THE EFFECTS OF PHYSICAL AND CHEMICAL PRE-BRINING TREATMENTS ON THE QUALITY OF SALT-STOCK PICKLES

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ABSTRACT

THE EFFECTS OF PHYSICAL AND CHEMICAL PRE-BRINING TREATMENTS ON THE QUALITY OF SALT-STOCK PICKLES

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The effect of treating cucumber wash water with chlorine dioxide was evaluated to determine if the storage life of the fresh fruit could be lengthened.

Under certain conditions, chlorine dioxide treated cucumbers remained free of visible mold one day longer than controls. Chlorine dioxide was found to be effective in reducing microbial populations in cucumber wash water and was found to be more efficient than chlorine or Anthium Dioxcide®. No appreciable effect upon the initiation of natural fermentation was noted.

The effects of piercing fresh cucumbers, vacuum impregnation of brine and the use of sorbate in cucumber brines were also studied.

Vacuum treatment resulted in increased initial brine penetration, rapid cure color development and increased bloater formation in the absence of sorbate. Piercing treatments increased initial brine penetration but were insufficient to significantly effect the finished salt-stock quality. Sorbate reduced the incidence of salt-stock defects in all treatments.

To My Wife Paula

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INTRODUCTION

Michigan is a leading state in the growing and manufacture of pickling cucumbers and pickle products, and thus, this commodity makes up an important part of the State's economy. Fresh cucumbers are either 1) processed at the time of harvest as "Fresh Pak" products, by pasteurization in individual containers, or 2) cured in bulk, via the natural fermentation process.

Salt-stock defects and fresh product deterioration contribute greatly to product devaluation and economic loss for the pickling industry. The length and condition of storage of fresh pickling cucumbers has a direct effect on the subsequent quality of the finished product. As the trend towards transporting fresh cucumbers from out-state growing areas to lengthen the packing season becomes ever more prominent, the importance of proper post harvest handling and storage becomes more critical. Brining techniques are also continually being improved in an effort to insure the consistent development of good quality salt-stock once the fruit are in brine.

Large populations of microorganisms build up quickly in hydrocooling, wash and flume waters that come in contact with cucumbers. Microbial populations of such water may

significantly increase the load of undesirable organisms on the green-stock cucumbers and consequently decrease their storage life. Molds are of particular importance because they remain active in cucumber brines and produce pectinolytic enzymes capable of causing softening spoilage. The use of chlorine as a germicidal agent in such water is not practical due to the pH and the high content or organic matter of the water. Chlorine dioxide has been used effectively to treat vegetable processing waters when chlorine has been ineffective. However, chlorine dioxide has not been evaluated for use in cucumber processing waters. The chlorine dioxide studies were initiated to possibly answer questions specifically related to the pickle packing industry. These questionable areas include the efficiency of chlorine dioxide in maintaining low microbial populations in cucumber processing water and on the fresh fruit. Whether an increase in the storage life of fresh cucumbers will be realized from the use of chlorine dioxide. And whether the use of chlorine dioxide has any effects upon the initiation of natural fermentations.

Despite much research in the area of cucumber fermentations, salt-stock defects such as softening and bloating still occur. The Cucumber Piercing and Vacuum Studies were initiated to evaluate the effect of such prebrining treatments on the quality of the finished salt-stock

pickles. The piercing and vacuum treatments were intended to increase initial brine penetration into the fresh fruit and to allow gases entrapped within the cucumber tissues to escape more efficiently.

Sorbate is known to reduce bloater formation and softening in cucumber fermentations due to its selective antimicrobial action against yeasts and molds. A sorbate treatment was added to the cucumber Piercing and Vacuum Studies to observe the interaction between sorbate and the piercing and vacuum treatments.

LITERATURE REVIEW

The Natural Fermentation Process

The brining and subsequent fermentation of cucumbers is an ancient method of preservation for this perishable commodity. Cucumbers are placed in a vessel and covered with a 5-10% salt brine. Next, the cucumbers are "headed down" by means of a false head which keeps the bouyant cucumbers beneath the surface of the brine. The brine draws water and other soluble cellular components out of the cucumber tissues via osmotic pressure. These cellular fluids dilute the cover brine, so additional salt must be added after 24-48 hr. to equilibrate the brine at the desired salt concentration. A comprehensive review of proper commercial brining techniques is provided by Etchells and Moore (1971).

The fermentation that ensues is dependent upon the naturally occurring microflora associated with the surface of the cucumbers and the soil. Of this complex heterogeneous mixture of yeasts, molds and bacteria, only the non-gas producing lactic acid forming bacteria are desirable organisms. These bacteria, which include Lactobacillus plantarum, Lactobacillus brevis, and Pediococcus cerevisiae, make up approximately .02% of the total initial microbial population

(Lingle, 1975).

A proper initial salt concentration favors the growth of the lactic acid forming bacteria and inhibits the growth of many competing organisms. The lactics utilize the sugars and other nutrients drawn from the cucumbers by the action of the brine and produce lactic acid as a by-product of metabolism. As the fermentation proceeds, lactic acid accumulates and the pH drops to a point where the lactic acid formers themselves are inhibited. At this point, the total acidity should range from .6 to 1.2 percent when calculated as lactic acid (Etchells, Fabian & Jones, 1945). Also, there should be little or no remaining sugar present in the brine at the completion of the fermentation as these sugars can be utilized by salt and acid tolerant yeast and reduce the quality of the salt-stock. Jones et al. (1940) describe the course of natural fermentations in terms of the microbiological and chemical changes which occur.

The total fermentation period may last from two to six weeks, depending upon the temperature, brining techniques and the variability of the naturally occurring microflora (Etchells, Fabian and Jones, 1945).

Following the completion of fermentation, the brine strength is gradually increased to 60-80^{o} salometer(s). The salt-stock can be held under these conditions for up to a year and are available for desalting and finishing when needed.

Although the preservation of cucumbers via natural fermentation appears to be a straightforward process, extensive research has proved it to be highly complex. This is evidenced by the incidence of salt-stock defects such as bloater formation and softening. Over the past forty five years, extensive investigations have been conducted on cucumber fermentations in an attempt to learn more about these spoilage problems. This research has helped the pickle packing industry make numerous advances toward understanding and solving brining problems. The results include manufacture of higher quality products in an economical fashion.

Bloater Formation and Control

Formation of bloaters during the fermentation of the larger sizes of pickling cucumbers constitutes a major source of product devaluation. The bloater is characterized by the presence of gas pockets within the cured cucumber tissues. Severe bloaters, termed balloon bloaters, are characterized by a large hollow cavity within the cucumber, with the carpel tissues compressed toward the perimeter of the fruit. The Bloater Chart of Etchells et al. (1974), pictures examples of various types and degrees of severity of typical bloated cucumbers. These cucumbers are unsuitable for many high quality pickle products such as spears, slices or whole dills and are usually diverted for use in less valuable products such as relish.

Etchells et al. (1968) describe the mechanism of bloater formation in the following manner. CO_2 formed in the brine during active fermentation accumulates until it becomes supersaturated. When this brine diffuses into the cucumbers, CO_2 is released from solution and accumulates in areas of structural weakness. As CO_2 accumulates around the carpel tissues, they are gradually pressed towards the side of the fruit which may become distended due to the excessive gas pressure. Fleming et al. (1973a) revised this theory somewhat by showing that CO_2 need not be supersaturated in the brine to cause bloating.

There are several variables that contribute to bloater formation. One would easily conclude from Etchells et al. (1968) theory of bloater formation that the presence and amount of CO_2 in the brine is a critical factor. Veldhuis and Etchells (1939) studied the composition of the gas evolved from cucumber fermentations and found that it consisted mainly of CO_2, with H_2 occasionally present. Furthermore, the gases found inside of bloaters from any given vat were found to be similar in composition to the gas evolved from that vat. Bloater formation was not observed in microbiologically inactivated brines (Fleming et al., 1973b). And finally, Fleming, Thompson and Monroe (1978) induced bloater formation by artificial carbonation of cucumber brines.

Brining techniques have an influence on the amount of CO_2 produced by a given fermentation and therefore on the incidence of bloater formation. Veldhuis and Etchells (1939) found that if the initial salt concentration was high, a gaseous fermentation ensued. Fleming et al. (1977) reported that a greater amount of CO_2 was evolved from fermentations as brining temperatures increased. And Jones et al. (1940) reported that addition of sugar to brines increased bloater formation. Proper brining techniques, adjusted to the climate and fruit size, are necessary to control excess CO_2 production and to promote a healthy lactic fermentation.

Yeast populations in cucumber fermentation have been shown to produce large amounts of CO_2 (Etchells et al., 1968). Yeasts are an endemic portion of the normal microbial populations of cucumber fermentations. Etchells and Bell (1950b), isolated and identified numerous species of surface and subsurface yeast from commercial fermentations. As mentioned previously, salt and acid tolerant yeasts may ferment any residual sugars after the completion of lactic fermentation.

Costilow (1957) found that bloater formation could be reduced by the addition of sorbate to cucumber brines. Sorbate is known to selectively inhibit the growth of yeasts, molds and some species of bacteria.

Bacteria of the genus Enterobacter were also reported to produce CO_2 as well as H_2 in cucumber fermentations by

Etchells et al. (1945). These organisms were thought to account for the H_2 evolved from some fermentations as well as the H_2 found inside of some bloaters.

Etchells et al. (1964) developed a pure culture fermentation process whereby populations of all naturally occurring microbes were drastically reduced by acidification of the cover brine with acetic acid. This was followed by raising the pH and buffering it at about pH 4.7 with sodium acetate. Next, the brine was inoculated with a starter culture of lactic acid bacteria. This process reduced the initial populations of yeasts, molds and all other unwanted organisms and insured a large population of lactics. Buffering the brine allowed the lactics to completely utilize all of the sugars present in the brine by holding the pH at a level tolerable for them. However, this procedure did not successfully eliminate salt-stock defects such as bloating. Further studies showed that lactics common to pickle brines were also capable of producing CO_2 in quantities sufficient to induce bloater formation (Etchells et al., 1968).

Removing CO_2 from cucumber fermentations by purging proved to be the most significant innovation of the 1970's in terms of reducing bloater formation. Purging is accomplished by bubbling N_2, air, combusted air, or inert gases through the brine. These gases all have much lower solubility in brine than does CO_2. Therefore, the bubbles remain intact

and as they pass through the brine, molecules of CO_2 absorb into these bubbles. All the gases are liberated from the brine as the bubbles reach the surface (Fleming et al., 1975).

Fleming et al. (1975) found that N_2 was the most satisfactory purging gas. N_2 was found to efficiently remove CO_2 from the fermentations studied without effecting the lactic fermentation. The reduction in bloater formation was substantial. The drawbacks of purging with N_2 were the expense and a delay in cure color development. None the less, Etchells, Bell and Fleming (1973), incorporated N_2 purging into their suggested procedure for pure culture fermentations as an important step in inhibiting bloater formation.

Due to the expense of N_2 purging, interest was generated for defining the maximum amount of CO_2 tolerable in cucumber brines before bloater formation was induced, Etchells et al. (1973). A satisfactory level has yet to be defined due to the variable solubility of CO_2 in brines of different strengths and temperatures. Also, the minimum amount of CO_2 necessary to cause bloating has not been determined. Etchells, Bell and Fleming (1973) suggested a value of not more than 20 mg CO_2 per 100 ml of brine. Fleming et al. (1978) suggested that CO_2 should not exceed 50% saturation. And Costilow et al. (1981) reported that values as high as 90 mg per 100 ml or 75% saturation have not resulted in excessive bloater formation in purged fermentations.

At first, air was deemed unsuitable for purging due to softening, off flavors and discoloration of the salt stock (Fleming et al., 1975). Softening was attributed to the growth of molds which was supported by the oxygen present in the air (Costilow et al., 1980). Off flavors and discoloration of the salt-stock were considered to be the results of oxidation when air bubbles became entrapped beneath the cucumbers and remained in contact with the fruit for some period of time (Fleming et al., 1975).

The disadvantages of purging with air were overcome by several modifications in purging apparatus and purging schedules. Costilow et al. (1977) developed a device called a sidearm purger. This device draws brine from the bottom of the vat, purges the brine and expels it at the surface of the vat all through an L shaped tube. The sidearm purger circulates the brine as it purges and the purging gas does not come in contact with the cucumbers, thus eliminating oxidation problems. Next, Costilow et al. (1981) recommended intermittent purging to allow dissolved oxygen to dissipate from the brine. It was surmised that molds deprived of oxygen for even short periods of time would be rendered nonviable. Finally, Costilow et al. (1981) recommended that the flow rate should not exceed 20 ft^3/hr when air was used as the purging gas. Extensive studies of commercial fermentations purged with N_2 and air with the above modifications concluded that there were no significant differences between

N_2 and air purged stock, Costilow and Uebersax (1981). These advances made the replacement of costly N_2 with air as a purging gas practical and further increased the economic benefits derived from purging.

Enzymatic Softening of Salt Stock Pickles

The enzymatic softening of fermented cucumbers represents a second type of spoilage commonly encountered by pickle briners. Like bloaters, soft pickles are unacceptable for many pickle products and at best they may be incorporated into low quality products such as relish. Fermented cucumbers are composed mainly of cellulose and pectic substances and the hydrolysis of these substances has been implicated as the main cause of softening (Etchells et al., 1958a).

Pectin is considered to be the main component responsible for the characteristic crispiness of pickles. Pectin is a component of cell walls and also functions as a cementing substance that binds cell walls together in plant tissues. It is a complex polymer consisting of 1,4 alpha linked galacturonic acid units. The carboxyl groups may be free, esterified with methyl groups or in a salt form. In plant tissues pectin is located within the middle lamella or may be bound to cell wall constituents (Fenema et al., 1976).

It is known that many microorganisms can produce enzymes capable of hydrolyzing pectic substances. Fruit tissues also produce active pectinolytic enzymes and these account

in part for the natural softening of fruit upon ripening.

These enzymes can be placed in two main classifications: 1) pectinmethylesterases, which are enzymes capable of catalyzing the deesterification of the methyl groups attached to the pectin molecule and 2) polygalacturonases (PG), which are enzymes capable of hydrolyzing pectin into galacturonic acid sub-units. Although the deesterification of pectin alone does not cause softening, some degree of deesterification must take place before complete hydrolysis of the molecule can occur.

Bell, Etchells and Jones (1950) found polygalacturonase activity in cucumber brines and showed a correlation between this enzyme activity and softening. Furthermore, they concluded that the enzyme had a pH optimum of about 4.0 and was active in cucumber brines with a pH range of 3.2 to 3.9, salt content of 10-20% and brine acidity of .2-1.2% lactic acid. Aerobes (Fabian and Johnson, 1938), yeasts (Etchells and Bell, 1950a), and molds (Etchells, Bell and Jones, 1955a) were investigated as possible sources of this specific polygalacturonase since all are known to produce pectinolytic enzymes under certain condition. However, only molds were found to produce a polygalacturonase that was active in cucumber fermentations and met the specifications listed previously. Etchells *et al*. (1958) observed pectinolytic activity from species of *Penicillium*, *Ascochyta*, *Fusarium*, *Cladosporium*, *Alternaria* and others isolated from commercial

brines. These organisms are part of the normal flora of the soil and the surface of the cucumber fruit. Their populations on any lot of cucumbers vary with harvest conditions, time of year, area, and length and conditions of post harvest storage (Etchells et al., 1973).

Cucumber blossoms were found to harbor large populations of molds. Etchells, Bell and Jones (1955) observed that cucumbers brined with a large number of attached blossoms resulted in a high incidence of soft stock. They also showed that softening could be induced by the addition of cucumber blossoms to cucumber fermentations. Thus, molds were indicated as the causitive agents in the softening of brined cucumbers.

As mentioned earlier, fruit tissues may also be a source of pectinolytic enzymes. Bell (1951) measured pectinolytic activity in cucumber fruits and plant parts. He found that staminate and pollinated pistillate flowers were strongly positive for pectinolytic enzyme activity. Seeds and ripe fruit were also positive. Unpollinated flowers, unripe fruit and stems were weakly positive to negative. Bell and Etchells (1950) observed that pectin esterase diffused out of cucumber fruit when placed in 13.2% salt brine. Hamilton and Johnston (1961) also studied polygalacturonase like enzymes produced by molds in commercial brines. They did not find pectinesterase production by molds and concluded that both pectinesterase produced by the cucumber, plus the

polygalacturonase produced by molds were responsible for the enzymatic softening of brined cucumbers.

Management of softening problems in cucumber brines has been focused upon removing or inactivating enzyme activity in brines. Etchells, Bell and Jones (1955b) suggested that attached blossoms be mechanically removed before brining, or perhaps development of new cucumber varieties which have less tendency to retain the blossoms. Etchells et al. (1955b) also suggested that the brines of small sized cucumbers, containing a majority of the softening enzyme, be drained off and replaced with a fresh brine after the first 24-36 hr. This procedure has been used with some degree of success, especially in southern brining areas. However, the disposal problems that spent brine presents to the industry at this time somewhat limits the practicality of this procedure.

For years, home recipes for brining cucumbers have included the addition of grape leaves to the fermentation. Etchells, Bell and Williams (1958) used a crude extract of grape leaves to inhibit pectinase activity in cucumber fermentations. The extract did not disturb the fermentation process nor add any off flavors. Also, it was noted that the degree of inhibition corresponded to the amount of grape leaf extract added and that pectinolytic activity returned when the inhibitor was removed. Thus, the grape leaf extract was thought to contain a competitive inhibitor of

pectinolytic enzymes. Bell, Etchells and Singleton (1965) noted similar inhibition effects from Sericea extracts. However, the addition of plant extracts in the large quantities required for commercial fermentations is impractical. And at this point in time, there is no commercially available purified enzyme inhibitor for cucumber fermentations.

Chavana (1976) studied the thermal inactivation of pectinase in cucumber brines. Pectinolytic enzyme produced by Penicillium janthinellum was found to be the most stable in terms of resistance to thermal denaturation. Heat stability of this enzyme was found to be affected by pH and salt concentration. However, it was concluded that a pasteurization treatment of 175^{o}F for 30 seconds would be sufficient to inactivate this enzyme. This procedure is also somewhat impractical for commercial brining of cucumbers. However, heat inactivation of pectinolytic enzymes may have some value in the reuse of brines and in the pasteurization of fresh pack pickles.

Bell and Etchells (1961) studied the effect of salt on pectinase activity. They found that the enzyme activity decreased as salt concentration increased. Briners have felt for many years that low salt concentrations in brines increased the incidence of softening and these findings lend some credence to such thought.

The addition of calcium chloride to cucumber brines has also been shown to enhance the texture of the cured salt

stock (Buescher, Hudson and Adams, 1979). Calcium and other divalent ions are known to form cross linkages between adjacent pectin molecules. Cross linked pectin molecules are thought to be more resistant to hydrolysis by polygalacturonase enzymes. Calcium chloride or calcium citrate are commonly added to commercial fermentations to enhance the texture of salt-stock.

Influence of Post Harvest Handling and Storage on Salt-Stock Quality

Post harvest handling and storage of cucumbers has a profound effect upon the quality of the finished salt-stock. It has been said that even the finest briner can not make a decent pickle from an old moldy cucumber. Indeed this is so; the best pickles are manufactured from fresh, undamaged fruit of good size and variety.

In order to increase the brining season many pickle packers are transporting green stock from out state areas. In Michigan, pickle packers commonly buy early season cucumbers from as far away as Texas. This means increased time from field to brine. Lee, Uebersax and Herner (1982) reported that increasing holding times and temperatures of fresh cucumbers prior to brining increased weight loss and respiration and decreased firmness. Also, bloater formation and softening were shown to increase dramatically in salt-stock as prebrining holding time and temperatures increased.

These changes are probably attributable to senescence of the fruit followed by microbial invasion.

Rough handling of fresh cucumbers may also contribute to quality loss in salt-stock. Marshall, Cargill and Levin (1972) reported that repeated drops of several feet may bruise internal cucumber tissues and render them more susceptible to bloater formation. Limiting the number of transfers made, reducing drop heights and the use of 3-4 inches of foam or 3-4 feet of cushion brine in bins and brining tanks were suggested to reduce such damage.

Abrasions, cuts and scrapes on fresh cucumbers can also be detrimental. These types of injuries can increase respiration rates and provide an avenue for invasion by microorganisms. Sarig et al. (1975) suggested that rough areas, exposed bolts and welds be eliminated from conveyor belts used for sorting and washing operations.

Mechanically harvested fruit have been shown to have higher respiration rates (Garte and Weichmann, 1974). This is due to the abrasions and scrapes the fruit sometimes endure as they come in contact with machinery. Broken or smashed cucumbers are also more common among machine harvested fruit. To cope with these problems, mechanical cucumber harvesters are being refined and new varieties of cucumbers are being developed specifically for mechanical harvesting (Van Ee et al., 1981; Baker et al., 1973).

Chlorine Dioxide

Chlorine dioxide is a germicidal compound similar in action to chlorine. A gas at room temperature, it is slightly soluble in water, to .29%, and will impart a yellowish tint and a prominent chlorine odor to an aqueous solution.

Bernarde et al. (1965) found chlorine dioxide to be an effective rapid acting germicidal agent against bacteria, viruses and spores. In sewage effluent, 2 ppm chlorine dioxide drastically reduced microbial populations within one minute. Also, the effectiveness of chlorine dioxide was noted to increase with time and temperature (White, 1972).

In organic free water (low demand) at low pH the germicidal power of chlorine dioxide was noted to be comparable to that of chlorine. However, under alkaline conditions or in the presence of a large amount of organic matter, chlorine dioxide was found to be far superior (Bernarde et al., 1965). Unlike chlorine, in aqueous solution, chlorine dioxide is a true dissolved gas. Therefore, it is not directly affected by pH, as is chlorine, which is dependent upon the presence of hypochlorous ions for its most effective germicidal power (White, 1972). Also, chlorine dioxide is a more selective oxidizing agent. It enters into far fewer side reactions than does chlorine which will combine readily with many

organic compounds via addition and substitution reactions (Ward, 1975). Therefore, chlorine dioxide can be used more efficiently in water high in pH or organic matter. And although chlorine dioxide is more costly than chlorine, it may be used cost effectively under certain conditions.

Due to some of the unique properties of chlorine dioxide mentioned above, its use has been found to be very advantageous for certain applications. In the city of Niagara Falls, the presence of phenolic wastes in river water resulted in off tastes and odors in municipal water supplies following chlorination. Chlorine reacts with phenolic compounds to form chlorophenols that are responsible for objectionable taste and odors in such water. Chlorine dioxide, which completely oxidizes phenols to odorless compounds, has been used since 1945 by the city of Niagara Falls to eliminate such problems in the municipal water supply (Ward, 1977).

Vegetable processors have also found use for chlorine dioxide in plant waters. Welch and Folinazzo (1959) investigated the use of chlorine dioxide for the control of microbial populations in recycled water at a vegetable canning operation. They found that five to ten ppm chlorine dioxide maintained acceptable levels of bacteria in plant waters and on finished product. Chlorine was found to be less effective under the conditions studied. Olin Water Services (1978) provides similar case histories

where chlorine dioxide was used effectively to control bacterial populations and odor problems in tomato flume water and potato processing waters. In both cases the use of simple chlorination was insufficient. Also, Baran et al. (1973) reported that microbial counts on whole turkeys were further reduced when five to seven ppm chlorine dioxide was added to chilling waters following processing in chlorinated water as opposed to when chlorine was used alone. In the range of concentrations used in the studies above, no off flavors, odors or bleaching of the products were noted.

As a gas, chlorine dioxide is unstable and explosive at concentrations above 10-15%. Also, as noted earlier chlorine dioxide is not highly soluble in water and concentrated solutions are not available. Therefore, development of appropriate technology was necessary before chlorine dioxide could be made available for widespread use. Some companies have developed on-site generation systems where chlorine dioxide is liberated from a precursor solution of $NaClO_2$ by mixing with water containing free residual chlorine. This residual chlorine can be provided by dissolving chlorine gas into the water supply or by liberating it from NaOCl with acidification (Olin Water Services, 1978).

Other companies have developed so called stabilized chlorine dioxide solutions. Anthium Dioxcide ®

(International Dioxcide, Clark, N.J.) is one such solution manufactured by passing ClO_2 gas through sodium carbonate peroxide solutions. The process is completed upon removal of the peroxides. Free chlorine dioxide is said to be released from this product when the pH is reduced or in the presence of free residual chlorine.

MATERIALS AND METHODS

Experimental Design of Research Conducted

Source of Fresh Cucumber

All of the cucumbers used in these studies were donated by Green Bay Foods Inc., and were obtained from the plant in Eaton Rapids, Michigan. Size 1B (7/8 to 1 1/16" dia.) cucumbers were used throughout the ClO_2 studies and size 3B (1 1/2 to 2" dia.) cucumbers were used exclusively in the Cucumber Piercing and Vacuum studies.

Chlorine Dioxide Studies

Effect of ClO_2, Cl_2, Anthium Dioxcide® and Blanching on the Microbial Populations of Blended Cucumbers

This study was designed to study the effects of ClO_2, Cl_2, and Anthium Dioxcide® on the microbial populations of cucumbers. Size 1B cucumbers, carefully selected to be free from visible disease or mechanical damage, were used in this study. Prior to treatment, they were rinsed to remove adhering soil. The treatments were applied by washing a sample size of one hundred fruit per treatment in 10L of water containing the following compounds for 15 min: ClO_2, Cl_2 and Anthium Dioxcide® in

concentrations of 2.5, 25, and 150 ppm each. A control treatment consisted of washing the cucumbers in pure water and a positive control consisted of blanching the cucumbers for five minutes at 70°C. Following the treatment, the cucumbers were stored at 20°C and 95% relative humidity. A subsample of 10 cucumbers was withdrawn at one, twenty-four and forty-eight hours after treatment. These cucumbers were then homogenized with water in a 1:5 ratio. Serial dilutions were prepared from these homogenates and used to estimate microbial populations. In this and subsequent studies the following media were used: (LBS) agar with .05% bromocresol green (pH 5.6) for lactics, Yeast Nitrogen Base agar (YNBA) acidified with tartaric acid for yeasts and molds and Plate Count agar (PCA) for an estimated total count. Three replications with duplicate plates within each replication were counted to estimate the microbial populations within each treatment. Additional analysis included measuring polygalacturonase activity and texture at one, twenty-four and forty-eight hours. Each treatment was carefully observed at the times of analysis for the appearance of visible mold growth.

Effect of ClO_2, Cl_2 and Anthium Dioxcide® on the Microbial Populations in Cucumber Wash Water

These two studies were designed to study the effect of ClO_2, Cl_2 and Anthium Dioxcide® on the microbial populations in cucumber wash water under two conditions:

i.e., high and low demand environments. In the first experiment, the cucumbers were carefully selected to be free from mechanical damage and rinsed to remove any adhering soil prior to treatment. The treatments consisted of washing twenty cucumbers each in one liter volumes of water containing 2.5, 25 and 150 ppm of ClO_2, Cl_2 or Anthium Dioxcide® for fifteen minutes. The cucumbers were removed and the water samples from each treatment were evaluated for microbial populations and compared to a control.

In the second study in this section, a high demand environment was generated by soaking one bushel of cucumbers in ten gallons of water for four hours. These cucumbers included broken and damaged fruit and adhering soil was not removed. Following this soaking procedure, the cucumbers were removed and the water was divided into ten lots. 2.5, 25 and 150 ppm of ClO_2, Cl_2 and Anthium Dioxcide® were added to the different water samples. Then, after fifteen minutes, the microbial populations of these water samples were enumerated.

Effect of ClO_2 on Natural Fermentations of Salt Stock Pickles

These two studies were initiated to determine whether or not the use of ClO_2 in cucumber wash waters had any effect upon the natural fermentation process. In the first study, clean undamaged 1B cucumbers were washed for

fifteen minutes in clean water containing 1 to 100 ppm ClO_2. ClO_2 concentration was measured before and after each treatment. Also, following the treatments the microbial populations of the water samples were estimated. Treated cucumbers were brined in 1 gallon jars with a 45^o salometer cover brine. Dry salt was added the following day to equilibrate the brines at 25^o salometer. Total acidity and pH were monitored on these fermentations on a daily basis for one week.

In the second study cucumber fermentations were monitored over a longer period of time. Also, an attempt was made to simulate wet tank or commercial cucumber wash water. This was done by washing ten bushels of cucumbers in twenty gallons of water. Continuous washing of cucumbers in this comparatively small volume of water generated a source of water that represented a high demand environment for ClO_2. This water was used for the following four treatments: 1) cucumbers were washed in this water for fifteen minutes and then dipped in clean water containing 2.5 ppm ClO_2. This treatment was designed to simulate a commercial situation whereby the fresh cucumbers would be sprayed with a solution containing ClO_2 following wet grading: 2) ClO_2 was added to the high demand water until a residual of approximately 2.5 ppm ClO_2 was achieved. This required approximately 25 ppm ClO_2 initially. Then the cucumbers were washed in this water for fifteen

minutes. This treatment was designed to simulate the effect of adding ClO_2 directly to cucumber wash water or wet tanks. Obviously, in a continuous system, ClO_2 would have to be constantly replaced as it is used up; 3) cucumbers were washed in the water, then rinsed with clean tap water. This treatment simulates common commercial practices at this time; 4) cucumbers were washed in the water and used directly without a final rinse of any kind.

A sample of fifty cucumbers from each treatment was held at $20^{o}C$ and $10^{o}C$ at 75% relative humidity and observed daily for the appearance of visible mold. Subsamples of ten cucumbers from each treatment of the stored fruit were taken daily and used for enumeration of mold populations. In addition two five gallon pails of cucumbers from each treatment were brined immediately following the treatments. These fermentations were monitored for six weeks by measuring pH and total acidity every other day. Each fermentation was also evaluated for polygalacturonase activity. Following the fermentation, the stock was evaluated for texture using the Instrom universal testing machine.

Cucumber Piercing and Vacuum Studies

Preliminary Salt Penetration Study

A 2X2X2X3X2 factorial design was employed. The variables included two depths and three frequencies of piercing, two diameters of piercing implements, a vacuum

treatment and two replications. A total of fifty two, one gallon jars were used to evaluate the effect of piercing and vacuum treatments on brine penetration as measured by NaCl concentration of the stock at zero and twenty four hours.

Piercing variables included depth and frequency of pierces as well as size, or diameter of the piercing implement. Two depths were used to evaluate whether salt penetration was enhanced by piercing into the carpel space or if piercing just through the skin would be sufficient. These two depths are referred to as radius and 1/2 radius, respectively. Three frequencies were incorporated into this study. These include eight, sixteen or thirty two punctures per 3B cucumber. Also, needles of two diameters were used as piercing implements to evaluate the effect of hole size on brine penetration. Piercing variables are illustrated in Figure 1. All piercing was done by hand so that the piercing angle was perpendicular to the cucumber surface and did not tear the skin upon withdrawal.

Vacuum treatment consisted of submerging the green stock in a 45^{o} salometer brine and pulling a vacuum equivalent to 25 inches of Hg for ten minutes.

A sample of two cucumbers from each jar was removed after the initial vacuum treatment and again after twenty four hours in brine. These samples were towel dried and stored frozen for later evaluation of NaCl concentration.

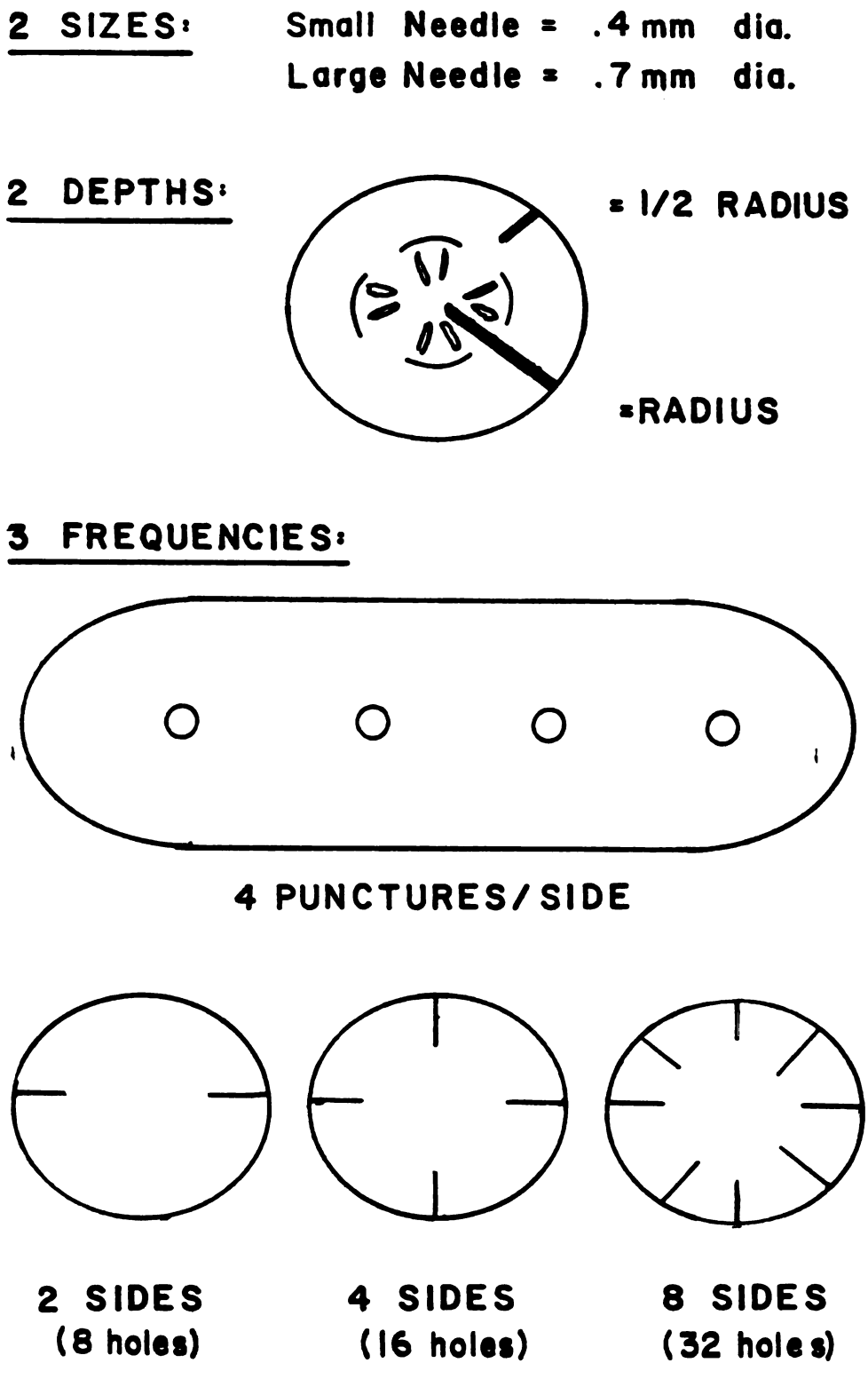

Figure 1. Piercing variables for preliminary salt penetration study.

In addition, after twenty four hours, a twenty ml sample of each brine was withdrawn and evaluated for polygalacturonase activity. All treatments were prepared in duplicate.

Five Gallon Pail, Vacuum X Pierce X Sorbate, Fermentation Study

A 2X2X2X2 factorial design was employed. Sixteen, five gallon pails of 3B cucumbers were brine following vacuum, piercing and sorbate treatments. Again, vacuum treatment consisted of submerging the green stock in a 45^{o} salometer brine and pulling a vacuum equivalent to twenty five inches of Hg for ten minutes. The piercing treatment consisted of piercing each treated cucumber sixteen times, to a depth of one half the radius with a .4 mm diameter needle. Sorbate treatment consisted of adding .1% sorbate to the brine. Following a six week fermentation period, the salt stock was evaluated for texture, defect classification and general appearance.

Analytical Procedures

Preparation and Measurement of Water Sanitizers

Working solutions of chlorine were prepared from commercial solutions of hypochlorite by dilution with deionized water. Initial chlorine concentrations, in ppm, were calculated on the basis that the stock solutions contained 5.25% available chlorine as stated on the label.

Working solutions of Anthium Dioxcide® were prepared from a concentrated solution of stabilized chlorine dioxide supplied by International Dioxcide, Inc., Clark, N.J. The stabilized chlorine dioxide was activated by lowering the pH to 4.0 with acetic acid per label directions. Initial concentrations of Anthium Dioxcide® were calculated on the basis that the stock solution contained 50,000 ppm chlorine dioxide as stated on the label.

Chlorine dioxide was prepared fresh daily by combining equal parts of chilled dilute solutions of hypochlorite, sodium chlorite and HCl, see Appendix I for formulation. This stock solution, containing 300-500 ppm chlorine dioxide, was diluted with deionized water to the desired working concentrations.

A spectrophotometric method (Wheeler et al., 1977) was used to determine the concentration of ClO_2 in water samples. The procedure is as follows: add 2.0 ml of a 3.3×10^{-4}M standard chlorophenol red solution to a 125 ml Erlenmeyer flask; add 1 ml of a pH 7.0 buffer and swirl to mix. The solution should immediately turn purple. Add 50 ml of a water sample containing up to .1 mg ClO_2 to the flask, mix and measure the absorbance at 575 nm. Absorbance at 575 nm was transformed to ppm ClO_2 using a standard curve where an absorbance reading of .70 equals zero ClO_2 and .1 absorbance is approximately 2.5 ppm ClO_2.

For samples containing greater than 2.5 ppm ClO_2 dilutions were prepared. For example, a 50 ml sample containing 4.0 ppm would be diluted to 100 ml and a subsample of 50 ml of this solution would be used to make a measurement. All samples requiring such a dilution to measure initial concentrations of ClO_2 were diluted in a similar manner to measure residual concentrations of ClO_2 after treatments.

Samples containing background interference such as suspended dirt were filtered through Whatman No. 1 paper prior to analysis.

The chlorophenol red solution was prepared by dissolving .1436 g of chlorophenol red in 100 ml of .01 N NaOH and diluting this solution to one liter with distilled deionized water. After standing overnight the solution was filtered through a .45-um Millipore filter. The chlorophenol red solution was standardized by titrating 10 ml of .01 N potassium dichromate in 25 ml sulfuric acid and 15 ml deionized water to a pink end point.

Polygalacturonase Activity Assay

Polygalacturonase (PG) activity of cucumbers and brines was measured using the procedure described by Bell et al. (1955), with modifications by Costilow et al. (1981). Brine samples had to be dialyzed to remove salt which reacts with the pectate solution to form a gel. Dialysis was accomplished by filling a twenty ml sample of brine

into a length of cellophane tubing and securing both ends. These tubes were then suspended in a container of tap water which was replenished with fresh water at a flow rate of about one gallon per five minutes. After three hours in tap water the samples were resuspended in a large volume of deionized distilled water for an additional hour. Samples were then transferred to sterile test tubes containing about five drops of toluene as a preservative. These samples could be capped and refrigerated for analysis at a later date or used immediately. For testing green stock the samples were first homogenized with water in a 1:5 ratio, filtered and used directly.

To perform the assay, 4 ml of a 1.2% sodium polypectate solution (see Appendix II for formulation) were added to a viscosity pipette, which was suspended in a 32^{o}C water bath, allowing ten minutes for the solution to come up to temperature. 1 ml of the dialyzed brine or fresh filtered cucumber homogenate was added and the two solutions were mixed by gently blowing air through the viscometer. The solution was drawn into the upper bulb of the viscometer and the time required for the solution to flow from the upper etch mark to the lower etch mark was measured with a stopwatch. A few drops of toluene were placed into the viscometer to prevent contamination. The viscometer was tightly stoppered to prevent evaporation of the toluene. The viscometer was incubated at 32^{o}C and the flow time

remeasured after 20 and 44 hours. The following formula was used to estimate polygalacturonase activity:

$$\% \text{ Loss in Viscosity after 20 hr.} = \frac{\text{initial flow time} - \text{20 hr. flow time}}{\text{initial flow time} - \text{flow time of } H_2O \text{ in the viscometer}} \times 100$$

Bell et al. (1955) provide a table that relates % loss in viscosity to activity units of polygalacturonase.

Cucumber and Salt-Stock Pickle Texture Evaluation

Two means of evaluating the texture of fresh and salt-stock cucumbers were employed. A Magness-Taylor fruit pressure tester (FPT) with a 7/16" diameter tip was used according to the procedure described by Bell et al. (1955). Firmness was recorded as the force in lbs. required to puncture the wall of the cucumber. In all cases, 10 cucumbers from each sample were measured to evaluate the firmness of the lot.

The Instron Universal Testing Machine (Model TTBM, Instron Corp., Canton, Massachusetts) with a 7/16" diameter probe was the second method of measuring the texture of cucumbers in these studies. As with the Magness-Taylor (FPT), firmness was measured as the force in Kg required to puncture the wall of the cucumber.

Microbiological Media and Methods

Lacto-Bacillus Selective Media (LBS) with .05% bromocresol green, acidified to pH 5.6 ± .05 was used to enumerate lactic acid forming bacteria (Costilow, Etchells & Anderson, 1964). Yeast and mold colonies were enumerated on Yeast Nitrogen Base Agar (YNBA) acidified with 10% tartaric acid. Total counts were estimated using plate count agar (PCA). See appendix II for formulation of media.

Microbial populations of cucumbers were estimated by homogenizing 10 cucumbers per sample in a 1:5 ratio with water. Serial dilutions were prepared from the homogenate and plated on the various media in duplicate.

To estimate the microbial populations of water samples, serial dilutions were prepared and plated in duplicate directly from the sample.

Brining and Fermentation Procedures

All cucumbers brined in five gallon pails followed the procedures described by Costilow et al. (1982) for pilot scale fermentations. The fresh cucumbers are washed and covered with a forty five degree salometer brine with .05% acetic acid to enhance CO_2 solubility in the brine. Dry salt was added as needed to equilibrate the brine at 25^{o} salometer. The pails were fitted with purging apparatus and purged with nitrogen gas on the following schedule:

immediately following brining for twenty minutes and thereafter fifteen minutes each morning and afternoon for approximately two weeks.

Measurement of total acidity and pH were used to monitor fermentations. Total acidity was estimated as percent lactic acid using .1N NaOH as a titrant and phenylphthaline as an indicator. A standard pH meter was used to measure pH. These measurements were taken daily.

Visual Evaluation of Salt-Stock Pickles

Following a six week fermentation period, salt stock from the 5 gallon pail, Pierce X Vacuum X Sorbate study were evaluated in the following manner. Thirty cucumbers were removed from each pail, sliced lengthwise, and grouped according to the following defect classifications: No Damage, Honeycomb, Lens, Balloon, or Soft Center. The "Bloater Chart" of Etchells *et al*. (1974) was used to help determine these classifications. These groupings were then rearranged by three judges until a consensus was reached.

Pierced stock was also observed for evidence of visible puncture marks.

Chlorine Ion Concentration of Salt Stock

Chloride ion concentration of pierced and/or vacuum treated salt stock was measured using an Orion Microprocessor Ionanalyzer 901 (Orion Research, Cambridge, Mass.), equipped with a selective ion electrode specific for chloride ions (Cl^-).

Two cucumbers from each sample to be tested were removed from the brine, the surface towel dried and then homogenized in a Waring blender with 1% nitric acid in a 1:5 ratio. The nitric acid served to extract the Cl^- from the cucumber tissues.

The Cl^- ion concentration was related directly to the NaCl concentration by calibrating the instrument with standard NaCl solutions (.1 to 10% NaCl), diluted with nitric acid in a 1:5 ratio. When the instrument is calibrated in this manner, the % NaCl of the sample is presented on the instrument read out (personal communication with Orion technical representative).

Statistical Analysis

The CDC 6500 computer, located at Michigan State University, was used in conjunction with the "Statistical Package for the Social Sciences" (SPSS), (Nie et al., 1975) to assist with the statistical analysis of the data herein.

Subprogram ANOVA was used to determine analysis of variance. Mean squares were reported after rounding and those with significant F tests were indicated with significant probability levels of $P \leq .05$ (*), $P \leq .01$ (**) and $P \leq .001$ (***).

Mean values were separated by L.S.D. and those which were not significantly different at $P \leq .05$ were indicated with like letters.

RESULTS AND DISCUSSION

Chlorine Dioxide Studies

Effect of ClO_2, Cl_2, Anthium Dioxcide® and Blanching on Microbial Populations of Blended Cucumbers

Since the cucumbers used in this experiment were all commercial run and of undetermined post-harvest age, the initial microbial populations measured varied widely. Therefore, microbial populations were analyzed as percent reduction as compared to controls within each lot of cucumbers used. As a positive control, blanching for five minutes at 70°C reduced the mold, yeast and lactics populations by greater than 100 fold. Although there were significant differences between some of the treatment means, none of the other treatments applied caused more than a ten fold reduction in microbial populations. In terms of microbial growth patterns, reductions of such magnitude may not be important.

Under the storage conditions employed in this study, 95% R.H. and 20°C, which are ideal for mold growth, it is not surprising then that the time before the appearance of visible mold growth and a corresponding decline in texture of the fresh fruit were not suppressed. It is probable that the rough, porous cuticle of the cucumber, at the

microscopic level, is capable of harboring vast numbers of microorganisms and protecting them from such water treatments as were applied in this experiment.

Trends noted in the data (Table 1) were first, that population reductions generally increased gradually as concentrations of ClO_2, Cl_2 and Anthium Dioxcide® were increased. Aside from blanching, the largest reductions occurred when using 150 ppm of either ClO_2, Cl_2 or Anthium Dioxcide® in the water treatment. Such concentrations are far too high for commercial application. In the commercially applicable range from 2.5 to 25 ppm, generally less than a one fold reduction in microbial populations of molds, yeasts and lactics was observed. At similar concentrations the different water treatment compounds had comparable effects.

The second trend noted, was that the population reductions decreased as the time following the treatment increased. Very little differences were noted between treatment means 48 hours following the treatments as compared to 24 hr estimates (Tables 1 and 2).

Polygalacturonase activity was not significantly different between treatments or control (Tables 3 and 4). The PG activity observed was probably due to a combination of enzymes of microbial origin and from the fruit itself.

As noted earlier there was a gradual decline in texture over time. Texture values varied very little between

Table 1. Mean values[1] for microbial populations of green-stock cucumbers treated with ClO_2, Cl_2, Anthium Dioxcide® or blanched at 70°C for 5 min. and then held at 20°C and 95% R.H. for 48 hours.

	% Reduction of Microbial Populations Versus Control								
	1 hr after treatment			24 hr after treatment			48 hr after treatment		
Treatment	Yeasts	Molds	Lactics	Yeasts	Molds	Lactics	Yeasts	Molds	Lactics
Control	0^{f}	0^{e}	0^{d}	0^{f}	0^{f}	0^{cd}	0^{d}	0^{b}	0^{c}
Chlorine Dioxide (ppm)									
2.5	66.7^{bc}	15.7^{cd}	6.6^{d}	56.3^{bc}	22.7^{cde}	25.7^{cd}	6.7^{c}	1.7^{b}	3.4^{c}
25	56.0^{cd}	20.0^{cd}	65.0^{bc}	43.3^{cd}	22.3^{cde}	70.0^{ab}	16.7^{bc}	8.7^{b}	22.0^{b}
1	78.7^{ab}	57.3^{b}	73.0^{ab}	37.0^{cde}	31.0^{cd}	39.3^{cd}	8.7^{b}	17.7^{b}	22.0^{b}
Chlorine (ppm)									
2.5	35.1^{e}	8.7^{cd}	10.0^{d}	31.0^{cdef}	12.0^{def}	16.7^{cd}	5.3^{c}	1.3^{b}	5.3^{b}
150	75.0^{bc}	53.3^{b}	66.7^{ab}	49.3^{cd}	37.0^{c}	33.3^{c}	30.0^{b}	6.7^{b}	15.3^{bc}
Anthium Dioxcide R (ppm)									
2.5	1.3^{f}	2.7^{d}	0.0^{d}	16.3^{def}	31.0^{f}	0.0^{cd}	0.0^{d}	30.7^{b}	0.0^{c}
25	39.7^{de}	8.0^{cd}	14.0^{d}	8.7^{ef}	9.3^{ef}	24.7^{cd}	4.0^{d}	6.0^{b}	9.7^{bc}
150	85.7^{ab}	35.0^{bc}	31.3^{cd}	81.0^{ab}	78.7^{b}	10.7^{cd}	10.7^{cd}	16.0^{b}	8.7^{bc}
Water Blanching 70°C, 5 min	99.9^{a}	99.9^{a}	99.9^{a}	99.9^{a}	99.9^{a}	99.9^{a}	99.9^{a}	99.9^{a}	99.9^{a}

[1] n=6, like letters within each column indicate no significant differences by LSD at P>.05.

Table 2. Analysis of variance of microbial populations of green-stock cucumbers treated with ClO_2, Cl_2, Anthium Dioxcide® or blanched at 70°C for 5 min. and then held at 20°C and 95% R.H. for 48 hours.

		% Reduction of Microbial Populations Versus Control								
		1 hr after treatment			24 hr after treatment			48 hr after treatment		
		Yeasts	Molds	Lactics	Yeasts	Molds	Lactics	Yeasts	Molds	Lactics
Source	df	Mean Squares[1]								
Treatment	9	3490***	2989***	3744***	2102***	3276***	2941***	2660***	2649***	1259***
Residual	20	154	302	357	365	154	345	77	355	138
Total	29	1190	1136	1408	1152	1123	1150	879	1067	899

[1]Significant F test indicated by *** ($p \leq .001$), ** ($p \leq .01$), * ($p \leq .05$).

Table 3. Mean values[1] for polygalacturonase activity and texture of green-stock cucumbers treated with ClO_2, Cl_2, Anthium Dioxcide® or blanched at 70°C for 5 min and then held at 20°C and 95% R.H. for 48 hours.

	PG Activity in % Loss in Viscosity			Firmness by Instrom (Kg force)		
	Time Following Treatment			Time Following Treatment		
Treatment	1 Hour	24 Hour	48 Hour	1 Hour	24 Hour	48 Hour
Control	25.3^{a}	21.0^{abc}	20.7^{a}	5.2^{a}	4.7^{abc}	3.7^{a}
Chlorine Dioxide (ppm)						
2.5	23.6^{ab}	30.7^{ab}	22.0^{a}	5.3^{a}	4.7^{abc}	3.6^{a}
25	18.3^{abc}	31.3^{ab}	19.7^{a}	5.3^{a}	5.9^{a}	5.0^{a}
150	18.3^{abc}	19.3^{abc}	25.0^{a}	5.4^{a}	5.3^{a}	4.7^{a}
Chlorine (ppm)						
2.5	20.6^{abc}	26.7^{abc}	29.0^{a}	5.2^{a}	4.7^{abc}	3.7^{a}
150	23.6^{ab}	21.0^{abc}	24.7^{a}	5.2^{a}	5.0^{ab}	3.4^{a}
Anthium Dioxcide®						
2.5	22.0^{ab}	21.7^{abc}	29.3^{a}	4.7^{a}	3.5^{bc}	3.3^{a}
25	23.6^{ab}	21.3^{abc}	26.7^{a}	5.3^{a}	5.0^{ab}	3.6^{a}
150	12.7^{bc}	17.3^{bc}	20.0^{a}	5.3^{a}	5.3^{a}	3.9^{a}
Water Blanch, 70°C 5 min.	11.0^{c}	14.0^{c}	25.3^{a}	5.4^{a}	2.5^{c}	0.6^{b}

[1]n=6 for PG activity and n=30 for firmness. Like letters within each column indicate no significant differences by LSD at P>.05.

Table 4. Analysis of variance of polygalacturonase activity and texture of green-stock cucumbers treated with ClO_2, Cl_2, Anthium Dioxcide® or blanched at 70°C for 5 min. and then held at 20°C and 95% R.H. for 48 hours.

		PG Activity, % Loss in Viscosity			Firmness by Instrom (Kg force)		
		Time Following Treatment			Time Following Treatment		
Source	df	1 Hour	24 Hour	48 Hour	1 Hour	24 Hour	48 Hour
				Mean Squares[1]			
Treatment	9	71.2	92.5	37.7	.12	2.98	4.12*
Residual	20	44.5	61.1	12.4	.19	0.86	1.30
Total	29	52.7	70.9	75.4	.17	1.54	2.18

[1]Significant F test indicated by *** ($p \leq .001$), ** ($p \leq .01$), * ($p < .05$).

treatments with the exception of the blanched lots which became extremely mushy by 48 hours after the treatment (Table 3). Although the blanching treatment successfully eliminated molds, yeasts and lactics, the blanched fruit are extremely vulnerable to bacterial decay, especially from spore formers.

Effect of ClO_2, Cl_2 and Anthium Dioxcide® on Microbial Populations in Cucumber Wash Water

In the first study, where cucumbers were sorted and rinsed prior to use, the initial microbial populations of the wash water were approximately 10^4 organisms per ml for the total count with nearly 10^3 molds and yeasts per ml. The water was practically free from soil and other visible debris. These conditions constituted a low demand environment.

Results showed that ClO_2 concentrations of 2.5 ppm were adequate to reduce total microbial populations by ten fold and nearly eliminate yeast and mold populations. Similar results with Cl_2 and Anthium Dioxcide® required initial concentrations of 25 ppm each (Table 5, Figure 2).

In the second study, where cucumbers were not sorted or rinsed prior to use, initial microbial populations were approximately 10^8 organisms per ml as a total count and just over 10^4 molds and yeasts per ml. There was also considerable soil and other organic material present in

Table 5. Effect of ClO_2, Cl_2 and Anthium Dioxcide® on microbial populations[1] in cucumber wash water.

Treatment	Microbial Populations (organisms/ml)	
	Total Count	Yeasts and Molds
Initial Population	1×10^4	8×10^2
Chlorine Dioxide (ppm)		
ClO_2, 2.5	2×10^2	1×10^1
25	3×10^1	0×10^1
250	2×10^1	0×10^1
Chlorine (ppm		
Cl_2 2.5	1×10^4	5×10^2
25.0	1×10^3	1×10^1
250	2×10^1	0×10^1
Anthium Dioxcide® (ppm)		
2.5	1×10^4	7×10^2
25.0	5×10^3	1×10^1
250.0	3×10^1	0×10^0

[1]Values represent means; n=6.

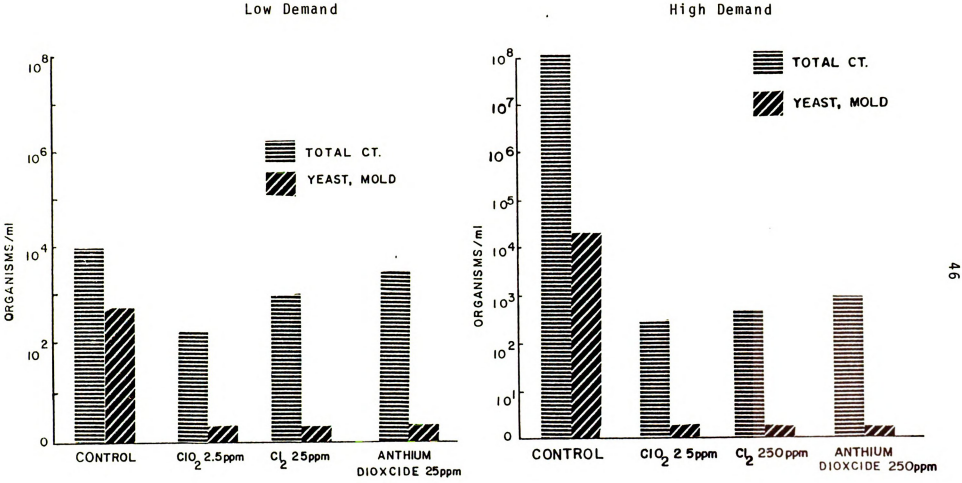

Figure 2. Effect of ClO_2, Cl_2 and Anthium Dioxcide® on cucumber wash water. (n=6)

these water samples, all of which increase the demand for the water treatment compounds. Under these conditions, ClO_2 concentration of 25 ppm was required to achieve an effective residual capable of significantly reducing microbial populations. In contrast, an initial concentration of 250 ppm of Cl_2 and Anthium Dioxcide® were necessary to get similar reductions in microbial populations (Table 6, Figure 3).

These studies indicate that the demand for ClO_2 varies depending upon the microbial and organic content of the environment, as is true with the other water treatment compounds. ClO_2 was also found to be up to ten times more efficient than either Cl_2 or Anthium Dioxcide® under the conditions in the two studies. This data suggests that ClO_2 may be used successfully in low concentrations to control microbial populations of water coming in contact with fresh cucumbers.

Effect of ClO_2 on Natural Fermentations

The first study in this section examined the effects of washing cucumbers in water containing 0-100 ppm ClO_2 on the microbial populations of the wash water and the effect of the treatment on the subsequent brining and fermentation of the fruit.

Table 7 shows the initial and residual levels of ClO_2 and the corresponding microbial populations of the water samples. The initial microbial count was relatively low,

Table 6. Effect of ClO_2, Cl_2 and Anthium Dioxcide® on microbial populations[1] in cucumber wash water.

Treatment	Microbial Populations (organisms/ml)	
	Total Count	Yeasts and Molds
Initial Population	$>10^8$	4×10^4
ClO_2, 2.5 ppm	1×10^7	6×10^3
25.0	5×10^2	1×10^1
250.0	6×10^1	0×10^1
Cl_2, 2.5 ppm	1×10^8	2×10^4
25.0	4×10^7	3×10^4
250.0	2×10^3	1×10^1
Anthium Dioxcide®		
2.5 ppm	1×10^8	4×10^4
25.0	3×10^7	2×10^4
250.0	5×10^3	1×10^1

[1]Values represent mean; n=6.

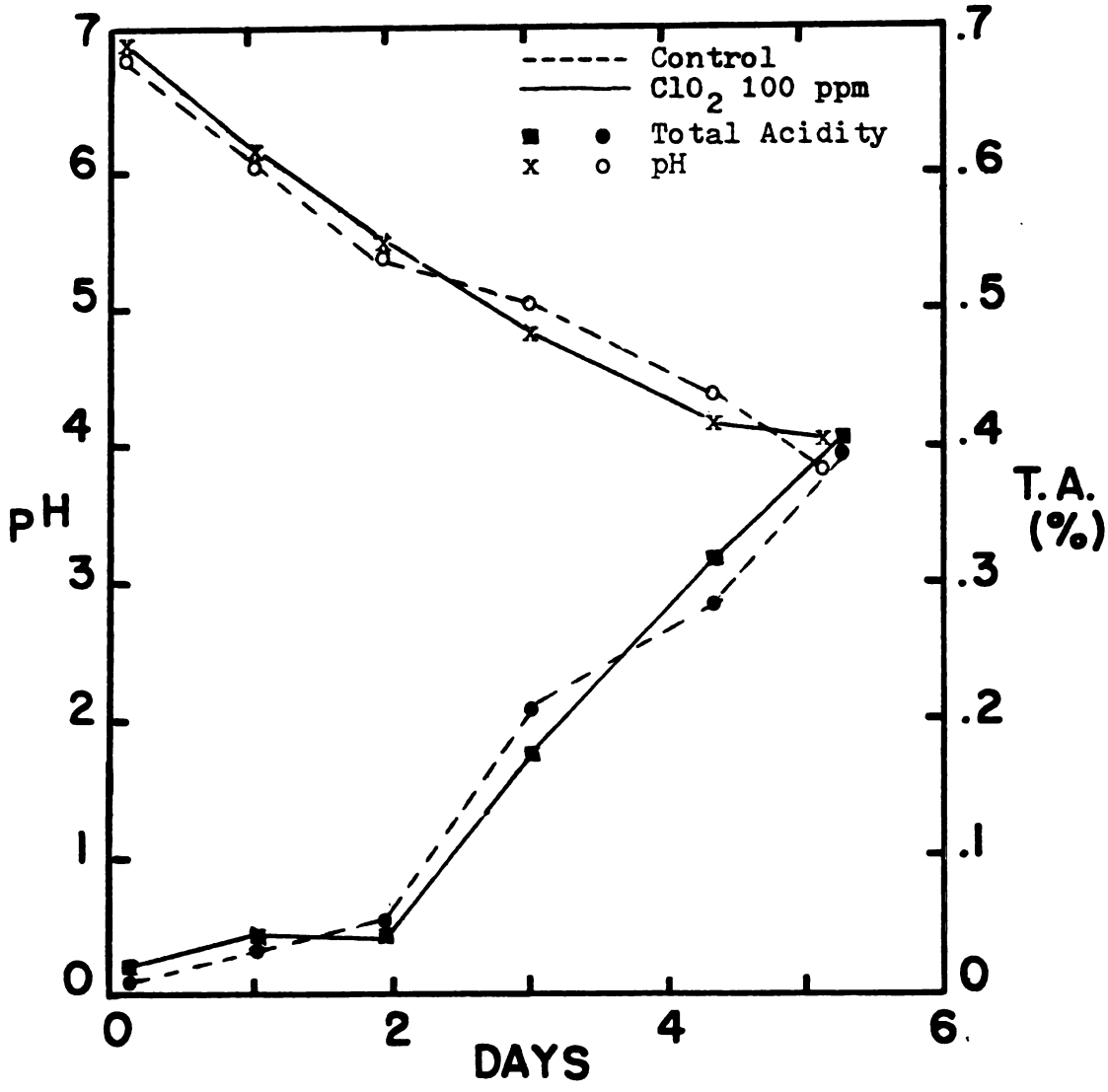

Figure 3. Total acidity and pH of cucumber fermentations following treatment of green-stock with 100 ppm ClO2 for 15 min. versus control. (n=4)

Table 7. Microbial populations[1] in cucumber wash water treated with ClO_2.

Chlorine Dioxide (ppm)		Organisms per ml			
Initial Concentration	Residual	Total Count	Mold	Yeasts	Lactics
0.0	0.0	10,000	70	1,000	250
1.0	0.0	5,000	10	110	0
3.5	1.0	3,000	10	0	0
6.0	2.0	500	0	0	0
10.0	4.0	250	0	0	0
25.0	20.0	180	0	0	0
50.0	44.0	70	0	0	0
100.0	96.0	100	0	0	0

[1]Values represent means, n=4.

approximately 10^4 organisms per ml, and there was little observable soil or organic matter in the water samples following the treatments. Under these conditions, low initial concentrations of ClO_2 were sufficient to obtain an effective residual capable of greatly reducing microbial populations in the water samples. The optimum efficiency was obtained when a residual of 2 ppm ClO_2 was reached. Under the conditions in this study, an initial concentration of 6 ppm ClO_2 was required to achieve the desired residual.

Figure 3 shows a plot of titratable acidity and pH for the control and the 100 ppm ClO_2 wash water treatment fermentations. There was no significant difference between the initiation and course of fermentation between the two treatments. Similar results were observed for the other levels of ClO_2 used in this study. It was concluded from this data that concentrations of ClO_2 as high as 100 ppm in cucumber wash water does not inhibit subsequent natural fermentation of the cucumbers.

The second study in this section monitored the fermentations of ClO_2 treated and untreated stock over a six week period. In addition, subsamples of treated and untreated stock were held at 10 and 20°C and 75% R.H. These subsamples were observed for the appearance of visible mold growth and the yeast and mold populations of each were measured intermittently.

The fermentations of all four treatments proceeded normally in terms of developed acidity and corresponding drop in pH. Figure 4 is a plot of total acidity and pH for the ClO_2 treated and untreated cucumbers which are essentially the same. Once again, this supports earlier findings that ClO_2 in cucumber wash water does not inhibit the natural fermentation process.

Moderate polygalacturonase activity was found in the brine of one of the replicates of treatment four (untreated) and this corresponded to slightly softer stock. Otherwise there were no significant differences of texture among salt-stock from the four treatments (Table 8).

Stock from treatments three and four (no ClO_2) held at 20^oC and 75% relative humidity showed profuse mold growth after two days of storage. Stock from treatments one and two (ClO_2 treated) did not show visible mold growth until the third day of storage. These results were consistent for both replicates in each of the treatments. However, at 10^oC and 75% relative humidity, stored fruit from all four treatments showed signs of visible mold growth on the sixth day. Also, there were no significant differences between the enumerated mold populations of the four treatments under any conditions in this study (Table 9). This may be due to the difficulty of accurately measuring the populations of molds with standard plating methods.

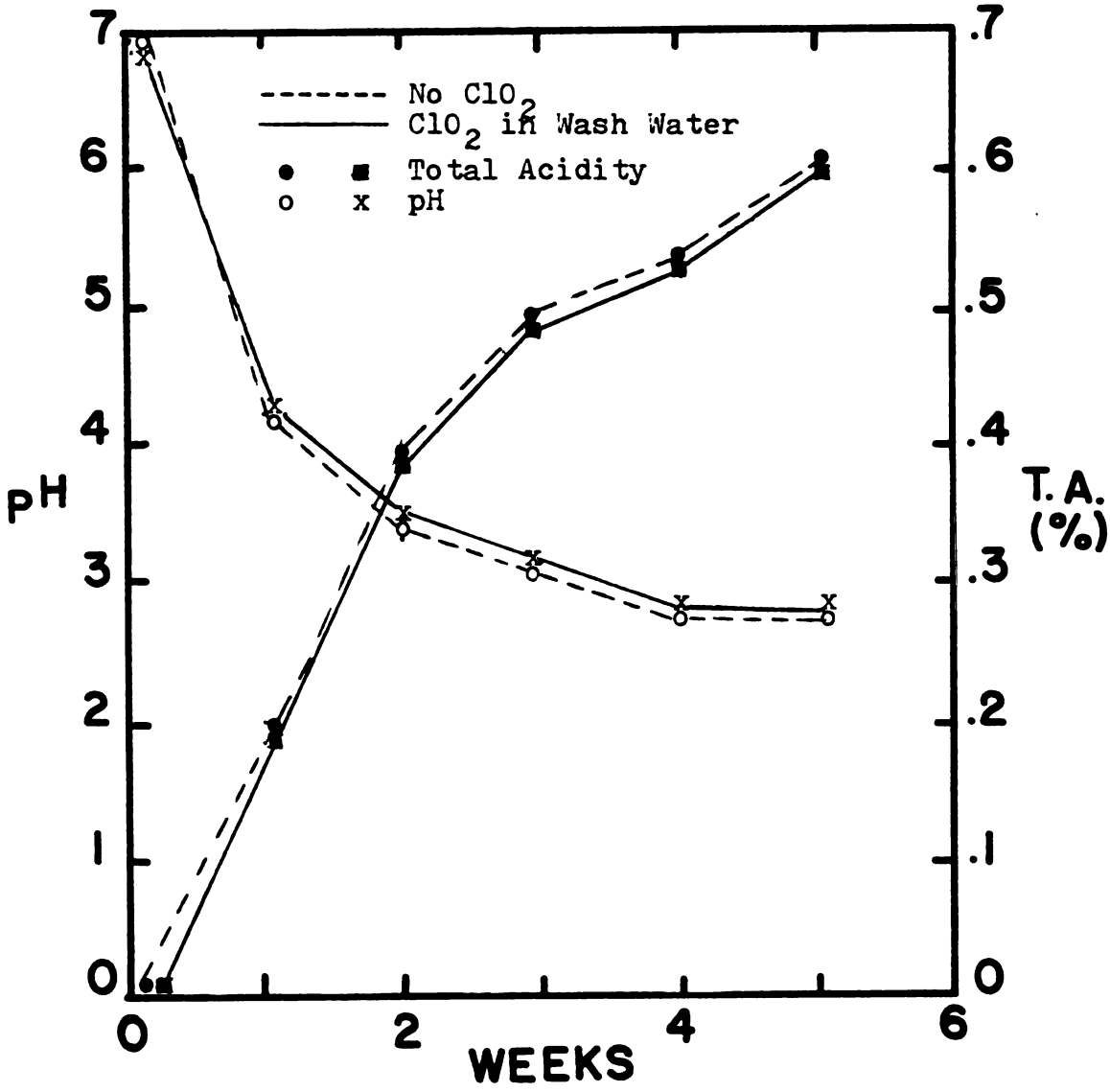

Figure 4. Total acidity and pH of cucumber fermentations following treatment of green-stock with 2.5 ppm ClO_2 for 15 min. versus control. (n=4).

Table 8. Firmness of salt-stock and PG activity of brine from cucumber fermentations following pre-brining treatment of green-stock with ClO_2.

Treatment[1]	Firmness[2]	PG Activity[3]
1	12.0+2.0	12.0%
2	12.2+1.4	12.8%
3	11.8+2.0	9.0%
4	11.0+2.6	15.0%

[1] Pre-brining treatments are as follows:
1) 2.5 ppm residual ClO_2 in wash water
2) 2.5 ppm ClO_2 rinse following washing
3) Tap water rinse following washing
4) Washed then brined directly

[2] Firmness is in Kg, measured by Instrom, n=60.

[3] Measured as % loss in viscosity after 44 hr, n=4.

Table 9. Mean values for microbial populations of green-stock cucumbers treated with ClO_2 and held at 10 and 20°C, 75% R.H. for up to 6 days.

Holding Time (Days)	10°C, 75% R.H.						20°C, 75% R.H.					
	Organisms/gram X 10^3											
	No ClO_2			ClO_2, 2.5 ppm			No ClO_2			ClO_2, 2.5 ppm		
	Yeasts	Mold	Lactics	Yeasts	Mold	Lactics	Yeasts	Mold	Lactics	Yeasts	Mold	Lactics
0	3.5	1.2	6.4	2.9	1.1	8.0	3.5	1.2	6.4	2.9	1.1	8.0
1	4.8	1.6	5.0	5.0	2.5	7.0	5.9	8.9	6.9	6.0	8.0	7.5
2	11.8	10.0	6.2	12.9	8.5	8.0	15.0	33.0*	8.0	11.0	19.0	9.2
3	14.0	19.0	8.0	15.5	18.6	7.0				23.0	40.0*	7.0
6	22.5	31.0*	9.9	20.2	33.5*	10.0						

*Indicates the appearance of visible mold on the samples. Values represent means where n=8.

Cucumber Piercing and Vacuum Studies

Preliminary Salt Penetration Study

Results of the preliminary salt penetration study indicates that both piercing and vacuum treatment of fresh stock significantly enhanced the initial penetration of the cover brine as measured by NaCl concentration. Vacuum treatment was superior in that NaCl concentrations in this stock were initially higher and remained significantly higher than in non-vacuum treated stock after twenty four hours in the cover brine. Polygalacturonase activity was very sporadic, occurring in only two samples, and apparently did not correlate to any of the treatments involved in the study (Tables 10 and 11).

The main effects of the piercing variables are as follows. First, the depth of piercing is not significant. Piercing just through the skin (1/2 radius), or all the way into the caprel space (radius) gave nearly identical results in terms of NaCl concentration. Secondly, increasing the frequency of piercing from four through thirty two holes per cucumber increased the NaCl concentration in a stepwise fashion. However, these increases were not great enough to be considered significant. Thirdly, the diameter of the piercing implement appeared to be the most critical factor for enhancement of brine penetration. The large size needle (.7 mm) increased NaCl concentration significantly

Table 10. Mean values[1] for NaCl concentration of green stock and PG activity of brine after vacuum[2] and piercing[3] treatments.

Piercing Variables			Vacuum			No Vacuum	
Size (Needle dia)	Depth	Freq.	I_o Salt (%)	24 Hr Salt (%)	PG[4] Activity	24 Hr Salt (%)	PG[4] Activity
		2 Sides	.14de	4.1^{b}	3.3^{b}	3.3^{jk}	6.4^{b}
	1/2 Rad	4 Sides	.24^{abc}	4.2^{ab}	4.8^{b}	3.4^{ij}	7.6^{b}
		8 Sides	.20^{bcde}	3.9^{de}	3.1^{b}	3.9^{de}	10.9^{ab}
Small .4 mm		2 Sides	.15^{cde}	3.4^{ij}	5.5^{b}	3.3^{jk}	6.6^{b}
	Radius	4 Sides	.14de	3.5^{hi}	21.9^{a}	3.8^{ef}	3.9^{b}
		8 Sides	.20^{bcde}	4.1^{bc}	3.0^{b}	4.0^{cd}	9.2^{b}
		2 Sides	.14de	2.9^{1}	3.3^{b}	3.2^{k}	4.5^{b}
	1/2 Rad	4 Sides	.28^{ab}	4.3^{a}	4.2^{b}	3.7^{fg}	3.5^{b}
		8 Sides	.23^{abcd}	3.9^{de}	5.0^{b}	3.3^{jk}	4.0^{b}
Large .7 mm		2 Sides	.24^{abc}	4.1^{bc}	5.2^{b}	3.6^{gh}	5.0^{b}
	Radius	4 Sides	.20^{bcde}	3.8^{ef}	4.3^{b}	3.4^{ij}	9.3^{b}
		8 Sides	.30^{a}	3.4^{ij}	5.4^{b}	3.3^{jk}	8.3^{b}
Control			.12^{e}	3.9^{de}	6.1^{b}	4.1^{bc}	3.2^{b}

[1] n=2, like letters within columns indicate no significant differences by LSD at P>.05.

[2] Vacuum = 25" Hg/10 min. in 40°S brine.

[3] Piercing = 2 sizes of needles (.4 mm and .7 mm dia.), 2 depths (7/16"=radius and 7/32"=1/2 radius) and 3 frequencies (2, 4 and 8 sides X 4 punctures per side).

[4] PG Activity = % loss in viscosity of pectin solution after 44 hr at 30°C.

Table 11. Analysis of variance of PG activity in brine and NaCl concentration immediately and twenty four hours following piercing and vacuum treatments of fresh cucumbers brined in 1 gallon jars with a 45°S cover brine.

Source of Variation	df	Mean Squares[1] NaCl at 0 Hr.	NaCl at 24 Hr.	PG Activity
Main Effects	5	.097***	.373	29.70
Vacuum	1	.466***	.656*	2.80
Size	1	.011*	.212	33.33
Depth	1	.000	.086	51.25
Frequency	2	.004	.455	30.54
Two-Way	9	.003	.135	24.40
Vacuum X Size	1	.011*	.000	6.75
Vacuum X Depth	1	.000	.089	28.83
Vacuum X Frequency	2	.004	.078	48.13
Size X Depth	1	.001	.027	.12
Size X Frequency	2	.001	.179	16.55
Depth X Frequency	2	.004	.292	27.27
Three-Way	7	.002	.396*	43.03
Vac X Size X Depth	1	.001	.566	100.92
Vac X Size X Freq	2	.001	.075	68.04
Vac X Depth X Freq	2	.004	.176	16.54
Size X Depth X Freq	2	.002	.853**	15.57
Four-Way Vac X Sixe X Depth X Freq	2	.002	.147	35.35
Explained	23	.023***	.267	32.18
Residual	24	.002	.152	34.25
Total	47	.012	.208	33.23

[1]Significant F tests indicated by *** ($p \leq .001$), ** ($p \leq .01$), * ($p \leq .05$).

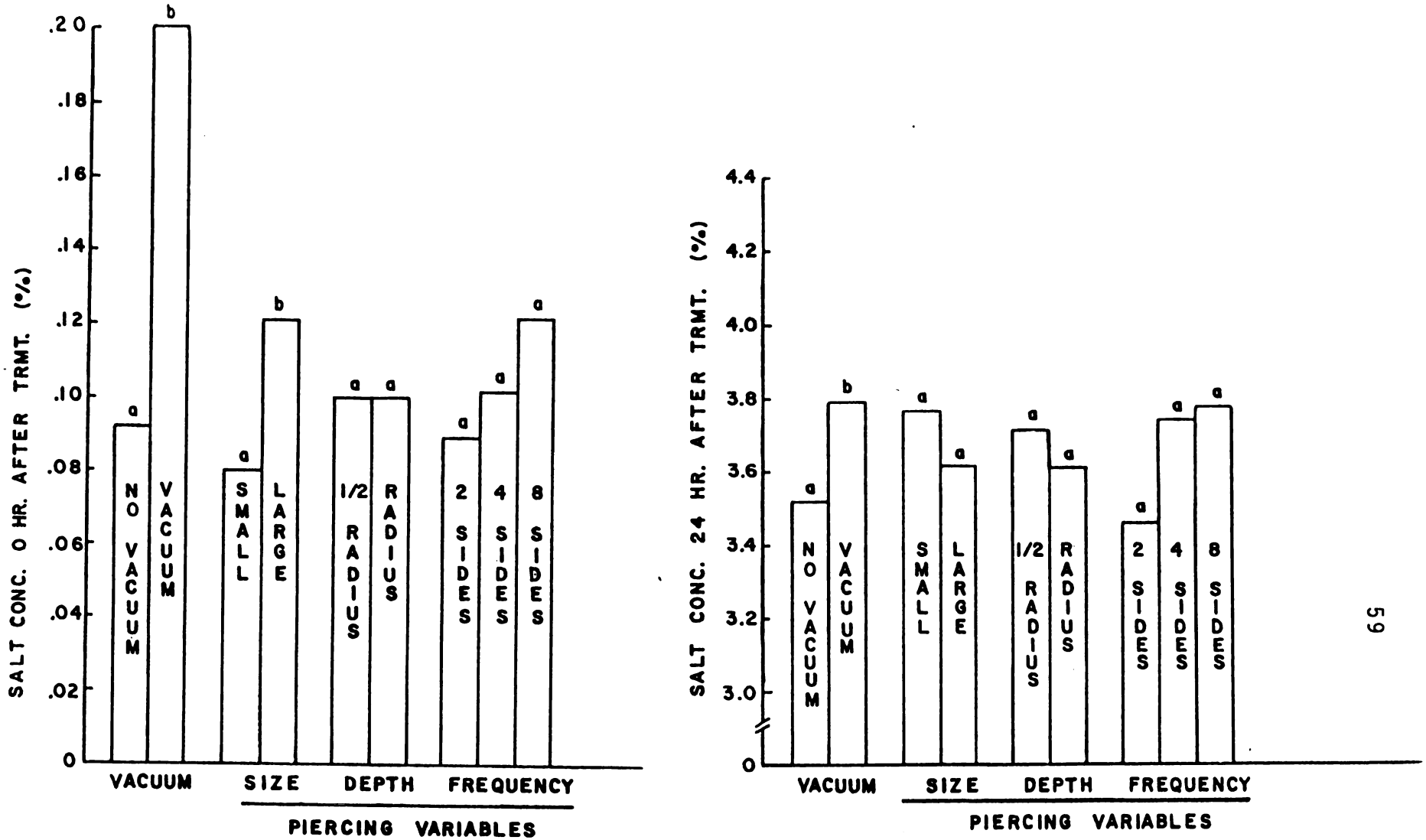

Figure 5. Main effects of vacuum and piercing treatments on the NaCl concentration of green-stock cucumbers. (n=26)

in comparison with the small needle (.4 mm dia.) (Figure 5). This is probably due to the resilience of the cucumber skin. The above findings indicate that the cucumber skin is the major barrier to brine penetration and that piercing of the green stock can speed brine penetration.

Subjecting cucumbers submerged in brine to a vacuum draws out gases entrapped within the cucumber tissues and creates a vacuum within the fruit itself. When the vacuum is released from the system, brine is drawn into the cucumber, replacing the lost gases. The development of cure color is reported to be dependent upon the depletion of gases from the cucumber tissues. This explains the rapid development of cure color in vacuum treated cucumbers. During the vacuum treatment, bubbles stream from the calyx. In pierced stock bubbles also stream from the piercing sites, indicating that the cucumber skin is also a barrier towards gas diffusion.

Five Gallon Pail, Vacuum X Pierce X Sorbate Fermentation Study

The five gallon pail fermentation study was initiated to determine the effect of increased initial brine penetration and increased gas diffusion potential from vacuum and piercing treatments on the overall quality of salt stock. An additional treatment which consisted of adding .1% sorbate to selected brines was utilized to evaluate the

interaction of this yeast and mold inhibitor with the vacuum and piercing treatments.

In this experiment the effects of vacuum treatment of green stock were dramatic. Most apparent was the rapid development of full cure color. The nearly instantaneous development of cure color is due to the removal of gases from the cucumber tissues as observed during vacuum treatment. Non-vacuum treated stock displayed incomplete development of cure color after six weeks in brine.

Also, in the absence of sorbate, vacuum treated stock had severe bloater damage. Since the addition of .1% sorbate to vacuum treated stock rectified this condition, the bloater damage of vacuum treated stock is assumed to be due to microbial action (Tables 12 and 13, Figure 6). It has been shown in the preliminary salt penetration study that vacuum treatment increased the penetration of cover brine. It is suspected by the author that microorganisms may be drawn into the cucumber along with the brine during the vacuum treatment. If so, the site of entry in non-pierced stock is most probably the calyx, the same site from which gases are drawn out of the cucumber during vacuum treatment. In addition, there are vascular tissues leading from the calyx to the carpel tissues. This is the most likely route to the carpel tissues for dissolved CO_2 or microorganisms and is likely where bloater formation begins.

Table 12. Mean values[1] for defects and texture of vacuum[2], pierced[3] and sorbate treated salt-stock.

		Defect Classification (%)					Texture
		No Damage	Honey-comb	Lens	Balloon	Soft Center	FPT (lbs.)
				No Sorbate			
No Vac	Fresh	23.3^{bc}	33.3^{a}	21.7^{a}	18.3^{c}	3.4^{ab}	24.0^{b}
	Pierce	26.7^{bc}	28.3^{a}	8.3^{ab}	33.3^{bc}	3.4^{ab}	23.2^{b}
Vacuum	Fresh	5.0^{c}	6.7^{b}	8.3^{ab}	71.7^{ab}	8.3^{ab}	20.5^{c}
	Pierce	6.7^{c}	3.3^{b}	13.3^{ab}	75^{a}	1.7^{b}	18.6^{c}
				.1% Sorbate			
No Vac	Fresh	83.3^{a}	11.7^{b}	1.7^{b}	3.3^{c}	0.0^{b}	23.5^{b}
	Pierce	70.0^{a}	11.7^{b}	8.3^{ab}	10.0^{c}	0.0^{b}	25.2^{ab}
Vacuum	Fresh	31.7^{bc}	5.0^{b}	10.0^{ab}	41.7^{abc}	11.6^{a}	24.9^{ab}
	Pierce	48.3^{ab}	8.3^{b}	21.7^{a}	15^{c}	6.7^{ab}	26.8^{a}

[1] n=60 (30 cucumbers/pail X 2 pails/treatment). Like letters within columns indicate no significant differences by LSD at P>.05.

[2] Vacuum = 25" Hg/10 min. in 40°S brine.

[3] Pierce = 1/2 radius depth, .4 mm dia. needle, 16 pierces/cucumber.

Table 13. Analysis of variance of visual defect classification and firmness of salt-stock following pierce, vacuum and sorbate treatments and a six week fermentation period.

Source of Variation	df	Mean Squares[1]					
		No Damage	Honey-comb	Lens	Balloon	Soft Center	FPT (lb)
Main Effects	3	3,500.63***	421.06	31.48	2.967.36**	50.69	20.20***
Pierce	1	17.36	6.25	25.00	.69	34.03	.49
Vacuum	1	3,117.36**	950.69**	44.44	4,784.03**	117.36	7.56
Sorbate	1	7,367.36***	306.25*	25.00	4,117.36**	.70*	52.56***
Two-Way	3	169.21	158.10	179.63	513.66	30.32	14.22**
Pierce X Vacuum	1	200.69	6.25	136.11	506.25	34.03	.04
Pierce X Sorbate	1	.69	34.03	177.78	367.36	.69	8.51*
Vacuum X Sorbate	1	306.25	434.03*	225.00	667.36	56.25	34.22***
Three-Way							
Pierce X Vac X Sorbate	1	250.69	.69	44.44	117.36	.69	.25
Explained	7	1,608.63**	248.31*	96.82	1,508.63*	34.82	14.79***
Residual	8	224.31	50.69	51.39	300.69	13.91	1.02
Total	15	870.32	142.91	72.59	864.40	23.29	7.45

[1]Significant F tests indicated by *** ($p \geq .001$), ** ($p \geq .01$), * ($p \geq .05$).

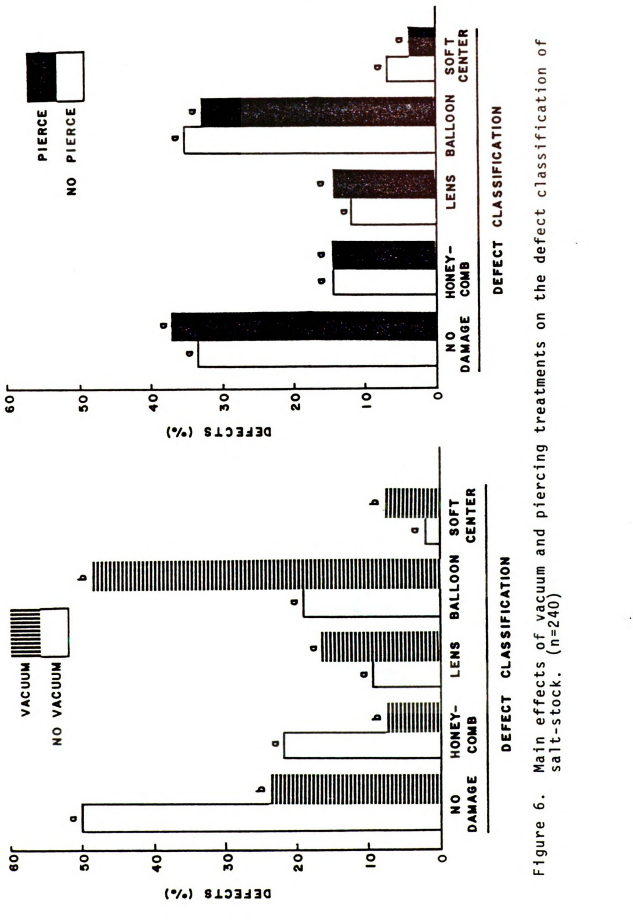

Figure 6. Main effects of vacuum and piercing treatments on the defect classification of salt-stock. (n=240)

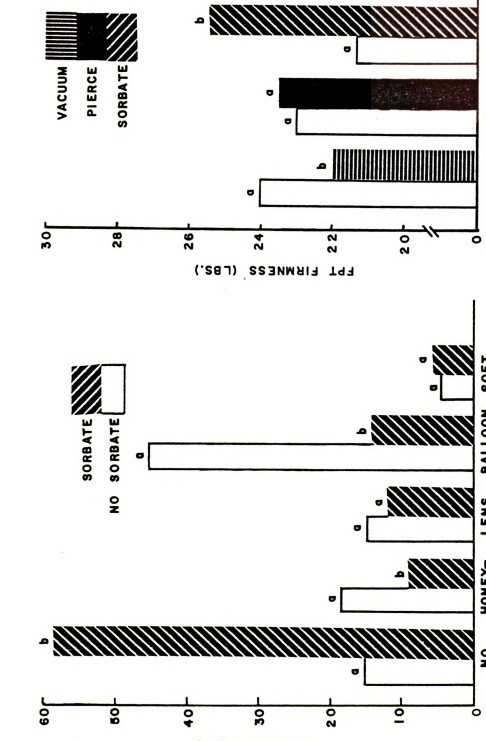

Figure 7. Main effects of sorbate treatment on the defect classification of salt-stock and the main effects of piercing, vacuum and sorbate treatments on the texture of salt-stock. (n=240)

The effect of piercing on the quality of salt stock in this experiment was not impressive. No significant differences were noted in terms of texture, defects or general appearance between pierced and nonpierced stock (Figure 6). This is attributed to the insufficiency of the piercing treatment used to increase brine penetration and particularly gas diffusion enough to affect the parameters measured in this study. A more severe piercing treatment that incorporates a larger size needle and a larger number of pierces per fruit may be necessary to decrease the incidence of salt-stock defects. The results from the preliminary salt penetration study indicate that needle size may be the most critical variable. As noted earlier, the resilient cucumber skin can close around the piercing site and negate the effects of the treatment if the diameter of the wound is too small.

The addition of sorbate to cover brines produced a higher percentage of nondamaged stock in all treatments (Figure 6). Texture was also enhanced by the addition of sorbate to cover brines (Figure 7). This is due to the selective antimicrobial action of sorbate which inhibits fungal growth and to a lesser extent bacterial growth. These results agree with earlier reports by Costilow (1957).

CONCLUSIONS

Chlorine Dioxide Studies

The effects of ClO_2, Cl_2 and Anthium Dioxcide® appear to be minimal in terms of reducing microbial populations of fresh clean cucumbers. It is assumed by the author that the natural flora of the fruits are sheltered from such treatments within the microscopic irregularities of the surface.

However, samples of cucumber wash water were shown to contain high numbers of undesirable organisms, up to 10^4 yeast and molds per ml. The water treatment compounds investigated herein proved to be effective in reducing the microbial populations of such samples. ClO_2 proved to be the most efficient of the three. Residual levels of 2 ppm ClO_2 were observed to practically eliminate yeast and mold populations from cucumber wash water. Initial concentrations of ClO_2 required to achieve a 2 ppm residual varied greatly according to the demand of the water samples. However, under conditions of both high and low demand, it was possible to achieve effective residuals of ClO_2 with approximately one-tenth the initial concentrations required with either Cl_2 or Anthium Dioxcide®.

It is assumed that if the large numbers of spoilage organisms in water that comes in contact with fresh cucumbers during washing, fluming or wet grading operations are reduced, then the total microbial load imposed upon the fresh fruit will have been reduced and the storage potential thereby maximized. In the laboratory, this was observed when lots of cucumbers were washed in water containing 10^4 yeasts and molds per ml versus lots washed in the same water containing residual ClO_2 and then stored.

Furthermore, the presence of ClO_2 in cucumber wash water was not observed to inhibit the natural fermentation process. Cucumbers treated with ClO_2 in concentrations as high as 100 ppm proceeded to ferment without inhibition.

These observations may indicate that ClO_2 may have some commercial application for the treatment of water that comes in contact with cucumbers. Such use should benefit the pickle packing industry by increasing the storage potential of fresh cucumbers and improving the sanitation of plant equipment.

However, due to the wide variation of ClO_2 demand in water samples observed in the laboratory, it is necessary to conduct further studies under commercial conditions to establish ClO_2 demand and optimal feed rates to maintain effective residuals of approximately 2 ppm. It is anticipated that the ClO_2 demand will vary for different operations on the commercial level, such as fluming and wet grading. Likewise, the improved storability of fresh fruit

treated with ClO_2 is dependent upon several variables such as temperature, humidity and the condition of the fruit prior to treatment. Again, further investigation under commercial situations is warranted.

Cucumber Piercing and Vacuum Studies

Vacuum treatment of green stock was shown to remove gases present in cucumber tissues and thereby produce a very rapid development of cure color. Vacuum treatment was also shown to increase the initial concentration of NaCl in the brined cucumbers. Gases were observed to be drawn out through the calyx and this is presumably the site at which brine is drawn back into the fruit following the release of the vacuum. Furthermore, a dramatic increase in salt-stock defects, particularly balloon bloaters, was noted in vacuum treated stock. Since the presence of sorbate in the brine reduced these defects, they were assumed to result from the action of microorganisms that were sensitive to inhibition by sorbate. This would be chiefly yeasts and molds. These data suggest to the author that microorganisms may be drawn into the fruit along with the brine following vacuum treatment.

Vacuum treatment appears to have very little potential for the control of salt stock defects. However, there is potential for the development of "instant cure color" for pickle products by the use of vacuum treatments. The

minimum amount of vacuum necessary to achieve this phenomenon would require further investigation. And to alleviate the development of severe defects in such products, it appears that an antimicrobial agent such as sorbate would have to be used in conjunction with vacuum treatments.

In the preliminary salt penetration study, piercing was shown to enhance brine penetration, but to a lesser extent than vacuum treatment. The diameter of the piercing implement proved to be the most important factor. Frequency of piercing was of less significance. And depth of piercing was totally nonsignificant. However, the piercing treatment applied in the five gallon pail fermentation study appeared to be insufficient to produce observable effects on the quality of the salt-stock in question. To accurately predict the potential for piercing to reduce the defects in salt-stock, further studies must be undertaken which utilize a more severe piercing treatment.

PG activity in brines was sporadic and did not correlate to the vacuum or piercing treatments.

The addition of sorbate increased the overall quality of the salt-stock for all treatments. This was attributed to the selective antimicrobial action of sorbate. The interaction between the sorbate and vacuum treatments suggested that the extensive bloater damage to vacuum treated stock, previously attributed to tissue stress, was of microbial origin.

APPENDICES

APPENDIX I

Preparation of ClO_2

ClO_2 was prepared according to the following formulation supplied courtesy of Olin Water Services, Kansas City, Ka.

I. Prepare these three solutions in 1 Liter volumetric flask:

a. 33.3 g of 5.0% sodium hypochlorite in 1 L of distilled, deionized water.

b. 16.1 g of 25% sodium chlorite in 1 L of distilled, deionized water.

c. 5.0 g of concentrated HCl in 1 L of distilled, deionized water.

II. Chill the above solutions to $34^{o}F$ and mix in the following manner. Working under a hood add 100 ml of the dilute HCl solution to a flask. Next add 100 ml of the sodium hypochlorite solution, followed by 100 ml of the sodium chlorite solution. This solution should contain from 350-500 ppm ClO_2 and is used to prepare dilute solutions of ClO_2 of the concentration desired.

APPENDIX II

Sodium Polypectate Preparation

Preparation of a 1.2% sodium polypectate in a sodium hydroxide-citric acid buffer solution at pH 5.0 is as follows. First, dissolve 1.33 gm of sodium hydroxide and 3.53 gm of citric acid in 1 L of distilled water. Heat this solution to 50^{o}C and slowly add 12 gm of sodium polypectate while stirring. Next, blend the suspension in a Waring Blendor for approximately 1 minute. Filter the blended solution through two thicknesses of cheese cloth and store refrigerated under toluene in a tightly stoppered bottle (Ball et al., 1955).

APPENDIX III

Microbiological Media (Lacto-Bacillus Selective Media (LBS))

	grams/liter
Trypticase*	10
Yeast Extract*	5
Potassium Phosphate, monobasic	6
Ammonium Citrate	2
Dextrose	20
Tween	1
Sodium Acetate Hydrate	25
Magnesium Sulfate	.575
Manganese Sulfate	.120
Ferrous Sulfate	.034
Agar*	15
Bromocresol Green	.005

Adjust pH to 5.6 with acetic acid and autoclave at 12 lbs. for 15 min. (Costilow et al., 1964).

Yeast Nitrogen Base Agar (YNBA)	grams/liter	
Yeast Nitrogen Base*	6.7	filter sterilize in
Dextrose	5.0	100 ml H_2O
Agar*	15.0	Autoclave in 900 ml H_2O

Mix both portions together and acidify with 10% sterile tartaric acid (10 ml/L), just before pouring plates.

Final pH should be 3.5 - 4.0 (Etchells et al., 1958a).

Plate Count Agar (PCA)	grams/liter
Tryptone*	5.0
Yeast Extract*	2.5
Glucose	1.0
Agar	15.0

Autoclave at 12 lbs. for 15 min. (Frazier et al., 1968).

*From Difco Laboratories, Inc., Detroit, Mi.

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