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Chapter 49

PICKLED VEGETABLES

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49.1 INTRODUCTION

The preservation of vegetables by pickling may be classified into three general methods: (1) salting or brining, (2) pasteurization, and (3) refrigeration. Organic acids and sodium chloride are primary preservatives for all types of pickles. Lactic acid is produced naturally in fermented products. Acetic acid in the form of vinegar is the usual acid added to pasteurized, unfermented, as well as finished, salt-stock pickles. Other preservatives such as sodium benzoate, potassium sorbate, and sulfur dioxide may be added to finished products.

Cucumbers, cabbage, olives, and peppers account for the largest volume of vegetables and fruits commercially pickled. Lesser quantities of onions, tomatoes, cauliflower, carrots, melon rinds, okra, artichokes, beans, and other produce are pickled also.

In salting or brining, the vegetables may or may not undergo a lactic acid fermentation, depending upon the concentration of salt used. Salt may be added in the dry form, as with cabbage, or as a brine solution, as with most other vegetables. The concentration of salt used varies widely among vegetables, depending upon tendency of the vegetable to soften during brine storage. Softening of brined cucumbers can be reduced or prevented by adjusting the level of salt to inhibit pectinolytic enzymes.[2,4] After removal from brine storage, the vegetables may be desalted if needed before being finished into various types of products such as dills, sweets, sours, hamburger dill chips, mixed vegetables, and relishes.[31] Finished salt-stock dill pickles contain a minimum of 0.6% acid, as lactic, according

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to USDA grade standards.[67] Extensive reviews are available on the brining and fermentation of cabbage,[59,64] cucumbers,[27,33] and olives.[32,69,70,71]

Pasteurized, fresh cucumbers and other vegetables (fresh-pack pickles) are heated to an internal temperature of 74 C and held for 15 minutes according to the original recommendations of Etchells and coworkers.[13,30,52] Although this heat process is still followed by some packers, the times and temperatures are varied by other packers today, depending upon product and risk factors. Fermented pickles such as whole genuine dills and hamburger dill chips may or may not be heated. If pasteurized, these products may be given a milder heat treatment than fresh-pack pickles, such as an internal product temperature of 71 C with no holding time. Fresh-pack pickles contain a minimum of 0.5% acetic acid according to USDA grade standards.[67]

Refrigerated pickles may or may not be fermented before refrigeration. Most commercially prepared and distributed refrigerated pickles sold today are not fermented but are acidified, and contain a preservative such as sodium benzoate. Some specialty products, however, are neither fermented nor acidified.

Fresh-pack pickles are considered acidified foods for regulatory purposes. According to the U.S. Food and Drug Administration, " 'Acidified foods' means low-acid foods to which acid(s) or acid food(s) are added; these foods include, but are not limited to beans, cucumbers, cabbage, artichokes, cauliflower, puddings, peppers, tropical fruits, and fish, singly or in any combination. They have a water activity (a_w) greater than 0.85 and have a finished equilibrium pH of 4.6 or below. These foods may be called, or may purport to be, 'pickles' or 'pickled—.' "[68]

49.2 NORMAL FLORA

Fresh produce contains a varied epiphytic microflora (Chapter 46). Pickling cucumbers were found to contain as high as 5.3×10^7 total aerobes, 1.9×10^4 aerobic spores, 9.8×10^5 total anaerobes, 5.4×10^2 anaerobic spores, 6.1×10^6 coliforms, 5.1×10^4 total acid formers, 4.6×10^3 molds, and 6.6×10^3 yeasts per gram of fresh, blended tissue.[18] The numbers increased during storage at higher temperatures (21 C) and humidity (>70% R. H.). Although some investigators have held the belief that the interior of sound, fresh cucumbers is sterile, others have found microorganisms, mostly gram-negative rods, within the healthy fruit.[49,63] In cucumbers, bacteria were more often near the skin and less often in the central core; in tomatoes, their frequency was highest near the stem-scar and central core, and decreased toward the skin.[63] Cabbage contains the greatest number of bacteria on the outer leaves, and lower numbers toward the center of the head.[59]

The floral changes during natural fermentation of brined vegetables may be characterized into four stages: initiation, primary fermentation, secondary fermentation, and post fermentation.[33] During initiation, the various gram-positive and gram-negative bacteria that were on the fresh vegetable compete for predominance. *Enterobacteriaceae*, aerobic spore formers, lactic acid bacteria, and other bacteria

may be active. Eventually the lactic acid bacteria gain predominance by lowering the pH, and the primary lactic fermentation occurs. During primary fermentation, five species of lactic acid bacteria are active, listed in approximate order of their occurrence: *Streptococcus faecalis*, *Leuconostoc mesenteroides*, *Pediococcus cerevisiae* (probably *P. pentosaceous* and/or *P. acidilactici* according to recent classification[7]), and *Lactobacillus plantarum*. Although all five species are active during fermentation of sauerkraut,[59] which contains relatively low concentrations of sodium chloride (ca. 2.25%), only the latter three species predominate in fermentation of cucumbers, which contain higher concentrations of sodium chloride (ca. 5 to 8%).[27] *Lactobacillus plantarum* characteristically terminates the lactic fermentation, apparently because of its greater acid tolerance.[58]

Green olives contain inhibitors of lactic acid bacteria[40,41,44] which are thought to influence fermentation of Spanish-type green olives.[23,45] Yeasts are not inhibited and predominate fermentation when the olives are neither properly lye treated nor heat-shocked before brining.[23]

Various species of fermentative yeasts also are active during primary fermentation. If fermentable sugars remain after primary fermentation, these sugars may give rise to secondary fermentation predominated essentially by yeasts. Fermentative yeasts grow as long as fermentable sugars are available; this may result in severe gaseous spoilage (bloater formation).[15,20,25] During post fermentation, growth of oxidative yeasts, molds, and bacteria may occur on brine surfaces of open tanks that are not exposed to sunlight.[16,55] Vegetable brining tanks are typically uncovered and are held outdoors to allow sunlight to reduce or prevent surface growth. No surface growth occurs in fermented and anaerobically stored green olives.[70] Attempts are being made to develop a suitable anaerobic tank for the cucumber brining industry.

Various attempts have been made toward the use of lactic starter cultures in sauerkraut, olives, cucumbers, and other products.[35] *Pediococcus cerevisiae* and *L. plantarum* have been used in pure culture or controlled fermentations of cucumbers[19,24] and olives.[23] Although the starter cultures have been used on a limited commercial scale for fermenting cucumbers over the past ten years, they have not received widespread application.

49.3 FLORA CHANGES IN SPOILAGE

49.31 Salt-Stock Vegetables and Genuine Dill Pickles

Vigorous activity in the cover brine by coliform bacteria, obligate halophiles, heterofermentative lactic acid bacteria, and fermentative species of yeasts is associated with gaseous fermentation and resulting bloater spoilage. Even homofermentative lactic acid bacteria such as *L. plantarum* and *P. cerevisiae* produce sufficient CO_2, when combined with CO_2 from cucumber tissue, to cause bloater formation in brined cucumbers.[38] Recent studies have shown that the major cause of CO_2 production by homofermentative lactic acid bacteria is a malolactic reaction,

with malic acid, which is normally present in pickling cucumbers, being degraded to lactic acid and CO_2.[48] Purging of fermenting cucumber brines with nitrogen has been shown to be effective in preventing bloater formation.[9,19,34,38] Purging is now widely used by the pickle industry. Air purging also is effective in preventing bloater formation,[9,34] but can result in cucumber softening due to mold growth,[10,34,42] reduced brine acidity due to yeast growth,[60] and off colors and flavors unless the purging regime is carefully controlled. Bloater formation has been attributed to growth of gas-forming microorganisms in the brine surrounding the cucumbers[22] or within the cucumber.[11,62]

Softening of brined vegetables is caused by pectinolytic enzymes of plant or microbial origin. Growth of film yeasts on brine surfaces may occur and result in loss of brine acidity. Accompanying mold growth on the brine surface can cause softening of sauerkraut, cucumbers, or olives. Heavy scum yeast and/or mold growth is usually the result of neglect of brined material during the curing and storage period. Softening of brined cucumbers may result from mold polygalacturonases that accompany the cucumbers, and especially cucumbers with flowers attached,[3,21] into the brine tank. This problem may be reduced by draining and rebrining of the tank ca. 36 hours after initial brining. Recycled brine may be treated to inactivate softening enzymes.[43,47]

49.32 Finished Pickle Products from Salt-Stock Vegetables (Not Pasteurized)

Fully cured, salt-stock vegetables are made into various types of finished pickle products by a series of operations involving leaching out most of the salt, souring with vinegar, and then sweetening with sugar. Preservation of these products is dependent upon sufficient amounts of vinegar alone (for sour pickles), or a combination of vinegar and sugar (for sweet pickles)[1]. If the amount of either ingredient is inadequate, fermentation usually takes place, principally by two groups of organisms, lactic acid forming bacteria and yeasts. Osmotolerant yeasts are the principal spoilage organisms in such products.[1] Molds and film yeasts may grow on the surface of the liquor chiefly as the result of faulty jar closure.

49.33 Pasteurized Pickle Products

Spoilage usually occurs in these products when they are improperly pasteurized and/or improperly acidified so that an equilibrated brine product of pH 3.8 to 4.0 is not achieved. Spoilage is due chiefly to acid forming bacteria, and to a lesser extent yeasts, that survive faulty heat treatment, or butyric acid bacteria when the product is not acidified adequately at the outset. Molds and film yeasts are factors in cases of poor jar closure.

49.34 Refrigerated Pickle Products

1. Fermented

These products are known as overnight dills, half-sour dills, genuine kosher dills, kosher new dills, sour garlic pickles, half-sour new pickles, fresh packed half-sour pickles, new half-sours, home-style new pickles, half-sour kosher new dills, and the like. The cover brine may or may not be acidified. The products are held in barrels for a few days or longer at room temperature and then refrigerated at 2 to 5 C. They may be distributed in bulk or consumer-size, glass containers. In some cases, they may be initially brined, held, and distributed in consumer-size containers. Under such conditions and at equilibrated brine strengths of 10 to 12 salometer (1 salometer = 0.264% salt by weight), microbial growth (chiefly coliforms, gas-forming and non-gas-forming lactics, and fermentative yeasts), and enzymatic activity (pectinolytic and cellulolytic), together with the curing process continues at a slow rate.[22]

Gaseous spoilage of this product is caused chiefly by the gas-forming microbial groups mentioned earlier. Gas production may be sufficient to reach 15 pounds pressure on the cap.

Softening problems may be even greater than for salt-stock cucumbers since these products are held at much lower concentrations of salt than are salt-stock cucumbers. Fresh, whole garlic cloves and other spices are normally added to such products. These spices may contain high levels of softening enzyme activity, which increases softening problems with these products. In a few months, the stored pickles may have lost much of their desired characteristic flavor, texture, and color and also may be bloated because of gaseous fermentation by the principal gas-forming microbial groups present.

Whether these pickles are made in bulk or in the retail jar, the fact remains that the very nature of the product makes it difficult to maintain good quality pickles for any reasonable length of time. The barreled product reaches the GMP recommended brine pH of 4.6 or below for acidified foods usually before refrigeration or shortly thereafter, and then slowly continues acid development. This recommended condition for brine product pH cannot be assured for the product made in the retail jar because there is no accepted uniform process by the packers wherein the product is acidified at the outset or where it is deliberately incubated for development of natural lactic acid fermentation.

2. Not Fermented

Most of these products for national distribution are acidified with vinegar to an equilibrium pH well below 4.6, contain 2 to 3% NaCl and are immediately refrigerated upon packing. They may contain sodium benzoate or other preservatives. Like the fermented, refrigerated product, the cucumbers are not heated either before or after packing. If properly acidified, refrigerated and preserved, these products will maintain acceptable quality for several months and do not present a public health concern. Recipes that do not contain vinegar or other acid in the initial cover liquor, however, should be viewed with great caution.

49.4 HUMAN DISEASE BACTERIA

There are no authenticated reports, to our knowledge, of human disease bacteria associated with standard, commercial pickle products prepared under "good manufacturing practices" of acid, salt, and sugar content (and combinations thereof) from brined, salted, and pickled vegetable brine-stock, including cucumbers. The Commissioner of the Food and Drug Administration stated that "No instances of illness as the result of contamination of commercially processed fermented foods with *Clostridium botulinum* have been reported in the United States."[68] Even so, certain types of microorganisms that may cause spoilage of the product may, at times, be encountered, such as molds, yeasts, and acid tolerant lactic acid bacteria. These organisms, usually under conditions associated with neglect, may reduce the quality (texture and/or flavor) of the product (prepared in bulk or retail container) and render it unusable. However, these organisms are not considered human pathogens.

Essentially the same pattern of consumer safety applies to fresh-pack (pasteurized) pickle products. These have continued to increase in popularity until these items now use over 40% of the annual cucumber crop in the USA. These pickles usually are prepared from raw cucumbers, but may include other vegetables in a mixture; also, vegetables other than cucumbers may be packed, such as various types of peppers, okra, carrots, green beans, and green cherry, pear shaped, or regular globe tomatoes. The process calls for the packed product to be acidified at the outset with a sufficient amount of food-grade organic acid (vinegar, acetic acid, or lactic acid) to result in an equilibrated brine product pH of 4.0 or below (preferably 3.8). Vinegar (10 to 20% strength) is usually the acidulant of choice of industry for cucumber pickle products. The basic pasteurization procedure has been used successfully by industry since ca. 1940.[13,28,30]

As far as fresh-pack pickle products are concerned, changes in formulation, calling for specifically reduced acidification, or lowering the salt content, or both of these, is probably the most significant and dangerous set of factors to tamper with (assuming that an adequate pasteurization procedure is used). For instance, arbitrarily reducing the vinegar (acid) and salt content of the cover brine of a given product to achieve some abnormally mild flavoring to appeal to some segment of the consuming public may inadvertently lead to a butyric acid-type spoilage problem involving the public health aspect of the product.

49.5 RECOMMENDED METHODS

49.51 Collection and Storage of Brine Samples

Brine or pickle liquor covering the vegetable material is required for examination. The size of container to be sampled may range from a small jar of pickles to a 1,000 bushel tank of fermented brine stock. Brine samples from containers, such as tanks and barrels, should be taken for bacteriological analysis as follows:

A suitable length of 3/16 inch stainless steel tubing, sealed at one end with lead or solder and perforated with several 1/16 inch holes for a distance of 6 to 8 inches from the sealed end, is inserted through an opening between the wooden boards comprising the false head down into the brine toward the mid-depth of the vegetable material. Withdraw brine through a sanitized, attached piece of rubber tubing into a 12 oz bottle. Fit the receiving bottle with a two-hole, rubber stopper and two short lengths of glass tubing, one for the rubber tubing leading from the stainless steel sampling tube, and the other for a suction bulb to start siphoning action. The length of the steel sampling tube is governed by the depth of the container to be sampled.

Withdraw and discard approximately 24 oz of brine before taking the final sample, about 10 ml, into a sterile test tube. Sterile vacuum tubes with rubber stoppers are suitable. If microbial changes during the fermentation are to be followed, start sampling at the time the material is salted or brined, and continue at regular intervals of 1 to 2 days during active fermentation. After sampling, wash the whole assembly thoroughly.

For tightly headed barrels such as those used for genuine dills and salted vegetables for nonpickle use, take the sample through the top or side bung.

For smaller containers, such as jars or cans of pickle products, shake thoroughly and take the sample from the center of the material by means of a sterile pipette. Wash the tops of the metal cans with alcohol, flame, and puncture. A beer can opener is useful for puncturing metal tops. If the containers show evidence of gas pressure, carefully release gas by puncturing the sanitized top with a flamed ice pick. Containers under heavy gas pressure may be refrigerated overnight to reduce the gas pressure prior to sampling.

Brine samples from actively fermented material should be examined as promptly as possible after collection to prevent changes in the microbial flora. The same is true for samples of packaged pickle products. If it is necessary to ship or store samples, this should be done under the best of refrigerated conditions; the elapsed time from collection to examination should not exceed 12 to 24 hours. When shipment by air is required, samples are collected in sterile, 16 × 150 mm tubes and fitted with plastic screw caps having rubber liners. Pulp and oil liners, or plastic liners such as teflon, may leak due to changes in air pressure.

Brine samples may be preserved for subsequent chemical determinations by the addition of toluene or Merthiolate, 1% aqueous solution, one to two drops per 10 ml of sample. Samples preserved with the above chemicals are unfit for human consumption and should be so marked.

49.52 Microscopic Examination

Microscopic examination of brine samples for bacteria and yeasts is helpful at times, particularly when carried out in conjunction with plate count observations.

1. Bacteria

Make direct counts for bacteria according to the following procedures:

Place 0.01 ml amounts of brine or liquor on slides, by using a Breed pipette,[6] and spread evenly over a 1 square cm area; fix with heat.

Stain according to the Kopeloff and Cohen modification of the Gram stain.[46] Count according to the Wang[72] modification of the Breed[6] technic.

Report results as "numbers of different morphological types of gram-positive and gram-negative bacterial cells per ml of brine."

2. Yeasts

Use the microscopic technic for determining yeast populations in fermenting vegetable brines, and various types of finished pickle products undergoing gaseous spoilage by the organisms, particularly where populations are in excess of 10^4 cells/ml of sample, and where yeast colonies are not required for isolation and study. The use of a vital stain permits differentiation of yeast population into viable and nonviable cells, and increases the usefulness of the direct counting technic.

The counting procedure is essentially the method of Mills[51] as modified by Bell and Etchells[1] for counting yeasts in high salt content brines and in high sugar content liquors:

Add 1 ml of brine or pickle liquor sample to 1 ml of 1:5,000 (0.02%) erythrosin stain.

Shake the sample stain mixture to obtain an even suspension.

Using a 3 mm diameter platinum loop, transfer enough of the mixture to the area under the cover glass of an improved Neubauer double-ruled hemacytometer to fill the chamber in one operation.

Allow cells to settle for approximately 5 minutes and count the yeast cells, using a microscope equipped with a 4 mm objective and 15× oculars.

Record cells stained pink as "dead yeast cells" and unstained cells as "live yeast cells."

The number of yeast cells per ml of brine or pickle liquor may be calculated thus:

$$\frac{\text{Number of yeast cells counted} \times \text{dilutions} \times 250{,}000}{\text{Number of large squares counted}} = \text{Numbers per ml}$$

If only one side of the hemacytometer counting chamber is used (25 large squares), the lowest yeast count obtainable is 20,000 per ml, while if both sides are counted (50 large squares), a population as low as 10,000 per ml can be counted.

Report yeast count as "total yeast cells," "live yeast cells," and "dead yeast cells per ml of sample."

49.53 Titratable Acidity and pH

Determine titratable acidity of a 10 ml sample of the brine or liquor by diluting the sample with 30 to 50 ml of distilled water and titrate with 0.1 N NaOH, using

phenolphthalein as the indicator. Alternatively, samples may be titrated to pH 8.2 with a pH meter. Report values for brined samples as grams of lactic acid per 100 ml of sample and for finished liquor samples as grams of acetic acid per 100 ml of sample.

For a 10 ml sample use the following calculations:

a. ml of 0.1 N alkali used × 0.090 = g of lactic acid per 100 ml
b. ml of 0.1 N alkali used × 0.060 = g of acetic per 100 ml.

When only a small amount of the original sample is available, use a 2 ml amount for titration purposes. Such small samples are not recommended. For the 2 ml sample, multiply the ml of 0.1 N alkali by 5, then by the above number for lactic or acetic acid.

Carry out pH determinations of the samples with a pH meter, checking the instrument frequently with a standard buffer in the pH range of the sample under test.

49.54 Determination of Salt Content of Brine

It is often helpful to know the approximate salt content in performing microbiological examinations of brines. Use a salometer, and test about 200 ml of brine. A chemical test for salt is required for small amounts of sample, or when a higher degree of accuracy is desired than that obtainable by the salometer.

The following method is recommended. Transfer 1 ml of sample to a flask and dilute with 15 to 20 ml of distilled water. Titrate with 0.171 N silver nitrate solution, 29.063 g per liter, using 3 to 5 drops of 0.5% dichlorofluorescein as the indicator. Agitate to keep the precipitate broken up until a light salmon pink color is developed. Report as "g of sodium chloride per 100 ml of the sample."

When 1 ml of sample is titrated, each ml of silver nitrate solution is equal to 1 g of sodium chloride per 100 ml.

49.55 Determination of Reducing Sugars

It is important that all fermentable sugars be converted to acid and other end products during brine storage of many vegetables, in order to preclude subsequent yeast growth during further storage of the brine stock or the finished product. With vegetables that contain primarily glucose and fructose, reducing sugar determination can give an indication as to when fermentation is completed.

The dinitrosalicyclic acid (DNS) reducing sugar determination described by Summer and Sisler[67] has been found to be suitable for analysis of cucumber brines. Brine components do not cause serious interference. The reagent is stable at room temperature for many months. Finally, the assay can be reliably performed with an inexpensive colorimeter or spectrophotometer. The standard curve does not go through zero due to some sugar destruction.[51] Samples must be read within the linear portion of the standard curve. A cucumber fermentation can be considered

complete if the brine contains less than 0.05% reducing sugar and there is no increase in acid for several days.

49.56 Determination of Softening Enzyme Activity

Softening enzymes in brines of fermenting cucumbers and other vegetables may be determined by the highly sensitive viscometric method of Bell et al.[5] The procedure, which has been widely used in the pickle industry for many years, is based on viscosity loss of a buffered polypectate solution. Brine samples, 25 ml, are dialyzed in running water for 3 hours and distilled water for 1 hour. One ml of the dialyzed sample is added to 5 ml of 1.2% sodium polypectate (Raltech Scientific Services, P. O. Box 7545, Madison, WI), which is dissolved in 0.018 M, pH 5.0 citrate buffer in an Ostwald-Fenske no. 300 viscometer. A drop of toluene is added to the sample to prevent growth during incubation. The flow time of the pectate solution is measured after sample addition and at 20 hr. The viscosity loss is calculated according to the following equation:

$$\text{Percent loss in viscosity} = \frac{A - B}{A - W} \times 100,$$

where A is the initial flow time in seconds, B is the flow time at 20 hours and W is the flow time for water. A table is provided to relate loss in viscosity to the units of pectate depolymerizing activity. Less than a 9% loss of viscosity in 20 hours is considered to represent weak to negative activity in brine samples.

Refer to Chapter 15 for isolation of pectinolytic organisms and characterization of pectinolytic enzymes.

49.57 Determination of Dissolved Carbon Dioxide

The advent of purging to remove CO_2 from fermenting cucumber brines and thus prevent bloater formation has given need for the determination of the concentration of dissolved CO_2 in the brine. For highly accurate determinations as may be required for research purposes, dissolved CO_2 is determined by the micro distillation procedure of Fleming et al.[37] A 10 ml brine sample is injected by syringe into a capped jar and into an acid solution. A small vial of standardized NaOH placed inside the jar traps the CO_2 as it distills from the acidified solution. After 24 hours at 37 C, the vial is removed, $BaCl_2$ is added, and the remaining base is titrated to the phenolphthalein end point with HCl. Values are expressed as mg CO_2/100 ml brine.

For quick estimates which may be required for quality control tank monitoring, dissolved CO_2 is determined with a Harleco CO_2 apparatus (Philadelphia, PA). Adaptation of this instrument for the determination of CO_2 in fermenting cucumber brines has been described.[36] This is a gasometric method based on the classical Van Slyke procedure. A 1 ml brine sample is placed in the instrument vial, a volumetric syringe is clamped into place, an acid solution is added, the apparatus and sample vial are shaken, and the gas volume displacement is read on the calibrated syringe scale. Carbon dioxide in the brine sample is calculated from scale readings of the brine compared to a CO_2 solution of known concentration and is expressed as mg CO_2/100 ml brine. It is suggested that brine samples be taken from brine tanks through a siphon tube (see section 49.51) and 8.5 ml syringed through a needle into a Vacutainer tube (10 ml draw, Becton-Dickinson, containing 0.5 ml of ca. 3N NaOH) to minimize CO_2 loss. The samples are then equilibrated to the same temperature as the known solution before analysis.

In both methods, the total CO_2 content of the solution is determined and is expressed as mg CO_2/100 ml brine, or as percent saturation.[39]

49.58 Microbiological Analyses

1. Aerobic plate count

   Use plate count agar or nutrient agar (Chapter 58) and incubate for 3 days at 32 C. Overlay the solidified plated samples with about 8 to 10 ml of the same medium to prevent or minimize spreaders.

2. Lactic acid-forming bacteria

   Lactic acid bacteria associated with pickled vegetables of the genera *Lactobacillus* and *Pediococcus* are selectively enumerated with *Lactobacillus* selection medium (LBS) (Chapter 58), appropriately modified. Overlay the plated samples with the same medium to permit earlier enumeration of colonies. Incubate at 32 C for about 4 days or until suitable colony enlargement. The incubator should be humidified to retard desiccation of the medium during incubation. Fructose, 1%, may be added to the medium to ensure greater enumeration of certain lactobacilli.[65] Bromcresol green (or brilliant green as in Chapter 58), 0.0075%, may be added to aid in colony counting, but may further retard growth of lactic acid bacteria in an already inhibitory medium. Cycloheximide, 200 ppm, should be added as needed to inhibit yeasts.

   Total lactic acid bacteria may be estimated by plating samples in MRS agar (Chapter 58) containing 0.02% sodium azide and incubating for 1 to 4 days at 30 C.[12]

   To differentially enumerate all species of lactic acid bacteria associated with vegetable fermentations, plate fermenting samples in noninhibitory medium such as tryptone-glucose-yeast extract agar.[57] After incubation at 32 C for 48 hours, isolate colonies for later identification on the basis of acid and gas production, cell morphology, and mucoid growth[56]; other chosen reactions may be used.

3. Salt-tolerant cocci

   Use nutritive caseinate agar, plus 0.1 glucose (Chapter 58) and incubate for 3 days at 32 C. Count colonies that are grayish white, entire, glistening and of moderate size, and similar colonies that are light orange to yellow in color. Subsurface colonies are lenticular to elliptical in shape. For morpho-

logical identification when lactose fermenting yeasts may be present, make stained preparations and examine under the microscope.

4. Total Enterobacteriaceae

Add 1% glucose to violet red bile agar (Chapter 58), which is referred to as MacConkey glucose agar.[53] Incubate for 24 to 48 hours at 32 C.

5. Coliform bacteria

Use violet red bile agar, incubate plates at 32 or 35 C for 24 hours and count all purplish red colonies surrounded by a reddish zone of precipitated bile, 0.5 mm in diameter or larger as in Chapter 25.

6. Yeasts and molds

Acidify sterile and tempered, 45 C, dextrose agar with 5% by volume sterile 10% tartaric acid; final pH 3.5 ± 0.1. Potato dextrose or malt agar (Chapter 58) may be used when acidified to pH 3.5 as above. Incubate for 3 to 5 days at 30 C.

Mold colonies are distinguished readily from yeasts on acidified dextrose agar, whereas, differentiation of subsurface yeasts and film yeasts presents more difficulty. Surface colonies of the common film forming yeasts associated with pickle products and vegetable brines, i.e., species of *Debaryomyces, Endomycopsis, Saccharomyces, Candida,* and *Pichia*,[16,54] are generally dull and very rough as contrasted to the usual round, raised, white, glistening colonies of the fermentative, subsurface yeasts, i.e., species of *Torulopsis, Brettanomyces, Hansenula, Saccharomyces,* and *Torulaspora*.[20,25,29] However, even when distinguishing colony characteristics of the two yeast groups exist, they are not considered sufficiently clear cut for separation. Because of this, the procedure outlined under 49.587 should be used. Film yeasts rapidly form a heavy wrinkled surface film at one or both salt concentrations. Certain species, such as *Saccharomyces halomembranis,* form heavier films at 10% salt than at 5%.[14,16,26,54]

Use yeast nitrogen base agar plates for estimation by surface streaking.

Alternatively, use antibiotic media (Chapter 17) for enumeration when problems with inability of some yeasts and molds to grow, or bacterial growth, are suspected.

7. Film yeasts

For an estimate, pick representative filamentous colonies from the yeast plates into tubes of dextrose broth containing 5 and 10% salt. Incubate 3 to 5 days at 32 C, and observe for heavy surface film. Two salt concentrations are suggested for use because some species develop heavier films at the lower salt strength (5%) whereas, with other species, the reverse is true.

8. Obligate halophiles

Use tubes of liver broth plus salt (Chapter 58). Prepare decimal dilutions, seal with sterilized, melted petroleum jelly, and incubate 7 days at 32 C.

Record positive tubes daily by noting the raising of the petroleum seal due to gas production and the absence of any distinctive odor.

This medium has proved satisfactory for detecting obligate halophiles sometimes found in brined and dry salted vegetables. The salt content of the medium should approximate that of the sample. No interference has been encountered by growth of coliforms or yeasts in this medium. This is probably due to the inability of either group to initiate satisfactory early growth in laboratory media even at moderately high salt concentrations in competition with the very fast growing obligate halophiles.

9. Butyric acid-forming bacteria

Neutralize the brine sample with an excess of sterile calcium carbonate. Heat a 50 to 100 ml sample in a water bath for 20 minutes at 80 C to kill vegetative cells. Prepare decimal dilutions and inoculate previously heated and cooled tubes of liver broth medium. Seal with melted petroleum jelly and incubate 7 days at 32 C. Examine tubes daily for production of gas and a strong butyric acid odor.

49.6 INTERPRETATION OF DATA

49.61 Salt-Stock Vegetables and Genuine Dill Pickles

Proper record keeping of salting procedures and chemical and microbiological data can greatly aid the commercial briner in assessing causes for success or failure in preserving the quality of brined vegetables. Records of chemical determinations of salt, titratable acidity, pH, fermentable sugars, dissolved CO_2, and softening enzyme activity are very useful in such assessments, depending upon the particular commodity.

In fermented vegetables it is important that the lactic acid fermentation become established early to preclude growth by spoilage bacteria. Acidity and pH data provide this information. Salt concentrations above 8% for cucumbers and olives or above 2.5% for cabbage may prevent or retard a desirable lactic fermentation. Unusually low salt concentrations may result in softening of the brined vegetables.

If the dissolved CO_2 concentration in the brine of fermenting cucumbers is allowed to exceed about 50% saturation (equals 54 mg/100 ml at 21 C and 6.6% NaCl) at any time during brine storage, bloater damage may result. Maintaining the brine CO_2 concentration below 50% saturation will greatly aid in reducing bloater damage.[39] Sporadic bloater damage may occur even in effectively purged brine-stock cucumbers. Such damage may be due to growth of bacteria within the brined fruit.[11] Since brines must be purged as long as fermentation occurs, it is important to monitor the level of fermentable sugars in the brine. When fermentable sugars are not detected and acid development has ceased, the fermentation is considered to be complete and purging can be safely discontinued.

Microbial softening enzyme activity of brines may indicate the cause of soft brine-stock pickles, especially if the cucumbers are held at relatively low brine

strengths, 5 to 8% NaCl. Higher salt concentrations will prevent softening by these enzymes,[2] but high salt levels present disposal problems in addition to adversely affecting the lactic fermentation. Recent studies have indicated that calcium chloride, ca. 0.2 to 0.4%, and other salts of calcium may inhibit the action of softening enzymes.[8] Calcium chloride is now being added to commercial cucumber brines. The extent of protection against softening offered by calcium has not been fully assessed.

The absence of softening enzyme activity in older brine-stock pickles does not necessarily mean that such activity was not the cause of the softening. Softening enzymes that accompany the cucumbers and attached flowers into the brine tank may exert their influence early in brine storage and then be dissipated or inactivated so as not to be detectable later.

Softening in the seed area of large cucumbers, commonly termed "soft centers," is thought to be due to natural polygalacturonase of overly mature cucumbers,[61] and not to microorganisms.

49.62 Finished Pickle Products from Salt-Stock Vegetables

These products normally contain a few thousand microorganisms per ml. These counts may be composed chiefly of spores of aerobic bacteria that remain inactive in the acid medium, and tend to decrease during storage. Fermentative yeasts and lactic acid bacteria may cause vigorous gas production, which causes the pickle liquor to become highly charged with gas and to possess a tang when tasted. Viable microorganisms, normally latent in properly fermented and preserved products, may cause gaseous spoilage in improperly finished products. Gaseous spoilage and cloudy cover liquor may result in hamburger dill chips, genuine dill pickles, Spanish-style green olives, and similar products if residual sugar remains.

49.63 Pasteurized Pickle Products

Properly acidified, packaged, and pasteurized pickle products are not subject to microbial spoilage. When spoilage occurs, it is usually due to underpasteurization. Some commercial packers minimize heat processing in order to maintain greater product quality. Minimal processing is done at the risk of spoilage. Spoilage results in recall of the product by the packer at his expense. No public health problem exists in pasteurized pickle products that have been properly acidified. After spoilage occurs, however, as evidenced by gas pressure and brine turbidity, there is no way to insure that the product was properly acidified initially. Lactic acid bacteria are normally found in such products. The spoiled product usually contains acid. But, it is not known at that point if the original product, particularly if fresh produce, was sufficiently acidified to prevent growth of *Clostridium* before growth of the lactic acid bacteria.

Improper acidification can also be a source of spoilage with potential public health significance as discussed in section 49.4. Improper closure can result in growth of aerobic microorganisms on the surface of the brine, and a reduction in acidity.

49.64 Refrigerated Pickle Products

Fermented, refrigerated products are considered a specialty item by the pickle industry. These products are highly perishable and variable in quality. In contrast, the acidified, unfermented, refrigerated pickles that have become so popular in recent years are more predictable as to shelf life and more uniform in quality.

49.65 Salted and Brined Vegetables for Nonpickle Use

In these products gaseous fermentation usually is associated with active development of coliform bacteria, yeasts, and obligate halophiles, all of which can tolerate the high salt concentrations, 15% and above, normally employed for preservation. One or more of the above groups may be present. Gas pressure may be sufficient to burst the barrels. The flavor and appearance of the material also may be altered by growth of the above groups.

Numbers of salt tolerant cocci may be found over an extended period in brines, particularly in those containing no appreciable amount of developed acidity. These organisms are extremely salt tolerant, but not acid tolerant. Their fermentation is not gaseous in nature, and no outstanding change in the product has been attributed to their presence, although small amounts of brine acidity may be produced under conditions providing reduced oxygen tension. When numerous colonies showing a decided acid reaction are found on the plates, they should be examined carefully; it is likely that they will not be acid producing bacteria of the lactic acid group, as might first be suspected, but rather acid producing cocci. This is particularly true in cases where the brine concentration is above 15% salt.

Growth of molds and film yeasts is likely to be a factor when there is air above the brine surface in the container. Casks should be kept filled with brine at all times, irrespective of storage temperature. Unrestricted growth by molds may soften the texture of vegetable material so it is unusable. Heavy scum growth is undesirable, principally from the flavor standpoint and the reduction of brine acidity. This may lead to spoilage by salt-tolerant organisms that are not acid-tolerant.

As mentioned above, refrigerated storage at about 1.7 C of these brined and salted products is preferred. Under such conditions, and at salt concentrations of 5% and above, microbial activity of the various groups may be restricted greatly. However, at salt concentrations of approximately 10% strength, the cocci may grow rapidly at about 1.7 C.

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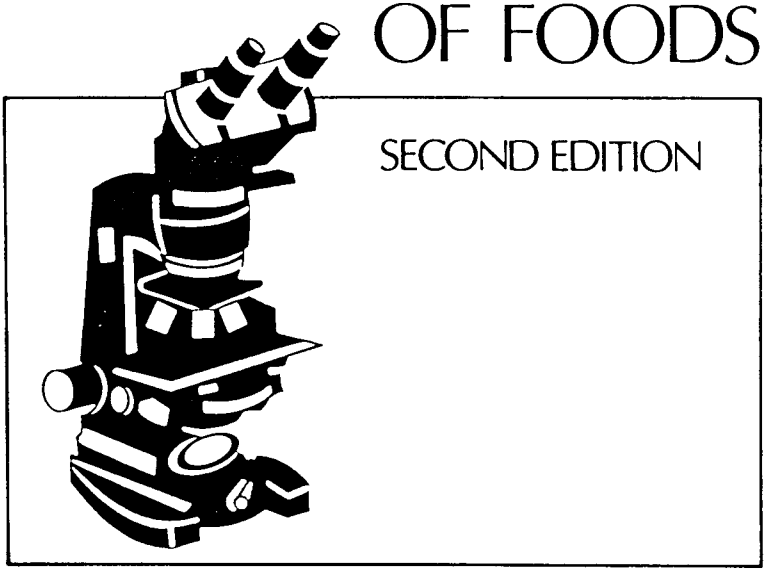
COMPENDIUM OF METHODS FOR THE MICROBIOLOGICAL EXAMINATION OF FOODS



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