorganisms can utilize the organic acids produced during primary fermentation, and thus initiate secondary fermentation with increases in brine pH and chemical reduction in the environment. Under these new conditions, opportunistic bacteria, such as *Cl. tertium* (Fleming et al. 1989), *Cl. bifermentans*, *Enterobacter cloacae* (Franco and Pérez-Díaz, 2012a, b, Franco et al. 2012), *Propionibacterium acidipropionici* and/or *Pectinatus sottacetonis* (Breidt et al. 2013, Caldwell et al. 2013) are able to metabolize lactic acid and other carbon sources, resulting in an increase in propionic acid and production of butyric acid (Fig. 5).

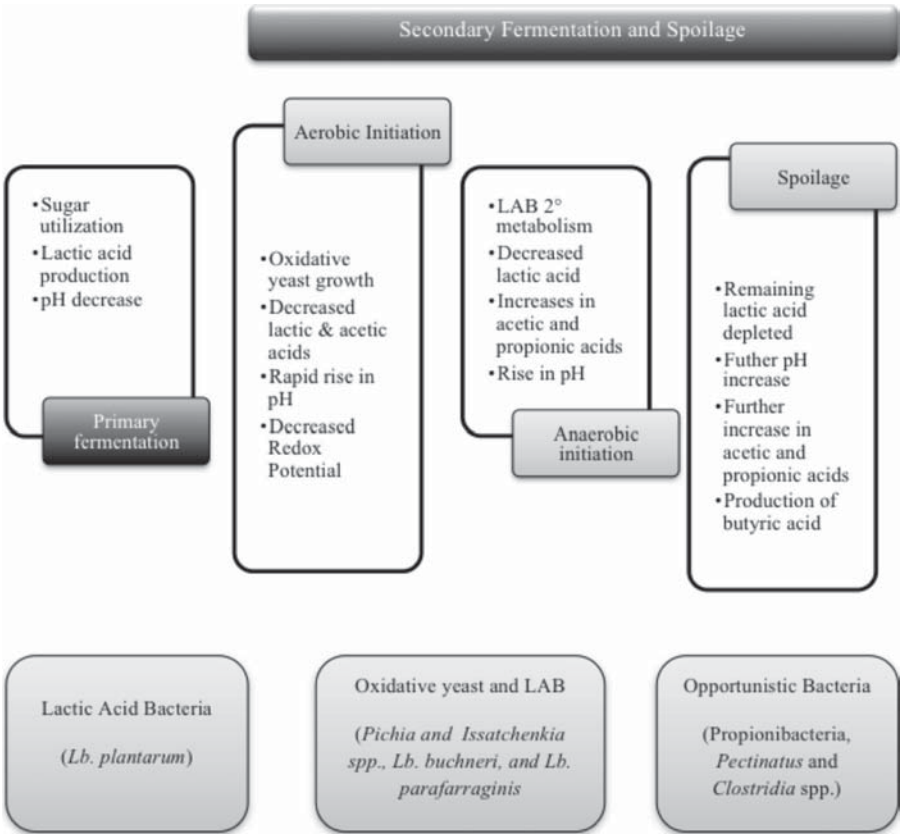

Fig. 5: Succession of Microbiota and Biochemical Changes Associated with Primary and Secondary Fermentations during Storage of Fermented Cucumber Pickles

5. Microbial Safety of Fermented Cucumbers

Acids and acidified foods are defined in the United States Code of Federal Regulations (21 CFR part 114) as having a pH value equal to or lower than 4.6. This pH is the upper limit that prevents *Cl. botulinum* spore outgrowth

and neurotoxin production (Ito et al. 1976). In addition, the regulation specifies that acid and acidified foods, such as fermented cucumbers, should be processed "to the extend that is sufficient" to destroy vegetative cells of microorganisms of public health concern.

Fermented cucumbers, characterized for having low pH values and the presence of organic acids, are considered safe because these conditions inhibit pathogen growth and eventually lead to bacterial death. So far, the safety record for fermented cucumbers has been excellent. During the last five years of reported data (2009-2013), no single food-borne outbreak related to fermented cucumbers was reported (CDC, FOOD Database). Proper control of pH below 4.6 at all times and studies confirming the 5-log reduction of vegetative pathogens of concern are reasons for this good record.

Food-borne pathogens, such as *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica* may be present on the surface of vegetables, including pickling cucumbers (Beuchat 1996, 2002, Brackett 1999, Taormina and Beuchat 1999). Of these, *E. coli* strains, in particular *E. coli* O157:H7, have been reported as the most acid-resistant bacteria in acidified, non-heated vegetables (Breidt et al. 2007). Breidt and Caldwell (2011) reported that a 5-log reduction of *E. coli* O157:H7 in fermented cucumber brine was possible in four days when brine pH was kept below 3.3, regardless of the holding temperature (10°C and above). These results were observed for both active fermentation and stable brines. When lower temperatures and higher pH were used, the authors reported that the 5-log reduction required longer periods of time, but was still achieved within the time frame of most commercial-scale fermentations (i.e. 23 days for pH 3.9 and 23°C). In contrast, a 5-log reduction in *L. monocytogenes* strains in home-prepared, fermented and refrigerated dill pickles required about 50 days, regardless of salt concentration (1.3–6.7 per cent), and, therefore, did not accomplish the pathogen kill required by the Code of Federal Regulations in a timeframe consistent with typical consumption patterns (Kim et al. 2004). Changing safety regulations, development of new technologies for fermentation, and the evolution of pathogenic organisms suggest that more research is needed in this area to ensure a continued excellent safety record for fermented cucumber pickles.

6. Chemical and Physical Changes During Fermentation, Bulk Storage and Processing

6.1. Quality of Fermented Cucumbers

The quality of fermented cucumber pickles is influenced by the presence or absence of physical defects, such as hollow cavities formed due to CO_2 buildup during brining, development of a cured appearance, texture properties and flavor. The greatest body of scientific literature is focused on

the prevention of hollow centers and softening of cucumber tissue during fermentation and bulk storage because these defects, when severe, can result in devastating economic losses for processors.

6.2. Physical Defects

The major physical defect that causes economic losses for processors of fermented cucumbers is the development of hollow cavities in the interior of whole fruits, commonly known as 'bloater formation'. Different patterns of hollow cavities, referred to as balloon, lens and honeycomb, and varying levels of severity of bloating can occur, depending on the conditions of brining (Etchells et al. 1974, Fleming 1979). Severe bloating is related to trapped gas volumes of 10–12 per cent and expansion volumes greater than 200 mL in 4 litres fermentation jars (Fleming et al. 1973a). Early studies showed that increased incidence of severely bloated cucumbers was related to high salt concentrations used for natural cucumber fermentation and was associated with higher gas content in the brines (Veldhuis and Etchells 1939, Jones et al. 1941, Jones and Etchells 1943). The excess gas accumulation occurred between 12–50 days after brining and was composed of both CO_2 and hydrogen between Day 12 and Day 23 (Veldhuis and Etchells 1939). It was later discovered that the unusual production of hydrogen gas occurs in brinestock fermented in 10.6–15.8 per cent NaCl as a result of an aberrant secondary fermentation by *Aerobacter* species, now known as *Enterobacter* species (Etchells 1941a, Etchells et al. 1945). In most cases of bloater formation, the gas inside the cucumber is primarily CO_2 (Etchells and Jones 1941) and excessive gas production during fermentation coincides with an increase in yeast count (Etchells 1941b). Addition of acetic or lactic acids and sugars to fermentation brines significantly increased the incidence of bloating (Jones et al. 1941, Veldhuis et al. 1941), due to shifting of the fermentation environment to one that was more favorable for yeasts. However, even in well-controlled and pure culture lactic acid fermentations, there are a number of sources of CO_2 which peak in concentration between two to seven days of fermentation and is sufficient to cause bloater defects (Etchells et al. 1975). The cucumber fruits themselves contain CO_2 and produce additional CO_2 through respiration for several days after brining (Fleming et al. 1973b). Thus, storage conditions of cucumbers prior to brining that result in higher accumulation of CO_2 or higher respiration rates may also contribute to bloater formation. LAB are another source of CO_2, which in combination with CO_2 from the cucumbers is enough to cause substantial bloating defects. *Lb. brevis*, a heterofermentative LAB that produces one mole of CO_2 for every mole of glucose or fructose consumed, produced severe bloating that was equivalent to that of yeasts, *Saccharomyces rosei* and *S. cerevisiae* (Etchells et al. 1968, Fleming et al. 1978). Interestingly, the decarboxylation of malic acid by homofermentative *Lb. plantarum* during the active part

of fermentation is also a source of CO_2 (McFeeters et al. 1982a) that may contribute significantly to bloater formation (Fleming et al. 1973b). All these sources contribute to the accumulation of CO_2 in the brine and fermenting cucumbers, and a strong relationship exists between the percentage CO_2 saturation of brine and bloater index as higher levels of saturation encourage diffusion of CO_2 into the cucumber fruits. Generally, a saturation of less than 50 per cent is associated with substantially reduced risk of bloater formation (Fleming et al. 1978).

The ability of the accumulated CO_2 to cause bloating of cucumbers depends on several factors. Cucumber cultivar (Wehner and Fleming 1984) and maturity influence the susceptibility of pickling cucumbers to bloating, with larger sized cucumbers exhibiting a higher incidence of bloating under similar fermentation conditions (Jones et al. 1941, Fleming et al. 1973a, Fleming et al. 1977). High NaCl concentration increases the susceptibility of cucumbers to bloating independently of the effects on microbiota discussed previously (Fleming et al. 1978). These effects are believed to be due to differences in the internal structure of cucumbers that influence the absorption of brine. High buoyancy forces increase bloater index as a result of the physical internal damage to the fruits, but an increase in hydrostatic pressure (influenced primarily by tank depth) decreases the cucumber susceptibility to bloating (Fleming et al. 1977).

Control of bloater defects is therefore a balance between the microbiological, chemical and physical conditions of the system. NaCl concentration, temperature and availability of oxygen significantly influence the composition of microbiota, which are substantial sources of CO_2. The sugar and malic acid content of cucumbers may vary due to cultivar, maturity and growing conditions (McFeeters et al. 1982b). The impact of these variations in substrates for CO_2 production will depend on the composition of the microbial community at any given time during cucumber fermentation. Natural fermentations in reduced NaCl brines (2.7 per cent) accumulated more CO_2 than those in 5.4 or 7.0 per cent of NaCl (Fleming et al. 1973b), perhaps due to the increased solubility of CO_2 at lower NaCl concentrations (Fleming et al. 1975) or possibly due to differences in microbiota. The solubility of CO_2, a governing factor in bloater defect formation (Corey et al. 1983b), decreases with increasing NaCl and temperature in fermented cucumber brines at pH 3.3 (Fleming et al. 1975), which influences the movement of gases in the cucumber (Corey et al. 1983b). It has been observed that less bloating occurs at lower fermentation temperatures (Etchells et al. 1975). Though pH does not substantially influence the solubility of CO_2, it does influence the proportion that exists as the gas vs. bicarbonate ion, which is relevant for efficient removal of CO_2 using nitrogen or air-purging techniques (Fleming et al. 1975). The microbiological and chemical factors that lead to CO_2 accumulation in the brine challenge the physical limitations of cucumber to resist damage caused by CO_2 trapped inside the cucumber

tissues. It was determined that the critical period for susceptibility to bloating was between one to 32 days (Fleming et al. 1978) and that physical pre-treatments that increase the ability for brine to move into the cucumber, such as piercing (Etchells and Moore 1971, Fleming et al. 1973a), peeling (Fleming et al. 1973a) and oxygen-exchange (Fleming et al. 1980), are effective in eliminating bloater formation. Since bloater defects occur frequently, even in controlled fermentations, purging systems were developed to remove CO_2 from brines, effectively eliminating the occurrence of bloaters (reviewed by Fleming 1979). Nitrogen purging is effective in reducing CO_2, eliminating bloating (Fleming et al. 1973a, 1977) and in reducing the incidence of bloaters from 60 per cent to less than 10 per cent for size 3B cucumbers in a commercial system employing a small-pore sized, gas diffuser (Costilow et al. 1977).

6.3. Color Changes and Cured Appearance Development

The appearance of fermented cucumber pickles is largely influenced by the exterior skin color and the change in mesocarp from opaque white in raw cucumbers to a translucent appearance in the completely fermented fruit (Figure 6). The intense green color of raw cucumber fruit changes to a dark olive-green during fermentation due to the conversion of chlorophylls to pheophorbides and to a lesser extent pheophytins (White et al. 1963). Differences in the skin color may occur due to cultivar selection and growing conditions (Shetty and Wehner 2002, Jasso-Chaverria et al. 2005, Gómez-López et al. 2006, Aghili et al. 2009) and/or undesirable changes associated with photooxidation during brining and storage that result in a lightening and yellowing of the surface color (Buescher and Hamilton 2000). Cucumbers and the salts used for brining contain trace amounts of metals, such as iron, zinc, and copper, which can promote oxidation of pigments (Eisenstat and Fabian 1953). Protection from light or addition of 100–200 ppm $CaNa_2EDTA$, a stable compound with metal-chelating capabilities, to cucumber fermentations inhibits the bleaching of color associated with photoxidation (Buescher and Hamilton 2000).

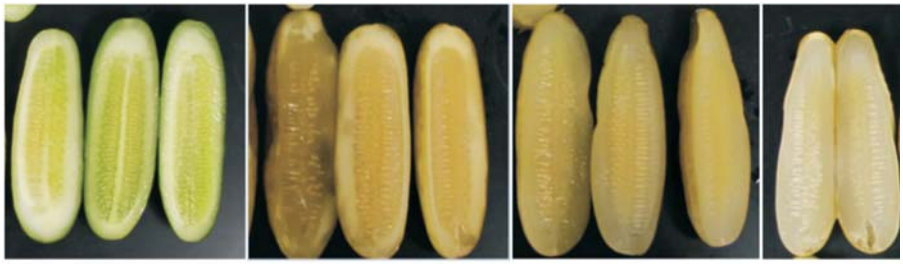

Fig. 6. Changes in Cucumber Fruit Appearance during Fermentation and Bulk Storage

In finished products, a red color defect can occur due to the metabolism of yellow 5 (tartrazine), a common additive in pickle products, by *Lb. casei* or *Lb. paracasei* during shelf storage of unpasteurized or improperly pasteurized pickles (Pérez-Díaz et al. 2007, Pérez-Díaz and McFeeters 2009). Other commonly observed, but not scientifically documented color defects can occur during storage and processing of fermented cucumber pickles, including bright green and blue-green surface pigmentation due to higher than normal levels of contaminant metals and browning/rust, color development that may be related to oxidation or polymerization reactions.

The development of a cured appearance is considered a desirable characteristic of fermented cucumber pickles and the cucumbers are not processed further until they are fully cured. The cured appearance is due to the absorption of brine into intercellular spaces that are filled with ~60–94 mL/kg gas in the raw cucumber (Veldhuis and Etchells 1939, Corey et al. 1983a, c). The rate of curing varies significantly with fermentation. These authors have observed fully cured cucumbers as early as eight days after brining as well as only partially cured cucumbers 30 days after brining under current commercial production conditions that employ air purging during fermentation (Johanningsmeier, unpublished data). Several factors may influence the rate of curing, including internal cucumber pressure, temperature, redox potential of the system and proteolytic activity from the cucumber tissue or fermentation microbiota. Application of vacuum or heat speeds the curing process (Veldhuis and Etchells 1939). Air-purged fermentation achieve a cured appearance more rapidly than unpurged or nitrogen-purged fermentation; higher fermentation temperatures favor faster curing rates; and oxygen-exchanged cucumbers rapidly absorb brine and appear fully cured within 24 hours of brining (Fleming et al. 1980). Additional research on development of cured appearance has been conducted in refrigerated and pasteurized cucumber pickles where the cured appearance is considered a quality defect. Pasteurized cucumber pickle products are recommended to have no more than 10 ins. Hg vacuum to prevent rapid curing of the product upon opening (Etchells and Jones 1944). In acidified cucumbers, curing is related to proteolytic changes in cell wall constituents during shelf storage (Howard and Buescher 1993, Mok and Buescher 2012, Buescher et al. 2013). Oxidizing agents slow the curing and reducing agents hasten it (Howard and Buescher 1993). Therefore, changes in redox potential during fermentation (Olsen and Pérez-Díaz 2009) and potential proteolytic activity of fermentation microbiota (Liu et al. 2010) may also influence the rate of curing. In most commercial facilities today, long-term bulk storage is desired, so the variations in the rate of curing have little impact. However, implementation of new fermentation technologies and a growing demand for fermented vegetables by consumers may drive a faster turnover of the product and stimulate more research in this area to better predict and control cure rates.

6.4. Texture Properties

Texture quality of fermented cucumber pickles is primarily associated with sensory attributes of crunchiness, crispness, firmness and fracturability (Breene et al. 1972, Ennis and O'Sullivan 1979, Rosenberg 2013). Because of the high degree of cucumber to cucumber variability and the costs associated with maintaining trained descriptive sensory panels, mechanical texture measurements have been developed for monitoring softening of fermented cucumbers at various stages of processing. The first such method that was widely adopted by the pickling industry was the use of a penetrometer with a 5/16″ tip (USDA Fruit Pressure Tester) to measure the entire fruit firmness of various cultivars after brining and storage (Jones and Etchells 1950). This method is quick, inexpensive, and a good indicator of whether the fermented fruits are firm enough to be processed into finished products. Since this method measures the force required to puncture through the exocarp (cucumber skin) and the mesocarp tissue below the surface (flesh), it does not differentiate between the small changes in the mesocarp that may relate to the sensory quality of sliced products. Texture profile analysis of fresh and brined cucumbers with and without skin showed that the sensory perception of crispness is more closely correlated with instrumental brittleness measurements for the cucumber tissue with skins removed (Jeon et al. 1973, 1975). Other methods that use a puncture test to measure mesocarp firmness also correlate well with fermented cucumber pickle sensory attributes of firmness (Thompson et al. 1982), crunchiness (Rosenberg 2013) and crispness (Yoshioka et al. 2009, 2010, Buescher et al. 2011, Rosenberg 2013). Significant differences in peak puncture force of hamburger dill chips were also related to consumer perception of texture quality with higher liking of texture clearly associated with higher peak puncture forces of pickle mesocarp (Wilson et al. 2015).

Many factors are known to influence the texture quality of fermented cucumbers. It has been shown that NaCl concentration, calcium addition, temperature, pH, bulk storage time, cucumber cultivar and maturity and the presence or absence of softening enzymes all influence the ability to maintain firm, fermented cucumber fruits for processing into pickle products (Table 2). These factors exert their effects in an interdependent fashion (Thompson et al. 1979, McFeeters et al. 1995), which makes it challenging to extrapolate the results of any one study to a commercial environment. That said, there are consistent patterns that have emerged from the many research studies performed to date. The concentration of NaCl used for brining and bulk storage is a key factor. Softening of cucumbers is consistently observed if NaCl concentrations of 1.8 per cent or less (equilibrated) are used for fermentation and bulk storage (Hudson and Buescher 1985, Fleming et al. 1987, 1996), and variable softening occurs with fermentation and storage in 2–2.8 per cent NaCl. However, the addition of 0.2–0.44 per cent $CaCl_2$ to fermentation is known to improve the retention

Table 2: Factors that Influence the Texture Quality of Fermented Cucumber Pickles

Brine Ingredient or Intrinsic Factor	**Function**	**Optimal Range**	**Legal Limit**
Sodium chloride con-centration	Selects for proper fermentation microbiota; influences microbial stability and texture quality during bulk storage	3–7 per cent for natural fermentation; 4–10 per cent for bulk storage	GRAS status; Limit is for chlorides in wastewater (230 ppm)
Calcium (usually added as $CaCl_2$)	Firming agent; protects against pH-induced softening and inhibits softening due to pectinolytic or cellulytic enzyme action	25–72 mM (0.28-0.8 per cent as $CaCl_2$) depending on NaCl concentration, cucumber size and downstream processing	36 mM (0.4 per cent) $CaCl_2$ in finished pickle products
Potassium sorbate	Anti-fungal to prevent spoilage yeasts in closed, anaerobic systems and inhibit molds in air-purged fermentations	6–20 mM (0.1–0.3 per cent) at pH 4.5 or below	
Acetic acid (as vinegar)	Reduces initial brine pH to give a selective advantage to lactic acid bacteria; fungal inhibitor; inhibits Enterobacteriaceae	27–50 mM (0.16–0.3 per cent) depending on purging routines and other brine ingredients	GRAS status
Alum	Firming/crisping agent used in desalting waters or finished products	Varies	Considered a processing aid
pH	Influences initiation of fermentation; induces softening at pH's below 3.5 and at neutral pH during extended hold times	3.5–4.3	None. Food safety standard: pH should reach less than 4.6 as quickly as possible and be maintained below this value throughout storage and processing
Tem-perature	Impacts initiation, rate and extent of fermentation; softening rates during storage increase with increasing temperatures	Storage conditions: As low as is feasible without freezing	Not Applicable
Pectin methyl-esterase (PME)	Cell wall enzyme that demethylates pectin	Unknown	Not Applicable

Contd...

Table 2: (***Contd.***)

Brine Ingredient or Intrinsic Factor	Function	Optimal Range	Legal Limit
Polygala-cturonase	Cell wall enzyme that cleaves polygalacturonic acid (demethylated pectin), resulting in texture quality loss	None	Not Applicable
Cellulase	Cell wall enzyme that cleaves cellulose, resulting in texture quality loss	None	Not Applicable

of firmness in cucumbers fermented and stored in low salt (1.8–2.6 per cent NaCl) brines (Buescher et al. 1979, Hudson and Buescher 1985, Fleming et al. 1987, McFeeters et al. 1995), including the prevention of soft center defects in size 4 (5.1–5.7 cm diameter) cucumber fruits when brined with $CaCl_2$ and NaCl to equilibrate at 0.44 and 1.8 per cent, respectively (Hudson and Buescher 1980). Even in fermentation brines containing higher NaCl concentrations for fermentation (5–7 per cent wt/vol) and storage (5–11 per cent wt/vol), the addition of 0.10–0.44 per cent $CaCl_2$ was found to improve firmness retention during bulk storage (Thompson et al. 1979, Tang and McFeeters 1983, Fleming et al. 1987, Buescher and Burgin 1988, Guillou et al. 1992, Guillou and Floros 1993) and in finished pickle products (Buescher and Burgin 1988). Conversely, simple addition of 0.2 per cent $CaCl_2$ to fermentation brines in the absence of NaCl was not able to maintain the cucumber firmness during bulk storage, despite implementation of controlled-fermentation procedures (Fleming et al. 1995). These results are consistent with the multiresponse optimization model which predicted that the optimal $CaCl_2$ concentration for brining and storage of cucumbers relying on a natural fermentation is 0.28 per cent in combination with 0.3–0.32 per cent potassium sorbate and 3–7 per cent NaCl (Guillou and Floros 1993). Increasing calcium concentrations from 0 to 25 mM (equivalent to 0.28 per cent $CaCl_2$) dramatically reduced the softening rate of fermented cucumber tissue during storage in 2 per cent NaCl with varying pH and temperature combinations (McFeeters et al. 1995). Thus, it is currently a common practice in the U.S.A. to add $CaCl_2$ (0.1–0.3 per cent, equilibrated) to brines for commercial production of fermented cucumbers (Pérez-Díaz et al. 2014).

Calcium ions added to fermentation brines help maintain cucumber firmness regardless of the form that is used for addition. Calcium acetate or calcium hydroxide used in buffered systems for controlled cucumber fermentation, $CaCl_2$, and sea salts that naturally contain calcium and magnesium (Yoo et al. 2006) have all been used to improve firmness retention during fermentation and storage. The natural level of calcium

in pickling cucumbers ranges from 1.8–8.3 mM (Hudson and Buescher 1985, McFeeters and Fleming 1989, McFeeters et al. 1995) and has been observed to significantly influence the softening rates of cucumbers stored in acid brines (McFeeters and Fleming 1989). To prevent softening during fermentation in low NaCl-brined cucumbers, calcium needs to be added at brining (Hudson and Buescher 1985, McFeeters et al. 1995). However, the softening rates during subsequent storage are mostly dependent on the concentration of calcium during storage (McFeeters et al. 1995). An empirical model was developed for the effects of pH (2.6–3.8), temperature (25–65°C), and calcium concentration (0–72 mM) on fermented cucumber softening rates during storage (McFeeters et al. 1995). This model suggests that a high concentration of calcium ions may be used to override the softening effects of low pH at temperatures up to 40°C (104°F). Although calcium chloride has been shown to inhibit PG-induced softening (Buescher et al. 1979, 1981a), it appears that it frequently aids in firmness retention that is independent of detectable PG activity (Buescher et al. 1981b). It was shown that increasing the $CaCl_2$ to 0.8 per cent for fermentation and bulk storage in NaCl brines resulted in a higher residual calcium concentration in the finished products (~31 mM) that helped maintain sensory crispness during shelf storage for one year without the use of alum in the desalting water (Buescher et al. 2011).

Alum, the common name for a variety of aluminum containing additives, such as potassium aluminum sulfate, sodium aluminum sulfate, ammonium aluminum sulfate or aluminum sulfate, is used during the desalting process or in finished pickle products as an ingredient to increase or maintain the pickle firmness and crispness. Despite the historical use of alum in pickling, a number of studies demonstrate that calcium is a more effective firming agent during storage of finished pickle products (Buescher and Burgin 1988) and that 31 mM calcium in finished products will achieve crispness retention better than the typical practice that includes 0.35 per cent wt/wt alum during desalting (Buescher et al. 2011). However, alum is still commonly added to desalting waters as insurance against texture quality losses as reflected by the range in aluminum content in fermented cucumber pickle products from 0–200 ppm (Pérez-Díaz et al. 2014).

In addition to the important role that salts play in texture quality, pH and temperature can have a significant impact on the ability to maintain high-quality texture attributes during storage in bulk or as finished products. Softening rates in fermented cucumbers increase with decreasing pH from 4.3 to 2.6 (McFeeters et al. 1995, Fleming et al. 1996). This relationship is also apparent in blanched cucumber tissue from pH 2–5 (McFeeters and Fleming 1991) and for *in vitro* cucumber pectin hydrolysis rates from pH 2–3.5 (Krall and McFeeters 1998), suggesting that the effect of pH in this region is independent of enzymatic or microbial mechanisms. The lowest softening rates were observed with the addition of 20 mM $CaCl_2$ to blanched cucumber mesocarp tissues with pHs of

3.5–4.5 (McFeeters and Fleming 1991). Addition of $CaCl_2$ to brined whole cucumber dramatically improved firmness retention for fruits stored at pH 3.3 as compared to those at pH 3.8 (Thompson et al. 1979), and the optimal pH for assuring both texture quality and microbial stability of fermented cucumbers in bulk storage was a pH of 3.5 (Fleming et al. 1996). Interestingly, *in vitro* acid hydrolysis of cucumber pectin was unaffected by 20 mM $CaCl_2$, indicating that some other mechanisms may be responsible for the softening of fermented cucumber pickles at low pH (Krall and McFeeters 1998). The effect of temperature appears to be additive and linear in most cases. Higher temperatures for storage result in greater softening rates (McFeeters et al. 1995). Temperature and pH can also influence enzymatic softening rates. However, this relationship is more complex as there are many isozymes of common softening enzymes, each with different temperature and pH optima.

Much less is known about the influence of cucumber cultivar and maturity on the texture after fermentation and bulk storage. However, a few studies have found differences in texture properties among cucumber cultivars using the raw fruits for evaluation (Breene et al. 1972, Yoshioka et al. 2009) and evaluation of cultivars after brining and storage (Jones and Etchells 1950, Jones et al. 1954). A study of 20 pickling cucumber cultivars showed that cultivar differences in crispness and firmness were only apparent after brining (Ennis and O'Sullivan 1979), indicating the challenges in evaluating the cucumber fruit suitability for brining. Therefore, brining trials are often conducted among seed companies, farmers and briners to select the best varieties for cultivation and pickling. Extended storage of pickling cucumbers prior to brining can also lead to changes in the microstructure, causing textural defects in the finished products (Walter et al. 1990), and the ideal conditions for storage were deemed to be 10°C and high relative humidity (Etchells et al. 1973).

Efforts to understand the root causes of softening of fermented cucumber tissues have focused on changes in the pectic substances of the middle lamella and the role of softening enzymes of native and fungal origin. Microscopic examination of soft and firm fermented cucumbers indicated that mesocarp tissue softening was related to swollen cell walls and dissolution of the middle lamella, resulting in decreased strength of the cell-to-cell junctions (Walter et al. 1985). Cucumber pectin isolated from cell walls was found to be ~74,200 Daltons with an average degree of polymerization of 402 (Tang and McFeeters 1983) and 34–64 per cent degree of methylation (Tang and McFeeters 1983, Hudson and Buescher 1985, McFeeters and Lovdal 1987, McFeeters et al. 1995, Krall and McFeeters 1998). Extensive demethylation occurs during fermentation, resulting in no (Tang and McFeeters 1983) to 14–15 per cent (Hudson and Buescher 1985, McFeeters et al. 1995) methylated residues in fermented cucumber pectic fractions. Changes in the degree of methylation of pectin were reflected in a decrease in acid soluble and an increase in EDTA soluble pectic fractions

(Hudson and Buescher 1985). However, the degree of pectin demethylation did not consistently relate to calcium concentration or cucumber tissue firmness among these studies. Cell-wall neutral sugar content is relatively low compared to other fruits and vegetables: 13–16 per cent of cell-wall by weight, comprised of primarily galactose (50 per cent) followed by xylose, glucose, mannose and arabinose (Tang and McFeeters 1983), which changes in composition to a small degree during maturation (McFeeters and Lovdal 1987). Softening of fermented cucumber tissue was related to losses in galacturonic acid, galactose, arabinose and rhamnose content using an alternative cell-wall extraction procedure that allowed detection of small changes in solubility of cell-wall polysaccharides (McFeeters 1992). Changes in the solubility of the pectin appear to be related to changes in cell-wall polysaccharides that influence texture quality, but the exact mechanisms of softening have not been fully elucidated.

Polygalacturonase, an enzyme that catalyzes the hydrolytic cleavage of pectic acid, is known to cause varying degrees of softening during storage of fermented cucumbers (Bell et al. 1950, Buescher et al. 1981a). Pectinase, PG and cell-free mold extracts caused softening of cucumbers and the extent of softening was dependent on NaCl concentration (Bell and Etchells 1961). Pectolytic enzyme activity can come from the cucumbers themselves, especially the seeds and ripe fruits (Bell 1951, Pressey and Vants 1975, McFeeters et al. 1980, Saltveit and McFeeters 1980, Cho and Buescher 2012), pollinated flowers that are not removed before brining (Bell 1951, Bell et al. 1958) and other fungal sources (Etchells et al. 1958a). Certain species of yeasts, such as *S. fragilis*, are unique in their ability to cause softening of cucumber brinestock (Bell and Etchells 1956), whereas an abundance of molds isolated from cucumbers and blossoms possess both PG and cellulase activity (Etchells et al. 1958a). Cellulases can also induce softening in fermented cucumbers during long-term storage (Buescher and Hudson 1984). The leaves of certain plants, including leaves from Scuppernong grapes, contain a natural inhibitor against softening enzymes (Bell et al. 1960, 1962, 1965a, b, Bell and Etchells 1961, Porter et al. 1961, Etchells et al. 1958b), which may be why many traditional home recipes suggest adding a couple of grape leaves to the ferment. In commercial fermentations that employ air-purging to reduce bloater defects, acetic acid can be added to reduce mold-induced softening of cucumbers (Potts and Fleming 1982).

6.5. Volatile Flavor Compounds in Fresh and Fermented Cucumbers

The characteristic aroma of fresh cucumbers is attributed to (E,Z)-2,6-nonadienal and E-2-nonenal, which are enzymatically synthesized from linolenic and linoleic acids by the action of lipoxygenase during tissue disruption (Fleming et al. 1968, Buescher and Buescher 2001). E-2-nonenal has been shown to have approximately 2 per cent of the odor impact of (E,Z)-2,6-nonadienal (Xu et al. 2012). Interestingly, significantly

less (E,Z)-2,6-nonadienal is produced in the exocarp as compared to the mesocarp and endocarp tissues, which explains why smaller-diameter fruits are associated with less (E,Z)-2,6-nonadienal production (Buescher and Buescher 2001). Exocarp tissues have higher levels of lipoxygenase, hydroperoxide lyase and unsaturated fatty acids; thus, the low (E,Z)-2,6-nonadienal production by exocarp tissues is attributed to limited substrate, enzyme inhibitors, or production with concomitant degradation (Pederson et al. 1964, Wardale et al. 1978, Wardale and Lambert 1980, Buescher and Buescher 2001). Palma-Harris et al. (2002) demonstrated that an increase in (E,Z)-2,6-nonadienal resulted in an increase in the intensity of 'fresh cucumber flavor' in refrigerated cucumber pickles as assessed by 24 trained panelists. These fresh cucumber flavors are not typically observed in fermented cucumbers due to the inactivation of lipoxygenase under acidic pH conditions (Wardale and Lambert 1980). Similarly, other conditions that lead to inactivation of lipoxygenase, such as freezing or pasteurization, also result in the observed loss of (E,Z)-2,6-nonadienal production (Buescher and Buescher 2001).

Fresh cucumber juice also contains ethanol, propanal, (E)-2-pentenal, hexanal, (E)-2-hexenal and (Z)-6-nonenal (Cho and Buescher 2011). Linoleic acid is believed to be the precursor of hexanal, (E)-2-heptenal, (E)-2-octenal, and E-2-nonenal; while propanal, (E)-2-hexenal, (E)-2-pentenal, ethanal and (E,Z)-2,6-nonadienal are formed from linolenic acid (Grosch and Schwarz 1971, Zhou et al. 2000). Cucumbers have a very high fatty acid α-oxidation activity, in which fatty acids are enzymatically broken down into $C_{(n-1)}$ long-chain fatty aldehydes and CO_2 (Borge et al. 1998). For example, pentadecanal is identified as the product of α-oxidation of palmitic acid in cucumber homogenate (Borge et al. 1998). Due to the high water activity of cucumber pickles, oxidation of these products involves enzymatic or microbial mechanisms; direct, non-enzymatic oxidation tends to be outcompeted by other modes of deterioration at high water activities (St. Angelo 1992).

The production of these flavor-active aldehydes is hypothesized to include breakage of double bonds in the unsaturated fatty acids by a dioxygenase-like reaction and the formation of hydroperoxide intermediates (Grosch and Schwarz 1971, Zhou et al. 2000). Among these compounds, (E,Z)-2,6-nonadienal and hexanal were identified in fermented cucumber brines with characteristic aromas of 'fresh cucumber' and 'green', respectively (Marsili and Miller 2000). Additionally, (E)-2-nonenal and (E)-2-octenal were identified by Cordero et al. (2010) in roasted hazelnuts with fatty/green and green aromas, respectively.

Although more often associated with raw fruits or refrigerated pickles, 'Green' is a term sometimes used to describe the aroma of freshly-fermented cucumbers. Eight volatile compounds are characteristic of the green odor emitted by plant leaves: (Z)-3-hexenol, (E)-3-hexenol, (E)-2-hexenol, (Z)-3-hexenal, (E)-3-hexenal, (E)-2-hexenal, hexanol and

hexanal (Hatanaka 1996). Forss et al. (1962) found that (E,Z)-2,6-decadienal was characterized by the flavor notes of 'green', 'plant-like', and 'like cucumbers'. After further review of literature, Forss et al. (1962) hypothesized that the cis-non-conjugated unsaturation is characteristic of a compound that produces a 'green' or 'plant-like' flavor. Interestingly, 2-nonenal and (Z,Z)-2,6-nonadienal were described as 'oily' and 'tallow', and (Z,E)-2,6-nonadienal as 'green' or 'like cucumbers' when evaluated near their threshold concentrations; but, as the concentration of the compounds increased, panelists gave the description of 'like cucumbers' to all three compounds (Forss et al. 1962).

A number of secondary reactions occur during fermentation of cucumber pickles, contributing to the flavor profile of finished products. Fermented cucumbers evaluated directly from the tankyard have been described as silage-like, sour, slightly sweet and green (Marsili and Miller 2000). These authors identified trans- and cis-4-hexenoic acid, low volatility compounds, as key flavor compounds with aromas characteristic of fermented cucumber brine by gas chromatography-olfactometry. Additional compounds with high odor impact values in fermented cucumber brines included 2-heptanol, cis-2,4-hexadienoic acid (tentative identification), phenyl ethyl alcohol, 2,6-nonadienal and 2-dodecen-1-al (tentative identification). While pure solutions of trans-4-hexenoic acid were characterized as being similar to authentic brine samples, addition of phenyl ethyl alcohol (a rose/floral note) to the pure solution resulted in a closer match. Phenylacetaldehyde was also present in brine samples and is produced by oxidation of phenyl ethyl alcohol (Marsili and Miller 2000). Murray and Whitefield (1975) reported the presence of 3-isopropyl-2-methoxypyrazine in fresh cucumbers. Since the compound does not change significantly during fermentation and has an odor threshold of only 2 ppt, it possibly plays a role in the overall aroma profile of fermented cucumbers (Zhou and McFeeters 1998, Whitefield and Last 1991).

Previous work by Zhou and McFeeters (1998) identified high levels of linalool in 2 per cent salt fermentation brines, but Marsili and Miller (2000) observed linalool in only 5 per cent of commercial brine samples with 8–10 per cent salt concentration, indicating linalool is really not a major aroma impact compound in industrial-scale fermented cucumbers. However, varying salt levels in fermentations could alter the microbiota, which could impact the production of by-products that contribute to flavor and aroma (Marsili and Miller 2000). Zhou and McFeeters (1998) compared volatile compounds found in fresh cucumbers to those in fermented cucumbers: ethyl benzene, o-xylene and benzaldehyde were the only compounds identified in fermented cucumbers that were not present in fresh cucumbers. Four compounds of interest were found in lower concentrations in fermented cucumbers as compared to fresh cucumbers: hexanal, (E)-3,7-dimethyl-1,3,6-octatriene, (E,Z)-2,6-nonadienal and 2-undecanone (Zhou and McFeeters 1998). More recently, Johanningsmeier

and McFeeters (2011) identified 314 volatile compounds in the brine of cucumbers fermented in 6 per cent NaCl, including hydrocarbons, aldehydes, alcohols, ketones, acids, esters, ethers, furans, pyrans, phenols, nitrogenous compounds and sulfur-containing compounds.

Oxidized flavor is an off-flavor of particular concern for pickle products. By definition, oxidation is simply the loss of electrons from a compound (Smith 2008). The oxidation of polyunsaturated fatty acids is of particular concern in flavor chemistry (Andreou and Fuessner 2009). Oxidation of unsaturated lipids is the process in which carbon-centered alkyl radicals and peroxy radicals are formed due to the presence of initiators, such as enzymes, light, heat, metals, metalloproteins and microorganisms. After initiation, propagation in the presence of oxygen leads to the formation of hydroperoxides as the primary products (St. Angelo 1992). In the presence of light, a non-free radical process known as photooxidation may form hydroperoxides due to the reaction of unsaturated fatty acids with singlet oxygen resulting from the excitation of oxygen by a photosensitizer, such as chlorophyll (Frankel 1991). For plant tissues, the polyunsaturated fatty acids found in greatest abundance are linoleic and linolenic acid (Baysal and Demirdoven 2007). After completion of cucumber fermentation, Pederson et al. (1964) observed a five-fold increase in free fatty acids and a reduction in phospholipids greater than 90 per cent of that found in the raw cucumber. Linoleic acid was found in the flesh, skin and seed of raw cucumbers at concentrations of 11, 38, and 26 mg/100 g. After fermentation, linoleic acid in the flesh, skin and seed increased to 215, 444, and 908 mg/100 g. Similarly, linolenic acid increased in the flesh, skin and seed from 10, 255, and 18 mg/100 g to 450, 545, and 1067 mg/100 g, respectively. The lipid content of cucumber flesh is much lower than that of the seed or skin, which suggests that some aromatic compounds might partition to the endo- or exocarp (Pederson et al. 1964). However, Zhou and McFeeters (1998) found that volatile compounds in fermented cucumber slurry and brine differed less than twofold in relative peak areas for the 37 identified compounds. Consistent with expectations, hexanal and ethyl-benzene were found in higher concentrations in the brine, while α-caryophyllene, 2-undecanone and (E,Z)-2,6-nonadienal were found in higher concentrations in fermented cucumber.

During fermentation and bulk storage, cucumbers are susceptible to oxidation due to the use of open tanks exposed to sunlight and the common practice of purging the tanks with air to mix the contents and remove CO_2 (Buescher and Hamilton 2000). Additionally, cucumbers and the salt used for brining contain trace amounts of metals, such as iron, zinc, and copper, which can play a role in promoting oxidation of pigments and flavor compounds (Eisenstat and Fabian 1953). Fermented cucumber homogenates incubated in the presence of oxygen exhibited significant increases in hexanal, (E)-2-heptenal, (E)-2-pentenal, (E)-2-hexenal and (E)-2-octenal that were highly correlated to oxidized odor intensity, as assessed

by 20 trained sensory panelists (Zhou et al. 2000). Furthermore, Zhou et al. (2000) determined that the aldehydes were not formed due to lipoxygenase activity since heat treatment to inactivate the enzyme prior to oxygen exposure did not result in a significant reduction in aldehyde production. However, recent studies show that several of these aldehydes decrease during long-term bulk storage of fermented cucumbers, giving rise to ketones and other compounds highly correlated with oxidized off-flavor in finished hamburger dill chips (Wolter 2013). Venkateshwarlu et al. (2004) modeled oxidative off-flavors commonly found in fish oil by mixing pure volatile flavor compounds in milk and evaluating the intensity of fishy and metallic off-flavors in these samples. A trained 16-member descriptive analysis panel determined that (E,Z)-2,6-nonadienal and 1-penten-3-one were the two main compounds characteristic of the off-flavors described as metallic and fishy. Of note, the fishy and metallic flavors were not present when the compounds were added to milk alone, confirming the importance of compound interaction in flavor production. In addition, the metallic odor of penten-3-one was enhanced in the presence of heptenal, while a synergistic relationship between (E,Z)-2,6-nonadienal and (Z)-4-heptenal contributed to fishy off-flavors (Venkateshwarlu et al. 2004). It is possible that in the earlier studies of oxidized off-flavor in fermented cucumber (Zhou et al. 2000), the unsaturated aldehydes were acting as potentiators for undetected compounds responsible for the oxidized flavor perceived by the sensory panel. More research is needed to fully understand the relationship between the changes in chemical composition and off-flavor development.

7. Sodium Chloride Reduction Strategies

7.1. Environmental Considerations

One of the biggest drivers of process change in cucumber fermentation has been the regulatory pressures to reduce salt discharge in the environment. In the US, there are many different regions with varying regulations depending on the state and location, with proximity to large municipal systems being more favorable. Nonetheless, waste treatment is required in one form or another, including removal of vegetable solids, adjustment of pH and control of hydraulic flow, BOD and chloride content.

7.2. Brine Recycling

Brine recycling is one way for the cucumber fermentation industry to reduce its salty effluent waste stream. The primary barriers to reusing the salty tank brine in the past were the carryover of softening enzymes and off-flavors. A diffusion plate assay for testing recycled brines for PG activity (Buescher and Burgin 1992) is used routinely for management of recycled brines. Various strategies were developed to inactivate or

remove the softening enzymes from brines prior to recycling, including coagulation and settling, heat inactivation, ultrafiltration, activated carbon and clay treatment (Geisman and Henne 1973, Little et al. 1974, Palnitkar and McFeeters 1975, Mercer et al. 1971, Buescher and Hamilton 2007). Today, because of its low investment and operating cost, clay treatment is the most commonly used process in commercial production. Pure-Flo B80 from Oil Dry-Corp has been a widely used bentonite based clay for the removal of softening enzymes in spent pickle brines. Even with active brine recycling programs in place, the disposal of large volumes of effluent waste water from the desalting process remains a challenge for large processing operations.

7.3. Salt Reduction in Cucumber Fermentation

Simple reduction of NaCl below the 6-7 per cent wt/vol concentration used in most commercial fermentations significantly increases the susceptibility to secondary fermentations and/or tissue softening, thereby increasing the risk of economic loss. However, research to develop new technologies that use less NaCl or even no NaCl have been developed to provide sustainable alternatives to the current process. A 'process-ready' bag-in-box fermentation technology was developed (Fleming et al. 2002a, b) that resulted in high-quality finished products (Johanningsmeier et al. 2002) and the ability to filter the brine for use in finished products (Fasina et al. 2002, 2003), such that the entire contents of the fermentation could be consumed with very little waste. This technology has not yet been widely adopted primarily because the investment in current infrastructure in tankyards was not conducive to an immediate shift to the new technology and investments would need to be made in new types of equipment. Guillou and Floros (1993) demonstrated that natural fermentations and bulk storage of cucumbers for 6 mo could be accomplished in 2.5-3.0 per cent NaCl in combination with 0.3 per cent $CaCl_2$ and 0.32 per cent potassium sorbate, but there is no public record of commercial trials with this process. Most recently, a fermentation process that relies entirely on calcium chloride as the only salt promises to eliminate sodium salt waste from the tankyard and reduce chloride levels by 60-80 per cent (McFeeters and Pérez-Díaz 2010). This process employs 1.1 per cent of $CaCl_2$ to aid in firmness retention, a *Lb. plantarum* starter culture, potassium sorbate to inhibit yeasts, and fumaric acid or sodium benzoate to stabilize the tanks for long-term bulk storage (Pérez-Díaz et al. 2015) and was capable of producing acceptable quality-hamburger dill chips on the commercial scale (Wilson et al. 2015). Commercial trials (2011-2014) have illuminated the challenges associated with scale-up to a production environment and active research in this area includes: 1) selection of appropriate starter cultures; 2) optimization of texture quality of finished products; 3) investigation of the suitability of this process in cold climates; and

4) adapting process-ready technologies for the current commercial environment to eliminate the desalting step.

8. Conclusions and Future Developments

Although cucumber fermentation remains largely a traditional process, it has proven to be a consistently safe process by which raw cucumbers are transformed into high-quality pickles that have a long shelf-life at ambient temperatures. Lactic acid bacteria, especially *Lb. plantarum*, which drives the fermentation, in community with yeast, both aerobic and anaerobic, confer the flavor and aroma characteristics of the end-product. Associated with these changes, the production of organic acids and decrease in pH results in a safe and innocuous final product. Conditions for natural fermentation that consistently result in high quality products that are microbially stable for many months include: 1) brining with NaCl to equilibrate with the cucumbers at 6–7 per cent (wt/vol); 2) purging the fermentation brines during active fermentation to remove CO_2 which can cause hollow cavities inside the whole cucumber fruits; and 3) excluding oxygen from the process as much as possible during fermentation and storage. However, to create more sustainable industrial processes, continued efforts are needed to further reduce the salt used for brining while delivering safe, high-quality fermented cucumber products to consumers.

Tremendous increases in demand for 'fresh-like' products containing natural ingredients and changing food consumption patterns have increased the desirability of products produced using LAB fermentations. As such, the process of LAB fermentations is being rediscovered in exciting ways all around the globe and the indigenous fermented foods that have played a vital role in the history of humankind's development are finding their way back into modern society as artisanal products. New molecular approaches to study the composition of microbiota and to select starter cultures targeted for fruits and vegetables, including those with health-promoting properties (probiotic and bioactive compound-generating), are being pursued to allow controlled fermentation processes and develop novel products as done for other fermented foods (cheese, yogurt, sausage, etc.). Lactic acid bacteria tailored to various environmental conditions and desired finished products will provide new potential for further development of cucumber fermentation.

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References

Aghili, F., A.H. Khoshgoftarmanesh, M. Afyuni and M. Mobli. 2009. Relationships between fruit mineral nutrients concentrations and some fruit quality attributes in greenhouse cucumber. *Journal of Plant Nutrition* 32(12): 1994-2007.

Andreou, A. and I. Feussner. 2009. Lipoxygenases – Structure and reaction mechanism. *Phytochemistry* 70: 1504-1510.

Baysal, T. and A. Demirdoven. 2007. Lipoxygenase in fruits and vegetables: A review. *Enzyme and Microbial Technology* 40: 491-496.

Bell, T.A. and J.L. Etchells. 1956. Pectin hydrolysis by certain salt-tolerant yeasts. *Applied Microbiology* 4(4): 196-201.

Bell T.A., J.L. Etchells and R.N. Costilow. 1958. Softening enzyme activity of cucumber flowers from northern production areas. *Food Research* 23(2): 198-204

Bell, T.A. and J.L. Etchells. 1952. Sugar and acid tolerance of spoilage yeasts from sweet-cucumber pickles. *Food Technology* 6(12): 468-472.

Bell, T.A. and J.L. Etchells. 1961. Influence of salt (NaCl) on pectinolytic softening of cucumbers. *Journal of Food Science* 26(1): 84-90.

Bell, T.A. 1951. Pectolytic enzyme activity in various parts of the cucumber plant and fruit. *Botanical Gazette* 113(2): 216-221.

Bell, T.A., J.L. Etchells and J.D. Jones. 1950. Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. *Food Technology* 4(4): 157-163.

Bell, T.A., J.L. Etchells, C.F. Williams and W.L. Porter. 1962. Inhibition of pectinase and cellulase by certain plants. *Botanical Gazette* 123(3): 220-223.

Bell, T.A., J.L. Etchells and W.W.G. Smart Jr. 1965b. Pectinase and cellulase enzyme inhibitor from sericea and certain other plants. *Botanical Gazette* 126: 40-45.

Bell, T.A., J.L. Etchells and A.F. Borg. 1959. The influence of sorbic acid on the growth of certain species of bacteria, yeasts and filamentous fungi. *Journal of Bacteriology* 7(5): 573-580.

Bell, T.A., J.L. Etchells, J.A. Singleton and W.W.G. Smart Jr. 1965a. Inhibition of pectinolytic and cellulolytic enzymes in cucumber fermentations by sericea. *Journal of Food Science* 30(2): 223-239.

Bell, T.A., L.W. Aurand and J.L. Etchells. 1960. Cellulase inhibitor in grape leaves. *Botanical Gazette* 122(2): 143-148.

Beuchat L.R. 1996. Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection* 59: 204-16.

Beuchat, L.R. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection* 4: 413-423.

Bevilacqua, A., M. Cannarsi, M. Gallo, M. Sinigaglia and M.R Corbo. 2009. Characterization and implications of *Enterobacter cloacae* strains, isolated from Italian table olives Bella di Cerignola. *Journal of Food Science* 75(1): M53-M60.

Binsted, R., J.D. Devey and J.C. Dakin. 1962. *Pickle and Sauce Making*, second editon. Food Trade Press, London.

Bobillo, M. and V.M. Marshall. 1991. Effect of salt and culture aeration on lactate and acetate production by *Lactobacillus plantarum*. *Food Microbiology* 8(2): 153-160.

Borge, G.I.A., E. Slinde and A. Nilsson. 1998. Fatty acid alpha-oxidation of tetradecylthioacetic acid and tetradecylthiopropionic acid in cucumber (*Cucumis sativus*). *Biochimica et Biophysica Acta, Lipids and Lipid Metabolism* 1394(2): 158-168.

Brackett, R.E. 1999. Incidence, contributing factors and control of bacterial pathogens in produce. *Post Harvest Biology and Technology* 15: 305-11.

Breene, W.M., D.W. Vis and H. Chou. 1972. Texture profile analysis of cucumbers. *Journal of Food Science*. 37(1): 113-117.

Breidt, F. 2006. Safety of minimally processed, acidified, and fermented vegetable products. Pp. 314-327 *In*: G.M. Sapers, J.R. Gorny and A.E. Yousef (Eds.). *Microbiology of Fruits and Vegetables*. CRC Press, Inc., Boca Raton, Florida.

Breidt, F. and J.M. Caldwell. 2011. Survival of *Escherichia coli* O157:H7 in cucumber fermentation brines. *Journal of Food Science* 76(3): M198-M203.

Breidt, F., E. Medina-Pradas, D. Wafa, I.M. Pérez-Díaz, W. Franco, H. Huang, S.D. Johanningsmeier and J. Kim. 2013b. Characterization of cucumber fermentation spoilage bacteria by enrichment culture and 16S rDNA cloning. *Journal of Food Science* 78(3): M470-M476.

Breidt, F., R.F. McFeeters and I. Díaz-Muñíz. 2007. Fermented vegetables. Pp. 783-793. *In*: M.P. Doyle and L.R. Beuchat (Eds.). *Food Microbiology*: *Fundamentals and Frontiers*, fourth edition. ASM Press, Washington, D.C.

Breidt, F., R.F. McFeeters, I. Pérez-Díaz and C.-H. Lee. 2013a. Fermented Vegetables. Chapter 33.*In*: M.P. Doyle and R.L. Buchanan (Eds.). *Food Microbiology*: *Fundamentals and Frontiers*, fourth editon. ASM Press, Washington, D.C.

Buescher, R. and C. Hamilton. 2000. Protection of cucumber pickle quality by $CaNa_2EDTA$. *Journal of Food Quality* 23(4): 429-441.

Buescher, R. and C. Hamilton. 2007. Adsorption of polygalacturonase from recycled cucumber pickle brines by pure-flo B80 CLAY1. *Journal of Food Biochemistry* 26(2): 153-165.

Buescher, R. and M. Hudson. 1984. Softening of cucumber pickles by cx-cellulase and its inhibition by calcium. *Journal of Food Science* 49(3): 954-955.

Buescher, R., M.J. Cho and C. Hamilton. 2013. Heat conditioning of cucumbers improves retention of sliced refrigerated pickle texture and appearance. *Journal of Food Biochemistry* 37(5): 564-570.

Buescher, R.H. and R.W. Buescher. 2001. Production and stability of (E,Z)-2,6-nonadienal, the major flavor volatile of cucumbers. *Journal of Food Science* 66(2): 357-361.

Buescher, R.W. and C. Burgin. 1988. Effect of calcium chloride and alum on fermentation, desalting, and firmness retention of cucumber pickles. *Journal of Food Science* 53(1): 296-297.

Buescher, R.W. and C. Burgin. 1992. Diffusion plate assay for measurement of polygalacturonase activities in pickle brines. *Journal of Food Biochemistry* 16: 59-68.

Buescher, R.W., C. Hamilton, J. Thorne and J.C. Mi. 2011. Elevated calcium chloride in cucumber fermentation brine prolongs pickle product crispness. *Journal of Food Quality* 34(2): 93-99.

Buescher, R.W., J.M. Hudson and J.R. Adams. 1981a. Utilization of calcium to reduce pectinolytic softening of cucumber pickles in low-salt conditions. *Lebensmittel Wissenschaft und Technologie* 14(2): 65-69.

Buescher, R.W., J.M. Hudson and J.R. Adams. 1979. Inhibition of polygalacturonase softening of cucumber pickles by calcium chloride. *Journal of Food Science* 44(6): 1786-1787.

Buescher, R.W., J.M. Hudson, J.R. Adams and D.H. Wallace. 1981b. Calcium makes it possible to store cucumber pickles in low salt brine. *Arkansas Farm Research* 30(4): 2.

Caldwell, J.M., R. Juvonen, J. Brown and F. Breidt. 2013. *Pectinatus sottacetonis* sp. nov., isolated from commercial pickle spoilage tank. *International Journal of Systematic and Evolutionary Microbiology* 63(10): 3609-3616.

Cho, M.J. and R. Buescher. 2011. Degradation of cucumber flavor aldehydes in juice. *Food Research International* 44(9): 2975-2977.

Cho, M.J. and R.W. Buescher. 2012. Potential role of native pickling cucumber polygalacturonase in softening of fresh pack pickles. *Journal of Food Science* 77(4): C443-C447.

Code of Federal Regulations (CFR). 2010. *Acidified Foods*. 21CFR114.

Cordero, C., E. Liberto, C. Bicchi, P. Rubiolo, P. Schieberle, S.E. Reichenbach and Q. Tao. 2010. Profiling food volatiles by comprehensive two-dimensional gaschromatography coupled with mass spectrometry: Advanced fingerprinting approaches for comparative analysis of the volatile fraction of roasted hazelnuts (*Corylus avellana* L.) from different origins. *Journal of Chromatography* A 1217(37): 5848-5858.

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

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

Corey, K.A., D.M. Pharr and H.P. Fleming. 1983b. Role of gas diffusion in bloater formation of brined cucumbers. *Journal of Food Science* 48(2): 389-393, 399.

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

Daeschel, M.A., R.E. Anderson and H.P. Fleming. 1987. Microbial ecology of fermenting plant materials. *FEMS Microbiology Reviews* 46: 357-367.

Daeschel, M.A., H.P. Fleming and E.A. Potts. 1985. Compartmentalization of lactic acid bacteria and yeasts in the fermentation of brined cucumbers. *Food Microbiology* 2(1): 77-84.

Daeschel, M.A., H.P. Fleming, and R.F. McFeeters. 1988. Mixed culture fermentation of cucumber juice with *Lactobacillus plantarum* and yeasts. *Journal of Food Science* 53(3): 862-864, 888.

Dakin, J.C. and M.P. Day. 1958. Yeast causing spoilage in acetic acid preserves. *Journal of Applied Bacteriology* 21(1): 94-96.

Das, A. and S. Deka. 2012. Fermented foods and beverages of the north-east India. *International Food Research Journal* 19: 377-392.

Deák T. 2008. *Handbook of Food Spoilage Yeast*, second edition, Boca Raton, Miami: CRC Press.

Demarigny, Y. 2012. Fermented food products made with vegetable materials from tropical and warm countries: microbial and technological considerations. *International Journal of Food Science and Technology* 47: 2469-2476.

Demo, J. 2002. Bulk tank technology: A pickle pumping system using an air-lift pump. *Pickle Pak Science* VIII (1): 44-46.

Di Cagno, R., R. Coda, M. De Angelis and M. Gobbetti. 2013. Review: Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiology* 33: 1-10.

Durán Quintana, M.C., F. González and A. Garrido. 1979. Aceitunas negras al natural en salmuera. Ensayos de producción de alambrado. Inoculación de diversos microorganismos aislados de salmueras de fermentación. *Grasas y Aceites* 30361-30367.

Eisenstat, L. and F.W. Fabian. 1953. Factors which produce bleaching of pickles-I. *The Glass Packer*. April 29-5178.

Ennis, D.M. and J. O'Sullivan. 1979. Sensory quality of cucumbers before and after brining. *Journal of Food Science* 44(3): 847-849.

Etchells J.L. and I.D. Jones. 1944. Procedure for pasteurizing pickle products. *Glass Packer* 23(7): 519-523, 546.

Etchells, J.L. and I.D. Jones. 1941. An occurrence of bloaters during the finishing of sweet pickles. *Fruit Products Journal* 20(12): 370, 381.

Etchells, J.L. and T.A. Bell. 1950a. Film yeasts on commercial cucumber brines. *Food Technology* 4(3): 77-83.

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

Etchells, J.L. and W.R. Jr. Moore. 1971. Factors influencing the brining of pickling cucumbers — Questions and answers. *Pickle Pak Science* 1(1): 1-17

Etchells, J.L. 1941a. A new type of gaseous fermentation occurring during the salting of cucumbers. *Univ Microfilms Abstr* III (2): 7-8.

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

Etchells, J.L., A.F. Borg and T.A. Bell. 1961. Influence of sorbic acid on populations and species of yeasts occurring in cucumber fermentations. *Applied Microbiology* 9(2): 139-144.

Etchells, J.L., A.F. Borg and T.A. Bell. 1968. Bloater formation by gas-forming lactic acid bacteria in cucumber fermentations. *Applied Microbiology* 16(7): 1029-1035.

Etchells, J.L., F.W. Fabian and I.D. Jones. 1945. The *aerobacter* fermentation of cucumbers during salting. *Michigan Agricultural Experiment Station Technical Bulletin*, No. 200. P. 56.

Etchells J.L., T.A. Bell, H.P. Fleming, R.E. Kelling and R.L. Thompson. 1974. Q-BAT *Instruction Sheet with Bloater Chart*. Advisory statement published and distributed by Pickle Packers International, Inc. St. Charles, IL.

Etchells, J.L., H.P. Fleming, L.H. Hontz, T.A. Bell and R.J. Monroe R.J. 1975. Factors influencing bloater formation in brined cucumbers during controlled fermentation. *Journal of Food Science* 40(3): 569-575.

Etchells, J.L., T.A. Bell and C.F. Williams. 1958b. Inhibition of pectinolytic and cellulolytic enzymes in cucumber fermentations by Scuppernong grape leaves. *Food Technology* 12(5): 204-208.

Etchells, J.L., T.A. Bell, R.N. Costilow, C.E. Hood and T.E. Anderson. 1973. Influence of temperature and humidity on microbial, enzymatic and physical changes of stored, pickling cucumbers. *Applied Microbiology* 26(6): 943-950.

Etchells, J.L., T.A. Bell, R.J. Monroe, P.M. Masley and A.L. Demain AL. 1958a. Populations and softening enzyme activity of filamentous fungi on flowers, ovaries and fruit of pickling cucumbers. *Applied Microbiology* 6(6): 427-440.

Fasina, O.O., H.P. Fleming and L.D. Reina. 2002. BAG-IN-BOX TECHNOLOGY: Membrane filtration of cucumber fermentation brine. *Pickle Pak Science* VIII (1): 19-25.

Fasina O.O., H.P. Fleming, E.G. Humphries, R.L. Thompson, and L.D. Reina. 2003. Crossflow filtration of brine from cucumber fermentation. *Applied Engineering in Agriculture*. 19(1): 107-113.

Fleming, H.P., W.Y. Cobb, J.L. Etchells, T.A. Bell. 1968. The formation of carbonyl compounds in cucumbers. *Journal of Food Science* 33(6): 572-576.

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., E.G. Humphries, O.O. Fasina, R.F. McFeeters, R.L. Thompson and F. Breidt Jr. 2002a. BAG-IN-BOX TECHNOLOGY: Pilot system for process-ready, fermented cucumbers. *Pickle Pak Science* VIII (1): 1-8.

Fleming, H.P., E.G. Humphries, R.L. Thompson and R.F. McFeeters. 2002b. Bag-in-Box Technology: Storage stability of process-ready, fermented cucumbers. *Pickle Pak Science* VIII (1): 14-18.

Fleming, H.P., J.L. Etchells, R.L. Thompson and T.A. Bell. 1975. Purging of CO_2 from cucumber brines to reduce bloater damage. *Journal of Food Science* 40(6): 1304-1310.

Fleming, H.P., L.C. McDonald, R.F. McFeeters, R.L. Thompson and E.G. Humphries. 1995. Fermentation of cucumbers without sodium chloride. *Journal of Food Science* 60(2): 312-319.

Fleming, H.P., M.A. Daeschel, R.F. McFeeters and M.D. Pierson. 1989. Butyric acid spoilage of fermented cucumbers. *Journal of Food Science* 54(3): 636-639.

Fleming, H.P., R.F. McFeeters and R.L. Thompson. 1987. Effects of sodium chloride concentration on firmness retention of cucumbers fermented and stored with calcium chloride. *Journal of Food Science* 52(3): 653-657.

Fleming, H.P., R.F. McFeeters and R.L. Thompson. 1996. Assuring microbial and textural stability of fermented cucumbers by pH adjustment and sodium benzoate addition. *Journal of Food Science* 61(4): 832-6.

Fleming, H.P., R.L. Thompson and D.M. Pharr. 1980. Brining properties of cucumbers exposed to pure oxygen before brining. *Journal of Food Science* 45(6): 1579-1582.

Fleming, H.P., R.L. Thompson and R.J. Monroe. 1978. Susceptibility of pickling cucumbers to bloater damage by carbonation. *Journal of Food Science* 43(3): 892-896.

Fleming, H.P., R.L. Thompson, J.L. Etchells, R.E. Kelling and T.A. Bell. 1973a. Bloater formation in brined cucumbers fermented by *Lactobacillus plantarum*. *Journal of Food Science* 38(3): 499-503.

Fleming, H.P., R.L. Thompson, J.L. Etchells, R.E. Kelling and T.A, Bell. 1973b. Carbon dioxide production in the fermentation of brined cucumbers. *Journal of Food Science* 38(3): 504-506.

Fleming, H.P., R.L. Thompson, T.A. Bell and R.J. Monroe. 1977. Effect of brine depth on physical properties of brine-stock cucumbers. *Journal of Food Science* 42(6): 1464-1470.

Food and Agriculture Organization of the United Nations.FAO. 2015. FAO Crop Production Data Base. Economic and Social Development Department. Available at http://faostat3.fao.org/home/E. Accessed April 2015.

Forss, D.A., E.A. Dunstone, E.H. Ramshaw and W. Stark. 1962. The flavor of cucumbers. *Journal of Food Science* 27(1): 90-93.

Franco, W. and I. Pérez-Díaz. 2012b. Role of selected oxidative yeasts and bacteria in cucumber secondary fermentation associated with spoilage of the fermented fruit. *Food Microbiology* 32: 338-344.

Franco, W. and I.M. Pérez-Díaz. 2012a. Development of a model system for the study of spoilage associated secondary cucumber fermentation during long term storage. *Journal of Food Science* 77(10): M586-M592.

Franco, W. and I.M. Pérez-Díaz. 2013. Microbial interactions associated with secondary cucumber fermentation. *Journal of Applied Microbiology* 114: 161-172.

Franco, W., I.M. Pérez-Díaz, S.D. Johanningsmeier and R.F. McFeeters. 2012. Characteristics of spoilage-associated secondary cucumber fermentation. *Applied and Environmental Microbiology* 78(4): 1273-1284.

Frankel, E.N. 1991. Recent advances in lipid oxidation. *Journal of the Science of Food and Agriculture* 54: 495-511.

Geisman, J.R. and R.E. Henne. 1973. *Recycling Brine from Pickling*. Ohio Report 58 (4): 76-77.

Gómez-López, M.D., J.P. Fernández-Trujillo and A. Baille. 2006. Cucumber fruit quality at harvest affected by soil-less system, crop age and preharvest climatic conditions during two consecutive seasons. *Scientia Horticulturae* 110(1): 68-78.

Grosch, W. and J.M. Schwarz. 1971. Linoleic and linolenic acid as precursors of the cucumber flavor. *Lipids* 6(5): 351-352.

Guillou, A.A. and J.D. Floros. 1993. Multiresponse optimization minimizes salt in natural cucumber fermentation and storage. *Journal of Food Science* 58(6): 1381-1389.

Guillou, A.A., J.D. Floros and M.A. Cousin MA. 1992. Calcium chloride and potassium sorbate reduce sodium chloride used during natural cucumber fermentation and storage. *Journal of Food Science* 57(6): 1364-1368.

Harris, L.J. 1998. The microbiology of vegetable fermentations. Pp. 45-72. *In*: B.J.E. Wood (Ed.). *Microbiology of Fermented Foods*, vol. 1, second edition. Blackie Academic &Professional, London.

Hatanaka, A. 1996. The fresh green odor emitted by plants. *Food Reviews International* 12(3): 303-350.

Howard L.R. and R.W. Buescher. 1993. Effect of redox agents on visual cure in refrigerated pickles. *Journal of Food Biochemistry* 17(2): 125-134.

Hudson, J.M. and R.W. Buescher. 1980. Prevention of soft center development in large whole cucumber pickles by calcium. *Journal of Food Science* 45(5): 1450-1451.

Hudson, J.M. and R.W. Buescher. 1985. Pectic substances and firmness of cucumber pickles as influenced by $CaCl_2$, NaCl and brine storage. *Journal of Food Biochemistry* 9(3): 211-229.

Hutkins, R.W. 2006. *Microbiology and Technology of Fermented Foods.* Blackwell Publishing, Ames, Iowa.

Ito, K.A., J.K. Chen, P.A. Lerke, M.L. Seeger and J.A. Unverferth. 1976. Effect of acid and salt concentration in fresh-pack pickles on the growth of *Clostridium botulinum* spores. *Applied and Environmental Microbiology* 32(1): 121-124.

Jasso-Chaverria, C., G.J. Hochmuth, R.C. Hochmuth and S.A. Sargent. 2005. Fruit yield, size, and color responses of two greenhouse cucumber types to nitrogen fertilization in perlite soil-less culture. *HortTechnology* 15(3): 565-571.

Jeon, I.J., W.M. Breene and S.T. Munson. 1973. Texture of cucumbers: Correlation of instrumental and sensory measurements. *Journal of Food Science* 38(2): 334-337.

Jeon, I.J., W.M. Breene and S.T. Munson. 1975. Texture of salt stock whole cucumber pickles: Correlation of instrumental and sensory measurements. *Journal of Texture Studies* 5(4): 411-423.

Johanningsmeier, S.D. 2011. Biochemical characterization of fermented cucumber spoilage using non-targeted, comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry: Anaerobic lactic acid utilization by lactic acid bacteria. Ph.D. Dissertation, North Carolina State University, Raleigh, NC.

Johanningsmeier, S.D. and R.F. McFeeters. 2011. Detection of volatile spoilage metabolites in fermented cucumbers using nontargeted, comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-TOFMS). *Journal of Food Science* 76(1): C168-C177.

Johanningsmeier, S.D. and R.F. McFeeters. 2013. Metabolism of lactic acid in fermented cucumbers by *Lactobacillus buchneri* and related species, potential spoilage organisms in reduced salt fermentations. *Food Microbiology* 35(2): 129-135.

Johanningsmeier, S.D., H.P. Fleming and F. Breidt FJ. 2004. Malolactic activity of lactic acid bacteria during sauerkraut fermentation. *Journal of Food Science* 69(8): M222-M227.

Johanningsmeier, S.D., R.L. Thompson and H.P. Fleming. 2002. Bag-in-Box Technology: Sensory quality of pickles produced from process-ready, fermented cucumbers. *Pickle Pak Science* VIII (1): 26-33.

Johanningsmeier, S.D., W. Franco, I.M. Pérez-Díaz and R.F. McFeeters. 2012. Influence of sodium chloride, pH and lactic acid bacteria on anaerobic lactic acid utilization during fermented cucumber spoilage. *Journal of Food Science* 77(7): M397-M404.

Jones I.D. and J.L Etchells. 1950. *Cucumber Varieties in Pickle Manufacture*. Canner 110(1): 34, 36, 38, 40.

Jones I.D. and J.L. Etchells JL. 1943. Physical and chemical changes in cucumber fermentations. *Food Ind* 15(1):62-64.

Jones, I.D., J.L. Etchells, O. Veerhoff and M.K. Veldhuis. 1941. Observations on bloater formation in cucumber fermentation. *Fruit Products Journal* 20(7): 202-206, 219-220.

Jones I.D., J.L. Etchells, and R.J. Monroe. 1954. Varietal differences in cucumbers for pickling. *Food Technology* 8(9): 415-418.

Jung, D.H. 2012. *Great Dictionary of Fermented Food.* Yuhanmunwhasa, Inc., Kayang-dong, Seoul. ISBN: 978-89-7722-570-1.

Kim, J. and F. Breidt Jr. 2007.Development of preservation prediction chart for long-term storage. *Korean Journal of Life Sciences* 17(12):1616-1621.

Kim, J.K., E.M. D'Sa, M.A. Harrison, J.A. Harrison and E.L. Andress. 2004. *Listeria monocytogenes* survival in refrigerator dill pickles. Paper 33C-1. Presented at the Institute of Food Technologists Annual Meeting, Las Vegas, NV, July 14, 2004.

Konisky, I. 1989. Colicins and other bacteriocins with established modes of action. *Annual Reviews of Microbiology* 36: 125-144.

Krall, S.M. and R.F. McFeeters. 1998. Pectin hydrolysis: Effect of temperature, degree of methylation, pH and calcium on hydrolysis rates. *Journal of Agricultural and Food Chemistry* 46(4): 1311-1315.

Kumar, R. Satish, P. Kanmani, N. Yuvaraj, A. Paari, V. Pattukamar and V. Arul. 2013. Traditional Indian fermented foods: a rich source of lactic acid bacteria. *International Journal of Food Science and Nutrition* 64(4): 415-428.

Little, L.W., J.C. Lamb, and L.F. Horney. 1974. Characterization and treatment of brine wastewaters from the cucumber pickle industry. Thesis. University of North Carolina at Chapel Hill.

Liu, M., J.R. Bayjanov, B. Renckens, Nauta A, and Siezen R.J. 2010. The proteolytic system of lactic acid bacteria revisited: A genomic comparison. *BMC Genomics* 11(36).doi: 10.1186/1471-2164-11-36

Lu, Z., E. Altermann, F. Breidt, P. Predki, H.P. Fleming and T.R. Klaenhammer. 2005. Sequence analysis of the *Lactobacillus plantarum* bacteriophage ΦJL-1. Gene 348: 45-54.

Lu, Z., F. Breidt, H.P. Fleming, E. Altermann and T.R. Klaenhammer. 2003a. Isolation and characterization of a *Lactobacillus plantarum* bacteriophage ΦJL-1 from a cucumber fermentation. *International Journal of Food Microbiology* 84: 225-235.

Lu, Z., F. Breidt, V. Plengvidhya and H.P. Fleming. 2003b. Bacteriophage ecology in commercial sauerkraut fermentations. *Applied and Environmental Microbiology* 69(6): 3192-3202.

Lu, Z., I.M. Pérez-Díaz, J.S. Hayes and F. Breidt. 2012. Bacteriophage ecology in an industrial cucumber fermentation. *Applied and Environmental Microbiology* 78(24): 8571-8578.

Lund, B.M., T.C. Baird-Parker and G.W. Gould. 2000. *The Microbiological Safety and Quality of Food*. pp. 160-161. Aspen Publishers, Inc. Gaithersburg, MD.

Malleret, C., R. Lauret, S.D. Ehrlich, F. Morel-Deville, and M. Zagorec. 1998. Disruption of the sole ldhL gene in *Lactobacillus sakei* prevents the production of both L- and D-lactate. *Microbiology* 144: 3327-3333.

Marshall, D.E., B.F. Cargill, and J.H. Levin. 1972. Physical and quality factors of pickling cucumbers as affected by mechanical harvesting. *Transactions of the ASAE*-1972: 604-608, 612.

Marsili, R.T. and N. Miller. 2000. Determination of major aroma impact compounds in fermented cucumbers by solid-phase microextraction-gas chromatography-mass spectrometry-olfactometry detection. *Journal of Chromatographic Science* 38: 307-314.

McDonald, L.C., H.P. Fleming and H.M. Hassan. 1990. Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. *Applied and Environmental Microbiology* 56: 2120-2124.

McFeeters, R.F., and I.M. Perez-Diaz. 2010. Fermentation of cucumbers brined with calcium chloride instead of sodium chloride. *Journal of Food Science* 75(3): C291-C296.

McFeeters, R.F. 1992. Cell wall monosaccharide changes during softening of brined cucumber mesocarp tissue. *Journal of Food Science* 57(4): 937-940.

McFeeters, R.F. and H.P. Fleming. 1989. Inhibition of cucumber tissue softening in acid brines by multivalent cations: Inadequacy of the pectin 'egg box' model to explain textural effects. *Journal of Agricultural and Food Chemistry* 37(4): 1053-1059.

McFeeters, R.F. and H.P. Fleming. 1991. pH effect on calcium inhibition of softening of cucumber mesocarp tissue. *Journal of Food Science* 56(3): 730-732, 735.

McFeeters, R.F. and L.A. Lovdal. 1987. Sugar composition of cucumber cell walls during fruit development. *Journal of Food Science* 52(4): 996-1001.

McFeeters, R.F., H.P. Fleming and M. Brenes Balbuena. 1995. Softening rates of fermented cucumber tissue: Effects of pH, calcium and temperature. *Journal of Food Science* 60(4): 786-788, 793.

McFeeters, R.F., H.P. Fleming and R.L. Thompson. 1982a. Malic acid as a source of carbon dioxide in cucumber fermentations. *Journal of Food Science* 47(6): 1862-1865.

McFeeters, R.F., H.P. Fleming and R.L. Thompson. 1982b. Malic and citric acids in pickling cucumbers. *Journal of Food Science* 47(6): 1859-1861, 1865.

McFeeters, R.F., T.A. Bell and H.P. Fleming. 1980. An endo-polygalacturonase in cucumber fruit. *Journal of Food Biochemistry* 4(1): 1-16.

McGrath, S. and D. van Sinderen. 2007. *Bacteriophage*: *Genetics and Molecular Biology*. Caister Academic Press, Northfolk, VA.

Mercer, W.A., J.W. Ralls, and H.J. Maagdenberg. 1971. *Reconditioning Food Processing Brines with Activated Carbon.* Chemical Engineering Progress Symposium Series 67: 435-438.

Mok, Y.N. and R. Buescher. 2012. Cure appearance development in pasteurized cucumber pickle mesocarp tissue associated with proteolysis. *Journal of Food Biochemistry* 36(5): 513-519.

Mundt, J.O. 1970. Lactic acid bacteria associated with raw plant food material. *Journal of Milk and Food Technology* 33: 550-553.

Mundt, J.O. and J.L. Hammer. 1968. Lactobacilli on plants. *Applied Microbiology* 16: 1326-1330.

Mundt, J.O., W.F. Graham and I.E. McCarty. 1967. Spherical lactic acid-producing bacteria of southern-grown raw and processed vegetables. *Applied Microbiology* 15: 1303-1308.

Murphy, M.G. and S. Condon. 1984. Comparison of aerobic and anaerobic growth of *Lactobacillus plantarum* in a glucose medium. *Archives of Microbiology* 138: 49-53.

Murphy, M.G., L. O'Connor, D. Walsh, and S. Condon. 1985. Oxygen-dependent lactate utilization by *Lactobacillus plantarum*. *Archives of Microbiology* 141:75-79.

Murray, K.E. and F.B. Whitefield. 1975. The occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables. *Journal of the Science of Food and Agriculture* 26: 973-986.

Olsen, M.J. and I.M. Pérez-Díaz. 2009. Influence of microbial growth on the redox potential of fermented cucumbers. *Journal of Food Science* 74(4): M149-M153.

Palma-Harris, C., R.F. McFeeters and H.P. Fleming. 2002. Fresh cucumber flavor in refrigerated pickles: Comparison of sensory and instrumental analysis. *Journal of Agricultural and Food Chemistry* 50(17): 4875-4877.

Palnitkar, M.P., and R.F. McFeeters. 1975. Recycling spent brines in cucumber fermentati ons. *Journal of Food Science* 40(6): 1311-1315.

Pederson, C.S. and M.N. Albury. 1969. The sauerkraut fermentation. *New York State Agricultural Experiment Station Technical Bulletin* 824, Geneva, New York.

Pederson, C.S., L.R. Mattick, F.A. Lee and R.M. Butts. 1964. Lipid alterations during the fermentation of dill pickles. *Applied Microbiology* 12(6): 513-516.

Pérez-Díaz, I., R.E. Kelling, S. Hale, F. Breidt and R.F. McFeeters. 2007. *Lactobacilli* and tartrazine as causative agents of red-color spoilage in cucumber pickle products. *Journal of Food Science* 72(7): M240-M245.

Pérez-Díaz, I.M., and R.F. McFeeters. 2009. Modification of azo dyes by lactic acid bacteria. *Journal of Applied Microbiology* 107: 584-589.

Pérez-Díaz, I.M., F. Breidt, R.W. Buescher, F.N. Arroyo-Lopez, R. Jimenez-Diaz, J. Bautista-Gallego, A. Garrido-Fernandez, S. Yoon and S.D. Johanningsmeier. 2014. Chapter 51: Fermented and acidified vegetables. *In*: F. Pouch Downes and K.A. Ito (Eds.). *Compendium of Methods for the Microbiological Examination of Foods*, fifth edition. American Public Health Association, Washington, DC.

Pérez-Díaz, I.M., R.F. McFeeters, L. Moeller, S.D. Johanningsmeier, J. Hayes, D.S. Fornea, L. Rosenberg, C. Gilbert, N. Custis, K. Beene and D. Bass. 2015. Commercial scale cucumber fermentations brined with calcium chloride instead of sodium chloride. *Journal of Food Science* 80(12): M2827-M2836.

Porter, W.L., J.H. Schwartz, T.A. Bell and J.L. Etchells. 1961. Probable identity of the pectinase inhibitor in grape leaves. *Journal of Food Science* 26(6): 600-605.

Potts, E.A. and H.P. Fleming. 1979. Changes in dissolved oxygen and microflora during fermentation of aerated, brined cucumbers. *Journal of Food Science* 44(2): 429-434.

Potts, E.A. and H.P. Fleming. 1982. Prevention of mold-induced softening in air-purged, brined cucumbers by acidification. *Journal of Food Science* 47(5): 1723-1727.

Pressey, R. and J.K.A. Vants. 1975. Cucumber Polygalacturonase. *Journal of Food Science* 40(5): 937-939.

Rosenberg, L.B. 2013. Texture retention in pickles produced from commercial scale cucumber fermentation using calcium chloride instead of sodium chloride. Thesis. North Carolina State University, Raleigh, North Carolina.

Ruiz-Cruz, J. and F. Gonzalez-Cancho. 1969. Metabolismo de levaduras aisladas de salmuera de aceitunas aderezadas 'estilo espanol'. I. *Asimilacion de los acidos lactico, acetico y citrico*. Grasas y Aceites 20(1): 6-11.

Saltveit, M.E. and R.F. McFeeters. 1980. Polygalacturonase activity and ethylene synthesis during cucumber fruit development and maturation. *Plant Physiology* 66(6): 1019-1023.

Sandhu, K.S., and F.C. Shukla. 1996. Methods for pickling of cucumber (*Cucumis sativus*) a critical appraisal. *Journal of Food Technology* 33: 455-473.

Schopf, J.W. and B.M. Packer. 1987. Early Archean (3.3-billion to 3.5-billion-year-old) microfossils from Warrawoona Group, Australia. *Aust. Sci.* 237(4810): 70-73.

Shetty, N.V. and T.C. Wehner. 2002. Screening the cucumber germplasm collection for fruit yield and quality. *Crop Science* 42(6): 2174-2183.

Singh, A.K., and A. Ramesh. 2008. Succession of dominant and antagonistic lactic acid bacteria in fermented cucumber: Insights from a PCR-based approach. *Food Microbiology* 25(2): 278-287.

Smith, J.G. 2008. *Organic Chemistry*, second edition. McGraw-Hill, New York.

St. Angelo, A.J. 1992. *Lipid Oxidation in Food.* American Chemical Society, Washington D.C.

Steinkraus, K.H. 1994. Nutritional significance of fermented foods. *Food Research International* 27: 259-261.

Tamang, J.P. 2010. Fermented Foods and Beverages. Pp. 41-84. *In*: J.P. Tamang and K. Kailasapathy (Eds.). *Diversity of fermented foods*. CRC Press, Boca Raton, Florida.

Tang, H.C.L. and R.F. McFeeters. 1983. Relationships among cell wall constituents, calcium and texture during cucumber fermentation and storage. *Journal of Food Science* 48(1): 66-70.

Taormina, P.J. and L.R. Beuchat. 1999. Behavior of enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa sprouts during the sprouting process as influenced by treatments with various chemicals. *Journal of Food Protection* 62: 850-6.

Thomas, T.R., L.L. McKay and H.A. Morris. 1985. Lactate metabolism by Pediococci isolated from cheese. *Applied and Environmental Microbiology* 49(4): 908-913.

Thompson, R.L., H.P. Fleming, D.D. Hamann and R.J. Monroe. 1982. Method for determination of firmness in cucumber slices. *Journal of Texture Studies* 13(3): 311-324.

Thompson, R.L., R.J. Monroe and H.P. Fleming. 1979. Effects of storage conditions on firmness of brined cucumbers. *Journal of Food Science* 44(3): 843-846.

Vaughan, R.H., K.T. Jakubczyk, J.D. McMillan, E. Higinio Thomas, B.A. Dave and V.M. Crampton. 1969. Some pink yeast associated with softening of olives. *Applied Microbiology* 18(5): 771-775.

Veldhuis, M.K. and J.L. Etchells. 1939. Gaseous products of cucumber pickle fermentations. *Food Research* 4(6): 621-630.

Veldhuis, M.K., J.L. Etchells, I.D. Jones and O. Veerhoff. 1941. Influence of sugar addition to brines in pickle fermentation. *Food Ind*. 13(10): 54-56; 13(11): 48-50.

Venkateshwarlu, G., M.B. Let, A.S. Meyer and C. Jacobsen. 2004. Modeling the sensory impact of defined combinations of volatile lipid oxidation products on fishy and metallic off-flavors. *Journal of Agricultural and Food Chemistry* 52(6): 1635-1641.

Walter, W.M. Jr., D.G. Epley and R.F. McFeeters. 1990. Effect of water stress on stored pickling cucumbers. *Journal of Agricultural and Food Chemistry* 38(12): 2185-2191.

Walter, W.M. Jr., H.P. Fleming and R.N. Trigiano. 1985. Comparison of the microstructure of firm and stem- end softened cucumber pickles preserved by brine fermentation. *Food Microstructure* 4(1): 165-172.

Wardale, D.A. and E.A. Lambert. 1980. Lipoxygenase from cucumber fruit: Localization and properties. *Phytochemistry* 19(6): 1013-1016.

Wardale, D.A., E.A. Lambert and T. Galliard. 1978. Localization of fatty acid hydroperoxide cleavage activity in membranes of cucumber fruit. *Phytochemistry* 17(2): 205-012.

Wehner, T.C. and H.P. Fleming. 1984. Evaluation of bloater resistance in pickling cucumbers using a brine carbonation method. *Journal of the American Society of Horticultural Science* 109(2): 261-265.

White, D. 2007. *The Physiology and Biochemistry of Prokaryotes.* Oxford University Press, Inc., New York, NY.

White, R.C., I.D. Jones and E. Gibbs. 1963. Determination of chlorophylls, chlorophyllides, pheophytins, and pheophorbides in plant material. *Journal of Food Science* 28(4): 431-436.

Whitefield, F.B. and J.H. Last. 1991. Volatile compounds in foods and beverages. *In*: H. Maarse (Ed). *Vegetables*. Dekker, New York.

Wilson, E.M., S.D. Johanningsmeier and J.A. Osborne. 2015. Consumer acceptability of cucumber pickles produced by fermentation in calcium chloride brine for reduced environmental impact. *Journal of Food Science* 80(6): S1360-S1367.

Wolter, E.M. 2013. Consumer Acceptability and Flavor Characteristics of Cucumber Pickles Produced Using an Environmentally-friendly Calcium Chloride Fermentation. Thesis. North Carolina State University, Raleigh, North Carolina.

Xu, Q., Y.L. Geng, X.H. Qi, X.H. Chen. 2012. Genetic analysis of the five major aromatic substances in cucumber (*Cucumis sativus* L.). *The Journal of Horticultural Science and Biotechnology* 87(2): 113-116.

Yoo, K.M., I.K. Hwang, G.E. Ji and B.K. Moon. 2006. Effects of salts and preheating temperature of brine on the texture of pickled cucumbers. *Journal of Food Science* 71(2): C97-C101.

Yoon, S.S., R. Barrangou-Poueys, F. Breidt and H.P. Fleming. 2007. Detection and characterization of a lytic *Pediococcus* bacteriophage from the fermenting cucumber brine. *Journal of Microbiology and Biotechnology* 17(2): 262-270.

Yoshioka, Y., H. Horie, M. Sugiyama and Y. Sakata. 2009. Quantifying cucumber fruit crispness by mechanical measurement. *Breeding Science* 59(2): 139-147.

Yoshioka, Y., H. Horie, M. Sugiyama, Y. Sakata and Y. Tamaki. 2010. Search for quantitative indicators of fruit texture for breeding in cucumber. *Acta Horticulturae* (871): 171-178.

Zhou, A. and R.F. McFeeters. 1998. Volatile compounds in cucumbers fermented in low-salt conditions. *Journal of Agricultural and Food Chemistry* 46(6): 2117-2122.

Zhou, A., R.F. McFeeters and H.P. Fleming. 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.