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CHAPTER 47

PICKLE PRODUCTS*

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47.1 INTRODUCTION

Brined, salted, and pickled vegetables are classified into the following groups:

47.11 Cucumber Pickles and Similar Pickle Products

47.111 Salt stock for cured pickle products

a. Cucumbers (and onions, peppers, tomatoes, cauliflower, carrots, cabbage, melon rinds, etc.)
b. Genuine dill pickles (from cucumbers or tomatoes)
c. Green olives (Spanish type), whole or stuffed
d. Naturally ripe (black) olives

47.112 Finished pickle products from brine cured stock

a. Sweets
b. Sours
c. Mixed
d. Relishes
e. Processed dills
f. Hamburger slices

47.113 Types of pasteurized pickles (not brine cured)

a. Dills (sliced or whole)
b. Sweets (sliced or whole)
c. Relishes (mixed vegetables)
d. Vegetables other than cucumbers (onions, peppers, green tomatoes, okra, carrots, etc.)
e. Green olives, not fully fermented

47.114 Overnight dill pickles (refrigerated)

a. Cucumbers (primarily)
b. Green tomatoes

*The methods described herein are based in part on procedures referred to in reference 26.

IS/A Committee Liaison: John H. Silliker

47.12 Brined and Salted Vegetables for Nonpickle Use

47.121 Brined

a. Okra (whole)
b. Celery (cut)
c. Sweet pepper hulls
d. Citron (peel)

47.122 Dry salted

a. Corn
b. Lima beans
c. Peas
d. Green snap beans
e. Okra (cut)
f. Celery (cut)

47.2 NORMAL FLORA

The normal microflora that usually predominates during the natural fermentation of brined cucumbers, other vegetables, and green olives is similar, particularly, if the fermentation takes place under suitable and comparable conditions of temperature, salt content, percentage of solids to brine (by weight). The lactic acid bacteria usually reach the ascendancy, but they may be preceded by growth of the coliform bacteria, and followed by fermentative yeast development, depending on the available brine nutrients. From the briner's standpoint, the desired brine microflora is established early and continued predominance by the homofermentative lactic acid bacterial species—initiated by *Pediococcus cerevisiae* followed by *Lactobacillus plantarum*. Gas forming (heterofermentative) lactics such as *L. brevis* and *Leuconostoc mesenteroides* may also be present in certain vegetable brines. The extent of their growth is highly dependent on brining conditions, essentially those described above. For example, Pederson et al.[37] reported good acid formation by *L. mesenteroides* in ten days in kraut at about 50 F and 2 to 2.5% salt by weight. In contrast, we have been unable to initiate growth of *L. brevis* in brined olives of the Manzanillo variety. However, neither species is expected to predominate in brine strengths employed for commercially brined cucumbers destined for brine stock purposes. Pure culture fermentation of cucumbers, olives and other vegetables has recently been proposed by Etchells et al.[14, 17, 18]

47.3 FLORA CHANGES IN SPOILAGE

47.31 Salt Stock Vegetables and Genuine Dills

Vigorous activity in the cover brine by coliform bacteria, obligate halophiles, heterofermentative lactic acid bacteria, and fermentative species of yeast is associated with gaseous fermentation. This may bring about a physical "spoilage" condition in salt stock cucumbers and dill pickles known as "bloaters" or hollow cucumbers. Although most of these groups of organisms are extremely salt tolerant, the coliform bacteria and halophiles

not found usually in brines having appreciable acidity. An exception may be found in cases of highly buffered material, such as dry salted peas or beans.

Luxuriant growth of film yeasts may occur at various salt concentrations, and will result in loss of brine acidity. When certain molds accompany this scum growth, the vegetable material may become soft and unusable. Heavy scum yeast and/or mold growth is usually the result of neglect of brined material during the curing and storage period. This is particularly true for stock brined the previous season.

The significance of the presence of the salt tolerant cocci and obligate halophiles is presented in section 47.65

47.32 Finished Pickle Products (Other than 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). If the amounts of either ingredient is inadequate, fermentation usually takes place, principally by two groups of organisms, lactic acid forming bacteria and yeasts. Molds and film yeasts may grow on the surface of the liquor chiefly as the result of faulty jar closure.

47.33 Pasteurized 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 yeasts and/or acid forming bacteria 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.

47.34 Overnight Dill Pickles (Refrigerated)

The barreled product may be stored for a few days at room temperature and then refrigerated at 36 to 40 F (ca 2 to 5 C). 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 nongas forming lactics, and fermentative yeasts), and enzymatic activity (pectinolytic and cellulolytic), together with the curing process continues at a slow rate.[16] 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 (mentioned above). 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 4.5 to 4.6 for low acid food 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 (to equilibrate at pH 4.5 to 4.6) or where it is deliberately incubated for development of natural lactic acid fermentation. 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. Our sampling of 50 one-quart jars of overnight dills from refrigerated counters or cases of large retail stores located in five geographical areas of the country indicated that every third jar was judged "not acceptable for commercial use." Twenty-five per cent more were placed in the "barely acceptable" to "poor" categories. A followup study on 23 jars in one of the large metropolitan production areas gave better results; jars placed "not acceptable" (½ of the number of the first study), but "barely acceptable" to "poor" ratings were given nearly 40% of the jars. Those jars of pickles placed as "fair," "good" and "excellent" amounted to 17, 13 and 17%, respectively.

47.4 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 brinestock—including cucumbers. 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 about 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, green cherry, pear shaped, or regular globe tomatoes, and the like. The process calls for the packed product to be acidifed at the outset with a sufficient amount of food grade organic acid (vinegar, acetic acid, and 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 for over 35 years.[10, 25]

As far as fresh pack (pasteurized) 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 consum-

ing public, might inadvertently lead to a butyric acid type spoilage problem involving the public health aspect of the product.

47.5 RECOMMENDED METHODS

The methods described here should prove useful to those concerned with, or responsible for, the examination of certain types of manufactured pickles, particularly those products undergoing spoilage as the result of microbial activity. The methods should prove helpful also to research investigators interested in conducting studies on predominating microbial changes occurring in certain brined and salted vegetables during natural fermentation and curing.[26] The methods are also helpful in following the pure culture fermentation of brined cucumbers,[18] green olives,[17] naturally ripe olives,[2] and other vegetables.

47.51 General Procedure

47.511 Collection, storage and preparation of brine samples

Brine or pickle liquor covering 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" stainless steel tubing, sealed at one end with lead or solder and perforated with several 1/16" 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 middepth 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 2 hole, rubber stopper and 2 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, screw cap test tube. 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 one to two 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 pipet. 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.

47.512 Storage of samples

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 and 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 test 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, of one to two drops per 10 ml of sample. Collect samples in standard 3 to 4 oz medicine bottles or in 16 × 150 mm test tubes, fitted with screw caps as described above. Shake well to distribute preservative. Caps having pulp-backed vinylite and teflon liners, or pulp backed foil liners should be used; those having cork or composition cork are not satisfactory for prolonged storage.

Samples preserved with the above chemicals are unfit for human consumption and should be so marked.

47.513 Preparation of the sample

Make suitable dilutions of the pickle liquor or brine in the usual manner, except for obligate halophiles. For this group make serial dilutions directly into the recommended liquid medium containing salt.

If poured plates using salt-containing media are desired, the dilution blanks should contain approximately the same salt concentration as the brine sample.

For actively fermenting brines, no specific number of dilutions can be suggested; however, as a guide, such brines, at 5 to 8% salt by weight, may be expected to contain the following populations per ml: acid forming bacteria, 10^6 to 10^9; yeasts, 10^4 to 10^7; obligate halophiles, 10^6 to 10^9; coliforms, 10^6 to 10^8; salt tolerant cocci; 10^6 to 10^7. The expected microbial populations in adequately pasteurized products are normally very low, and composed of resistant spore forming bacteria that remain dormant in the acid liquor. For such products, dilutions of 1 : 10 and 1 : 100 usually suffice. For improperly pasteurized products that are fermenting, the dilutions should cover the estimated range of population suggested for acid-formers and yeasts in fermenting brines. For raw products that are not properly washed, the spore count may reach counts of 1 to 2 × 10^5/ml even after pasteurization.

47.514 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.

a. Technic for 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 pipet,[5] and spread evenly over a 1 sq cm area; fix with heat.

Stain according to the Kopeloff and Cohen modification of the Gram stain.[32] Count according to the Wang[39] modification of the Breed[5] technic.

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

b. Technic for yeasts

Use the microscopic technic for determining yeast populations in fermenting vegetable brines, and various types of finished pickle products undergoing gaseous spoilage by these 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[34] as modified by Bell and Etchells[4] 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 per cent) 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."

47.515 Titratable acidity and pH

Determinations of titratable acidity and pH of the samples are extremely useful in providing information supplementary to bacteriological analysis.

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. 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 calaculations:

a. ml of 0.1 N alkali used × 0.090 = gm of lactic acid per 100 ml.

b. ml of 0.1 N alkali used × 0.060 = gm 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 acids.

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.

47.516 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 gm 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 "gm 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 gm of sodium chloride per 100 ml.

47.52 Procedure According to Type of Product: Cucumber Pickles and Similar Pickle Products

The three main classes of products under this heading are salt stock vegetables and genuine dills, finished or packaged pickle products made from salt-stock, pasteurized pickles made from fresh stock, and the unheated "overnight" dills (refrigerated). The cucumber is the principal vegetable involved, although substantial amounts of other vegetables, such as onions, peppers, cauliflower, okra, carrots, and green tomatoes, may be used in mixed pickles, relishes, or as individual products.

47.521 Salt stock vegetables and genuine dills

Use the plating technic with differential solid media and decimal dilutions in 0.85% saline diluent. Place decimal dilutions of samples in Petri plates, in duplicate, and fill with medium as follows:

a. Aerobic plate count

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

b. Lactic acid forming bacteria

Use lactobacillus selection medium (BBL), modified carefully to pH 5.6 ± 0.05, plus bromocresol green. Overlay the solidified plates to favor reduced oxygen tension. Lactobacilli colonies appear green to black with a yellow halo.

c. Salt tolerant cocci

Use nutritive caseinate agar, plus 0.1 glucose (Chapter 2) and incubate for three 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 morphological identification when lactose fermenting yeasts may be present, make stained preparations and examine under the microscope.

d. Coliform bacteria

Use brilliant green lactose bile agar, violet red bile agar or desoxycholate lactose agar (Chapter 2). Incubate for 18 to 24 hrs at 32 C.

e. Yeasts and molds

Use dextrose agar, acidified, and incubate three to five days at 28 to 30 C, or yeast nitrogen base agar plates for estimation of yeasts and molds by the streaking technique (Chapter 2).

f. Film yeasts

For an estimate, pick representative filamentous colonies from the yeast plates into tubes of dextrose broth containing 5 and 10 per cent salt. Incubate three to five 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 per cent) whereas, with other species, the reverse is true.

g. Obligate halophiles

Use tubes of liver broth plus salt (Chapter 2). Prepare decimal dilutions, seal with sterilized, melted petroleum jelly, and incubate seven 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.

h. 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 seven days at 32 C. Examine tubes daily for production of gas and a strong butyric acid odor.

47.522 Finished pickle products

Liquor of the sample should be examined for total number of microorganisms, acid forming bacteria, yeasts and molds, film yeasts, and butyric acid forming bacteria, using methods in 47.521.

In undisturbed containers, the surface growth of molds and film yeasts may be obvious. Carefully remove the film after recording the extent of growth, since if shaken up with the sample it will complicate the counts for acid formers and yeasts when the latter groups are present. Examine for coliform bacteria, salt tolerant cocci and halophiles. The test for butyric acid bacteria normally is not required due to acidity of these products.

47.523 Pasteurized types of pickles

There are probably a dozen or more different types of cucumber pickles that fall into this classification (pasteurized), such as various types of fresh dills, fresh sliced cucumber pickles and low acid sweet pickles (from salt-stock). Also, many noncucumber products are included: dill tomatoes, sweet and hot peppers, okra, green beans, and fresh vegetable relishes that are prepared from uncured stock.

Examination of the liquor from the products of this class should be made. See section 47.522.

47.524 Overnight dill pickles (refrigerated)

Examination of the liquor from the products of this class should be made as in section 47.522.

47.525 Salted and brined vegetables for nonpickle use

a. Salted vegetables

Most of the vegetables after blanching are preserved according to the dry salt method,[24] using a ratio of 1 : 5 with respect to salt and vegetable weight, and stored in tightly headed wooden casks or metal or plastic containers, preferably at refrigerator temperatures within the range of 1.7 to 4.4 C (35 to 40 F). Vegetables treated in this manner are usually green peas, corn, snap beans, cut okra, small onions, and cut celery. These salted vegetables can be used in the preparation of soups, mixed vegetable products, and strained vegetable products.

b. Brined vegetables

Products such as whole okra, whole celery, and sweet red peppers usually are brined at about 20% salt concentration (equilibrated) rather than by the dry salt method. These are used in the same manner as salted vegetables.

c. Microbiological examination

Brine samples from both types of these vegetables (47.525a and 47.525b) should be examined for aerobic plate count, lactic acid forming bacteria, salt tolerant cocci, coliform bacteria, yeasts and molds, film yeasts, obligate halophiles, and butyric acid forming bacteria by the directions set forth in section 47.521.

47.53 Summary of Procedure

A summary of the bacteriological methods described herein is presented in Table 1. This information is suggested for use as a guide in the examination of certain brined, salted and pickled vegetables and vegetable products.

Some new, some revised, and some different microbiological procedures for cucumber and cucumber product studies have been proposed by Etchells et al.[20] The reader should consult the original reference for details and application of these methods, and for greater detail in specialized instances, consult the related publications.[6, 11–15, 19, 21, 22, 27–31, 36]

47.54 Discussion of the Use of Culture Media and Types of Microorganisms

47.541 Nutritive caseinate agar (Chapter 2) plus 0.1% glucose

Use this medium to save time, effort, and glassware to detect and enumerate several types of bacteria on the same Petri plate. It can be used for enumeration of total bacterial count and salt tolerant cocci, for determining population trends of acid producing bacteria in dill pickles,[31] salt stock,[23] improperly pasteurized fresh cucumber pickles,[22] and in the storage of salted and brined vegetables.[27, 28]

Since this medium contains less agar than usual solid media, no more than 15 ml to insure solidification and prevent dropping of agar when plates are inverted. During hot weather, cool plates prior to inversion and incubation.

Acid forming bacterial colonies show a zone of precipitated casein and a yellow halo in the presence of bromcresol purple. The degree of casein precipitation and color change may vary with the activity and type of acid former.

Surface growth is usually poor. Subsurface colonies are generally elliptical in shape and range in size from 0.5 to 2.5 mm.

Yeasts, other than lactose fermenters, do not grow well on this medium and tend to give a slightly alkaline reaction. In case of doubt, make stained preparations.

While nutritive caseinate agar is not considered a differential medium for salt tolerant coccus forms, the numbers of these organisms in brines of high salt concentration may be estimated. They are indicated by two predominating types of colonies, one grayish white, entire, glistening, and of moderate size, and a similar colony that is yellow to light orange in color. In high salt content, nonacid brines, these organisms are the principal types found on this medium. Due to sensitivity to acid, they are not found usually in active fermentations of the acid type. Deep subsurface colonies may give an acid reaction but, upon prolonged incubation, become alkaline. In highly buffered, salted vegetables, bordering on the range of salt tolerance for acid forming bacteria, care should be exercised that deep colonies of cocci are not recorded as true acid producing bacteria of the lactic group.

47.542 Lactobacillus selection medium (Chapter 2)

This medium should be prepared with 0.0075% bromcresol green dye to aid colony counting, and adjusted carefully to pH 5.6 ± .05 with glacial acetic acid, rather than adding the fixed amount (1.32 ml acetic acid per liter). The modified medium was used successfully[6] for separating relatively low populations of lactic acid bacteria occurring on pickling cucumbers from exceedingly high populations of other microbial groups; thus, the medium is highly selective for the lactobacillus group.

47.543 V-8 medium (Chapter 2)

For determining numbers of lactobacilli, Fabian et al.[30] have shown this medium to give good results. The colonies are green to black with a yellow halo, and develop to a large size in the presence of lactose. The bromcresol green is said to be inhibitory to most of the nonacid formers.

47.544 Brilliant green lactose bile agar (Chapter 2)

This medium is preferred because of the ease of determining the coliform type of colony. Subsurface colonies of the coliform group are deep red against a blue green background. This medium is sensitive to light and should be prepared just prior to use. When this is not convenient, the medium should be stored in the dark. For more complete identification, representative colonies should be streaked on Levine's eosin methylene blue agar.

47.545 Dextrose agar (acidified)

This medium is more inhibitive to the lactic acid types of bacteria and is preferred over malt agar for detecting yeasts in fermenting vegetable brines.[11] Occasionally, yeasts that will not grow on this medium are found in high salt concentrations, 15 to 20 per cent.[21] By reducing the tartaric acid to 3 ml per 100 ml, growth can often be obtained. However, this modification should not be used when the salt concentration is known to be below 15 per cent, since acid forming bacteria will grow enough to make counting of yeasts very difficult.

Mold colonies are distinguished readily from yeasts on this medium, whereas, differentiation of subsurface yeasts and film yeasts present 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*),[13, 36] 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*).[15, 19, 23] However, even when distinguishing colony characteristics of the two yeast groups exist, they are not considered sufficiently clear cut for separation. Because of

Table 1: Guide to the Bacteriological Examination of Salted, Brined, and Pickled Vegetable Products

Microbial group involved	Culture Medium (Chapter 2)	Classes of products in which microbial group is likely to be present*	Remarks concerning microbial groups
Total count	Plain or dextrose agar	All classes of products 47.111, 47.112, 47.113, 47.114, 47.121 and 47.122	For determination of general microbial populations; in pasteurized products they help to indicate the effectiveness of the treatment.
Acid forming bacteria	LBS agar, modified; V-8 medium	47.111 Fermenting salt stock vegetables and genuine dills 47.112 Finished pickle products 47.113 Pasteurized pickle products 47.114 Overnight dill pickles (Refrigerated)	Acid fermentation; salt tolerant up to 15 per cent; not likely to be found in brined and salted vegetables above this concentration (47.121 and 47.122).
Salt tolerant cocci	Nutritive caseinate agar	47.12 Brined and salted vegetables for nonpickle use other high salt vegetables without appreciable acidity	No outstanding characteristics of fermentation reported; group salt tolerant but sensitive to acid; can grow at refrigerator temperature (1.7 C) at approximately 10 per cent salt.
Coliform bacteria	Brilliant green lactose bile agar, violet red bile agar, or desoxycholate agar	47.111 Fermenting salt stock vegetables and genuine dills 47.113 Types of pasteurized pickles (not brine cured) 47.114 Overnight dill pickles (refrigerated) 47.121, 47.122 Brined and dry salted vegetables for nonpickle use	Gaseous fermentation; group salt-tolerant but not acid tolerant; most likely absent from finished pickles due to acid content; same is true for brines when appreciable acid is present.
Obligate halophiles	Liver broth plus salt	47.12 Brined and salted vegetables for nonpickle use Other vegetable brines at high salt concentration	Gaseous fermentation; group requires 5 to 15 per cent salt in culture medium and reduced oxygen tension; sensitive to acid; general information or behavior not well known.
Fermentative yeasts, film yeasts and molds	Dextrose agar (acidified), dextrose broth plus salt;** yeast nitrogen base agar for streak plates	All classes of products (47.111, 47.112, 47.113, 47.114; 47.121 and 47.122) for yeasts Molds and film yeasts on liquid surface of products exposed to air and sheltered from sunlight.	Yeasts: gaseous fermentation; acid and salt tolerant; molds and film yeasts: acid and salt tolerant; both groups utilize acid of products and require free oxygen for growth.
Butyric acid group	Liver broth medium without salt	Uncommon in brined and salted vegetables; examination should be made if malodorous fermentation is detected in all classes of products (47.11 and 47.12), particularly 47.114 Overnight dill pickles (refrigerated).	Causes malodorous, gaseous fermentation; not particularly acid or salt tolerant; active fermentations rare in properly brined or salted vegetables.

*Refer to outline for more detailed classification of products listed under 47.11 and 47.12
**For culturing film forming yeasts in general.

this, the procedure outlined under 47.521f 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 per cent salt than at 5 per cent.[11, 13, 21, 36]

47.546 Yeast nitrogen base agar plates for estimation of yeasts and molds by streaking technique[15, 20] (Chapter 2)

Prepare the following in distilled water, equal amounts in separate containers of:

a. 4% agar (Bacto-Difco) + 4% glucose (dextrose)

b. double strength yeast N base broth (Difco Laboratories, Detroit, Michigan) = 1.3% or 1.3 grams of the dehydrated N base powder per 100 ml H_2O.

Sterilize the above containers at 15 lb pressure for 12 minutes. Cool to 50 C, and mix the contents of the two containers, add sterilized tartaric acid (5%) at the rate of 3 ml per 100 ml of mixed agar and N base. This equals 7.5 ml of 5% tartaric acid per 250 ml of media.

Pour plates using 25 to 30 ml of agar per plate; allow to solidify, invert, incubate 24 hours at 30 C, observe for any contaminating colonies, then store inverted in refrigerator until used. Streak the sample, or suitable dilutions of such, on surface with platinum loop (.01 ml capacity). Incubate three to five days at 28 to 32 C, and count.

47.547 Liver broth plus salt (Chapter 2)

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.

47.548 Liver broth medium (Chapter 2)

This medium has proved useful in detecting saccharolytic, and putrefactive, mesophilic anaerobes. While a positive test is presumptive evidence of mesophilic, spore forming, gas producing anaerobes of the butyric acid forming types, more specific bacteriological tests are required on positive tubes before identification can be made. Also, spore formation in the sample may be negligible due to high acid production in the presence of readily fermentable carbohydrates, and even though previous activity by this group may have been quite high, negative results usually will be obtained in old brines. Positive results in this medium indicate that these types of bacteria were responsible for the malodorous fermentation.

47.6 INTERPRETATION OF DATA

47.61 Salt Stock Vegetables and Genuine Dills

47.611 Significance of observations

The acid fermentation resulting from active growth of lactic acid bacteria is to be expected at brine concentrations below 12 to 15 per cent strength.[23] The acidity developed in the brine, in combination with the salt, results in preservation of salt stock cucumbers, genuine dills, olives, and other brined vegetables.

Yeast counts of viable cells of subsurface species in fermenting cucumber brines may average four to five times that obtained by the plating technic. Clusters of viable cells, no doubt, are responsible for this difference, as each clump of cells forms a single colony on a plate, while the actual number of cells is recorded by the microscopic method.

It should be emphasized that neither microscopic nor plate counting technics give a true picture of the populations of gas forming, subsurface species of yeasts in fermenting brines or pickle samples obtained from containers contaminated with film yeasts originating from luxuriant surface growth. This is applicable to small containers (jars), since large fermenting tanks (1,000 to 6,000 gal capacity) can be sampled in such a way that surface yeasts are not a problem.

47.62 Finished Pickle Products

47.621 Significance of observations

A total count of a few thousand organisms per ml normally is found in unspoiled pickle products. These counts are composed chiefly of resistant, aerobic spore forms that remain inactive in the acid medium of the pickle liquor and tend to decrease during storage. Active yeast fermentation in the product usually is characterized by vigorous gas production which causes the pickle liquor to become highly charged with gas and to possess a tang when tasted. Gas production may be sufficient to blow lids from jars, to break jars, or to distend or burst sealed cans.[16, 25] Also, whole pickles may become "bloaters" (hollow) due to the gaseous fermentation by yeasts and/or gas producing types of acid producing bacteria.[16, 25] The acid content of the liquor may be increased due to growth of acid producing bacteria.

Extensive mold and film yeast growth (on the surface of brine or liquor) usually result in a reduction in acidity of the liquor, and, in advanced stages, the vegetable may be completely softened by such growth.

47.63 Pasteurized Types of Pickles

According to Etchells and Jones,[25] pasteurization to an internal product temperature of 165 F, for 15 minutes, followed by prompt cooling, is required for pickle products that do not contain sufficient amounts of added vinegar and sugar to stop fermentation by certain organisms. Esselen et al[1, 7-9, 33] applied the mathematical method of process calculation, as described by Ball,[3] to the derivation of pasteurization times for pickles. The times and temperatures derived relate to the degree of sterilization or heat units given the product rather than to an internal product temperature per

se. Even so, the original pasteurization process described and recommended some 30 or more years ago is still followed by industry.[22, 25] Pasteurization times are based upon procedure wherein the jars are held in a processing tank, or a steam or hot water pasteurizer, at the indicated pasteurization temperature and time. At the end of the pasteurization period, the jars are cooled promptly below 100 F.

A series of experiments were conducted in commercial pickle plants involving the pasteurization of fresh pack dill pickles.[35] Internal product temperatures in the range of 160 to 170 F with an equilibrated acidity of 0.60% acetic acid or greater prevented spoilage by natural fermentation, and produced pickles of good quality. At temperatures less than 160 F, acidities of up to 1.00% acetic acid did not prevent spoilage. Increasingly higher internal product temperatures, from 170 through 200 F, resulted in correspondingly increased amounts of bloater damage to the internal structure of the cucumber. Faster heating rates decreased pickle firmness, particularly for those located in the upper part of the jar. Tightness of pack greatly influenced the heating rate of the fresh pack dill pickles.

The significance of organisms found in these products is essentially that described in section 47.522. Since the acidity is often lower than in finished pickles, and the products may be made from fresh uncured vegetables, it is essential that spoilage types of organisms be detected, and that any improper heat processing be recognized promptly and corrected.

47.64 Overnight Dill Pickles (Refrigerated)

In the pickle industry, the overnight dill is considered a specialty item, and for many years was prepared in bulk, usually by small packers located in or near large metropolitan areas of the country. In recent years, these pickles also have been prepared in quantity directly in consumer size glass containers which are then supposed to be stored, distributed, and retailed under refrigerated conditions (36 to 40 F). Details on the preparation of this product have been described by Schucart[38] and by Etchells et al.[16]

One important characteristic emphasized by Schucart over 30 years ago, for overnight dills made in bulk (barrels), was their "perishability". He also mentioned the low salt and vinegar content of the product and the strong spicing or seasoning, especially with respect to fresh garlic. We can say that, based on our recent studies (unpublished) on the quality of refrigerated overnight dills in glass jars from retail outlets in several metropolitan areas of the country, the same "perishability" characteristic, mentioned so long ago, still exists, plus a high degree of variability both as to the product's generic name (such as Half Sours, 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), as well as regarding the preparation of the product by individual companies, particularly as to their use of acidification, use of preservatives or other chemical additives, use of whole spices, use of spice emulsions, salt content, size and quality of green cucumbers used, type of jar closure used, ratio of cucumbers to brine on a per cent/weight basis, and

the presence or absence of proper refrigeration facilities for the product during preparation, distribution and storage, and display at retail outlets.

47.65 Salted and Brined Vegetables for Nonpickle Use

47.651 Significance of observations

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, as 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 per cent 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 reduction of brine acidity. This may lead to spoilage by salt tolerant organisms that are not acid tolerant.

As mentioned above, refrigerated storage (about 1.7 C) of these brined and salted products is preferred. Under such conditions, and at salt concentrations of 5 per cent and above, microbial activity of the various groups may be restricted greatly. However, at salt concentrations of approximately 10 per cent strength, the cocci may grow rapidly at about 1.7 C (35 F).

47.7 REFERENCES

1. Anderson, E. E., L. F. Ruder, W. B. Esselen, E. A. Nebesky, and M. Labbee. 1951. Pasteurized fresh whole pickles. II. Thermal resistance of microorganisms and peroxidase. Food Technol. 5:364–368
2. Balatsouras, Georges D. The Chemistry and Technology of Naturally Black Olives. Food and Agriculture Organization of the United Nations, Lecture Series, Athens, Greece. 49 pp
3. Bali., C. O. 1928. Mathematical Solution of Problems of Thermal Processing of Canned Food. University of California Publications, Public Health, I, 245 pp
4. Bell, T. A. and J. L. Etchells. 1952. Sugar and acid tolerance of spoilage yeasts from sweet-cucumber pickles. Food Technol., 6:468–472

5. Breed, R. S. 1911. The determination of the number of bacteria in milk by direct microscopic examination. Zentralblatt für Bakteriologie und Parasitenkunde. II. Abt, **30**:337–340
6. Costilow, R. N., J. L. Etchells, and T. E. Anderson. 1964. Medium for producing cells of lactic acid bacteria. Applied Microbiol. **12**:539–540
7. Esselen, W. B., E. E. Anderson, I. S. Fagerson, and M. Labbee. 1952. Pasteurized fresh whole pickles. V. Factors influencing pasteurization requirements. The Glass Packer **31**:326–327 and 346–347
8. Esselen, W. B., E. E. Anderson, L. F. Ruder, and I. J. Pflug. 1951. Pasteurized fresh whole pickles. I. Pasteurization studies. Food Technol. **5**:279–284
9. Esselen, W. B., I. S. Fagerson, I. J. Pflug, and E. E. Anderson. 1951. Pasteurized fresh whole pickles. III. Heat penetration in fresh pack pickles. The Glass Packer, **31**:175–178
10. Etchells, J. L. 1938. Rate of heat penetration during the pasteurization of cucumber pickles. Fruit Products J. **18**:68–70
11. Etchells, J. L. 1941. Incidence of yeasts in cucumber fermentations. Food Res. **6**:95–104
12. Etchells, J. L. and T. A. Bell. 1950. Classification of yeasts from the fermentation of commercially brined cucumbers. Farlowia **4**:87–112
13. Etchells, J. L. and T. A. Bell. 1950. Film yeasts on commercial cucumber brines. Food Technol. **4**:77–83
14. Etchells, J. L., T. A. Bell, H. P. Fleming, R. E. Kelling, and R. L. Thompson. 1973. Suggested procedure for the controlled fermentation of commercially brined pickling cucumbers—the use of starter cultures and reduction of carbon dioxide accumulation. Pickle Pak Sci. **3**:4–14
15. Etchells, J. L., T. A. Bell, and I. D. Jones. 1953. Morphology and pigmentation of certain yeasts from brines and the cucumber plant. Farlowia **4**:265–304
16. Etchells, J. L., A. F. Borg, and T. A. Bell. 1968. Bloater formation by gas-forming lactic acid-bacteria in cucumber fermentations. Applied Microbiol. **16**:1029–1035
17. Etchells, J. L., A. F. Borg, I. D. Kittel, T. A. Bell, and H. P. Fleming. 1966. Pure culture fermentation of green olives. Applied Microbiol. **14**:1027–1041
18. Etchells, J. L., R. N. Costilow, T. E. Anderson, and T. A. Bell. 1964. Pure culture fermentation of brined cucumbers. Applied Microbiol. **12**:523–535
19. Etchells, J. L., R. N. Costilow, and T. A. Bell. 1952. Identification of yeasts from commercial cucumber fermentations in northern brining areas. Farlowia **4**:249–264
20. Etchells, J. L., R. N. Costilow, T. A. Bell, and H. A. Rutherford. 1961. Influence of gamma radiation on the microflora of cucumber fruit and blossoms. Applied Microbiol. **9**:145–149
21. Etchells, J. L., F. W. Fabian, and I. D. Jones. 1945. The *Aerobacter* Fermentation of Cucumbers During Salting. Michigan Agricultural Experiment Station Technical Bulletin No. 2000, 56 pp.
22. Etchells, J. L. and I. D. Jones. 1942. Pasteurization of pickle products. Fruit Products J. **21**:330–332
23. Etchells, J. L. and I. D. Jones. 1943. Bacteriological changes in cucumber fermentation. Food Indust. **15**:54–56
24. Etchells, J. L. and I. D. Jones. 1943. Preservation of Vegetables by Salting or Brining. U.S. Department of Agriculture, Farmers' Bulletin No. 1932, 14 pp.
25. Etchells, J. L. and I. D. Jones. 1944. Procedure for pasteurizing pickle products. The Glass Packer **23**:519–523
26. Etchells, J. L. and I. D. Jones. 1946. Procedure for bacteriological examination of brined, salted and pickled vegetables and vegetable products. A. J. of Pub. Health **36**:1112–1123
27. Etchells, J. L., I. D. Jones, and W. M. Lewis. 1947. Bacteriological Changes During the Fermentation of Certain Brined and Salted Vegetables. U.S. Department of Agriculture Technical Bulletin No. 947, 64 pp
28. Fabian, F. W. and H. B. Blum. 1943. Preserving vegetables by salting. Fruit Products J. **22**:228–236
29. Fabian, F. W., C. S. Bryan, and J. L. Etchells. 1932. Experimental Work on Cucumber Fermentation. Michigan Agricultural Experiment Station Technical Bulletin No. 126, 60 pp

30. Fabian, F. W., R. C. Fulde, and J. E. Merrick. 1953. A new V-8 medium for determining lactobacilli. Food Res. **18**:280–289
31. Jones, I. D., M. K. Veldhuis, J. L. Etchells, and O. Veerhoff. 1940. Chemical and bacteriological changes in dill pickle brines during fermentation. Food Research, **5**:533–547
32. Kopeloff, N. and P. Cohen. 1928. Further studies on a modification of the Gram stain. Stain Technol. **3**:64–69
33. Labbee, M., W. B. Esselen, and E. E. Anderson. 1952. Pasteurized fresh whole pickles. IV. Enzymes and off-flavors in fresh-pack pickles. The Glass Packer **31**:252–253 and 281–282
34. Mills, D. R. 1941. Differential staining of living and dead yeast cells. Food Res. **6**:361–371
35. Monroe, R. J., J. L. Etchells, J. C. Pacilio, A. F. Borg, D. H. Wallace, M. P. Rogers, L. J. Turney, and E. S. Schoene. 1969. Influence of various acidities and pasteurizing temperatures on the keeping quality of fresh-pack dill pickles. Food Technol. **23**:71–77
36. Mrak, E. M. and L. Bonar. 1939. Film yeasts from pickle brines. Zentralblatt für Bakteriologie und Parasitenkunde. II. Abt. **100**:289–294
37. Pederson, C. S. and M. N. Albury. 1969. The Sauerkraut Fermentation. New York Agricultural Experiment Station Bulletin No. 824, 84 pp.
38. H. S. Schucart. 1942. Practical observations on the manufacture of kosher style dill pickles. Fruit Products J. **21**:206–212
39. Schu-Hsien Wang. 1941. A direct smear method for counting microscopic particles in fluid suspension. J. Bact. **42**:297–319