CHAPTER III

10A

BRINED, SALTED, AND PICKLED VEGETABLE PRODUCTS

The methods described here * should prove useful to those concerned with the examination of certain types of manufactured pickles, particularly those products undergoing spoilage as the result of microbial activity. The methods also should prove helpful to research workers interested in conducting studies on predominating microbial changes occurring in certain brined and salted vegetables during natural fermentation and curing.

CLASSIFICATION

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

I. Cucumber Pickles and Similar Pickle Products

A. *Salt Stock for Cured Pickle Products*

1. Cucumbers (and onions, peppers, tomatoes, cauliflower, melon rinds, etc.)
2. Genuine dill pickles (from cucumbers or tomatoes)

B. *Finished Pickle Products from Brine-Cured Stock*

1. Sweets
2. Sours
3. Mixed
4. Relishes
5. Artificial or processed dills

C. *Types of Pasteurized Pickles (Not Brine-Cured)*

1. Dills (sliced or whole)
2. Sweets (sliced or whole)
3. Relishes (mixed vegetables)
4. Vegetables other than cucumbers (onions, peppers, tomatoes, etc.)

* In large part revised from the original publication by Etchells, J. L., and Jones, I. D. Procedure for Bacteriological Examination of Brined, Salted and Pickled Vegetables and Vegetable Products. A.J.P.H. 36:1112-1123 (Oct.), 1946.

II. Brined and Salted Vegetables for Nonpickle Use

A. *Brined*

1. Okra (whole)
2. Celery (whole)
3. Sweet pepper hulls

B. *Dry-Salted*

1. Corn
2. Lima beans
3. Peas
4. Snap beans
5. Okra (cut)
6. Celery (cut).

In "Recommended Methods for the Microbiological Examination of Foods," 1958, pp. 14-30, American Public Health Assoc., Washington, DC.

GENERAL PROCEDURE

I. 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 vat of fermenting salt stock. Brine samples from large containers, such as vats 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 6-8" from the sealed end) is inserted, through an opening in the false head, into the brine toward the center of the vegetable material. Withdraw brine through a previously attached piece of rubber tubing into a 12-oz juice bottle. The receiving bottle is fitted 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 steel sampling tube is governed by the depth of the container to be sampled.

Withdraw approximately 24 oz of brine before taking the final sample (about 10 ml) in a sterile test tube. If microbial changes during the fermentation are to be followed, start sampling at the time material is salted or brined and continue at regular intervals of 1 to 2 days during active fermentation.

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

For smaller containers, such as jars or cans of pickle products, shake thoroughly and take from center of the material by use of a sterile pipette. Wash the tops of 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, release gas carefully by puncturing top with a flamed ice pick.

II. Storage of Samples

Brine samples from actively fermenting material should be examined as promptly as possible after collection to prevent changes in the microbial flora present. 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 preferably not exceed 12 to 24 hr. Samples collected in sterile 16 x 150 mm test tubes, fitted with plastic screw caps having pulp-backed vinylite and teflon liners, are useful where shipment is required.

Brine samples can be preserved for subsequent chemical determinations by addition of sodium, 2, 4, 5 trichlorphenolate (Dow) to make a dilution of about 1:10,000 as described by Veldhuis.[1] Collect samples in standard 3- to 4-oz medicine bottles and add about 10 drops of a 10 per cent aqueous solution of the chemical. Shake well to distribute preservative. Caps having pulp-backed vinylite and teflon liners, or pulp-backed foil liners should be used, as those having cork or composition cork are not satisfactory for prolonged storage. If samples are to be tested for enzyme activity, use 10–15 drops of toluene as a preservative instead of sodium, 2, 4, 5, trichlorphenolate.

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

III. 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 may be

expected to contain the following populations per ml: acid-forming bacteria, 10:1,000 million; yeasts, 10:100 million; obligate halophiles, 10:1,000 million; coliforms, 1:10 million; salt-tolerant cocci, 1:10 million. 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.

IV. 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 portions of brine or liquor on slides, by using a Breed [2] pipette, and spread evenly over a 1-sq cm area. Prepare and count according to the Wang [3] modification of the Breed [2] technic. Stain according to the Kopeloff and Cohen [4] modification of the Gram stain (B4). Report results as numbers of different morphological types of Gram-positive and Gram-negative bacterial cells per ml of brine.

B. *Technic for Yeasts*

The microscopic technic can be used to advantage for determining yeast populations in fermenting vegetable brines and various types of finished pickle products undergoing gaseous spoilage by these organisms. It provides a rapid and reliable method for detecting and following yeast activity, particularly where populations are in excess of 10,000–20,000 per ml of sample, and where yeast colonies are not required for isolation and study. Furthermore, the use of a vital stain permits differentiation of yeast population into viable and nonviable cells, and increases the value of the direct counting technic.

Yeast counts of viable cells of subsurface species in fermenting cucumber brines may average 4–5 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 are recorded in the microscopic method.

It should be emphasized that neither microscopic nor the plate counting technics give a true picture of the populations of gas-forming, subsurface species of yeasts in fermenting brine or pickle samples obtained from containers also contaminated with film yeasts originating from luxuriant surface growth.

The procedure is essentially the method of Mills [5] as modified by Bell and Etchells [6, 7] 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 (B1).

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 chamber in one operation.

Allow cells to settle for approximately 5 min and count yeast cells, using a microscope equipped with a 4-mm objective and 15 x 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:

$$\frac{\text{Number of yeast cells counted x dilution x } 250{,}000}{\text{Number of large squares counted}} = \text{numbers per ml.}$$

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 (a) total yeast cells, (b) live yeast cells, and (c) dead yeast cells, per ml of sample.

V. Titratable Acidity and pH

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

Determine titratable acidity on a 10-ml sample of the brine or liquor by diluting sample with 30–50 ml of distilled water, bring just to boiling, cool, and titrate with 0.1 *N* NaOH, using phenolphthalein as the indicator. Report values for brined samples in terms of gm of lactic acid per 100 ml of sample and for finished liquor samples in terms of gm of acetic acid per 100 ml of sample.

When a 10 ml sample is titrated the following may be used in making the calculations:

A. ml of 0.1*N* alkali used x 0.111 = gm of lactic acid per 100 ml.
B. ml of 0.1*N* alkali used x 0.074 = gm of acetic acid per 100 ml.

Where only a small amount of original sample is available, a 2-ml sample may be used for titration purposes, although such small samples are not recommended.

Make pH-determination with a glass electrode, after aeration of sample to remove dissolved carbon dioxide.

VI. Determination of Salt Content of Brine

Knowing the approximate salt content is often helpful in making the microbiological examination of brines. This can be obtained by a salometer using about 200 ml of brine. For small amounts of sample, or where a higher degree of accuracy is desired than that obtainable by the salometer, a chemical test for salt is required. The following method is recommended for use: Transfer 1 ml of sample to a flask and dilute with 15–20 ml of distilled water. Titrate with 0.171*N* silver nitrate solution (C4) (29.063 gm per liter) using 3–5 drops of 0.5 per cent dichlorofluorescein (C6) [8] as the indicator. Agitate to keep the precipitation broken up until a light salmon-pink color is developed. Report as gm of sodium chloride per 100 ml of 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.

PROCEDURE ACCORDING TO TYPE OF PRODUCT

I. 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; and pasteurized pickle made from fresh stock. The cucumber is the principal vegetable involved, although substantial amounts of other vegetables, such as onions, peppers, cauliflower, and green tomatoes, may be used in mixed pickle, relishes, or as individual products.

A. *Salt-Stock Vegetables and Genuine Dills*

The determination of numbers and types of microorganisms should be carried out by use of the plating technic with differential media and with decimal dilutions in differential liquid media. Place decimal dilutions of samples in Petri plates, in duplicate, and pour with media with respect to the following:

1. *Total Count*—Use nutritive caseinate agar (46) and incubate for 3 days at 32° C. The same set of plates may be used for counting the lactic acid-forming bacteria and the salt-tolerant cocci.

2. *Lactic Acid-Forming Bacteria*—Use nutritive caseinate agar (46), incubate 3 days at 32° C, and count those colonies showing a zone of precipitated casein and a yellow halo around the colony. The V-8 medium (72) may be used for differentiation of these types of bacteria. Lactobacilli colonies appear green to black with a yellow halo.

3. *Salt-Tolerant Cocci*—Use nutritive caseinate agar (46) 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. To effect identification when yeasts are present make stained preparation and examine under microscope.

4. *Coliform Types of Bacteria*—Use brilliant green lactose bile agar (12), violet red bile agar (73) or desoxycholate lactose agar (16). Incubate for 18–24 hr at 32° C.

5. *Yeasts and Molds*—Use dextrose agar, acidified, (17) and incubate 3–5 days at 32° C.

6. *Film Yeasts*—For an estimate, pick representative colonies from the yeast plates into tubes of dextose broth (18) containing 5 and 10 per cent salt. Incubate 3–5 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 per cent) whereas with other species the reverse is true.

7. *Obligate Halophiles*—Use tubes of liver broth plus salt (33). Prepare decimal dilutions, seal with 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 absence of any distinctive odor.

8. *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 min at 80° C to kill vegetative cells. Prepare decimal dilutions and inoculate previously heated and cooled tubes of liver broth medium (32). Seal with melted petroleum jelly and incubate 7 days at 32° C. Record positive tubes daily as evidenced by production of gas and a strong butyric acid odor.

9. *Significance of Observations*—The acid fermentation resulting from active growth of lactic acid bacteria, is to be expected at brine concentrations below 15 per cent strength.[9] The acidity developed in the brine, in combination with the salt, results in preservation of salt stock and genuine dills.

Activity by coliform types of bacteria, obligate halophiles, and yeasts is associated with a gaseous fermentation. This may bring about a condition in salt-stock cucumbers and dill pickles known as "bloaters" or hollow cucumbers. Although these groups of organisms are extremely salt tolerant the coliform bacteria and halophiles are not usually found in brines having appreciable acidity. An exception may be found in cases of highly buffered material, such as dry-salted peas.

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 so soft as to be unusable. Heavy scum yeast and/or mold growth is the result of neglect in looking after brined material during the curing and storage period.

The significance of the presence of the salt-tolerant cocci and obligate halophiles is presented under Brined and Salted Vegetables for Non-pickle Use.

B. *Finished Pickle Products*

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. In these finished products, preservation is dependent upon sufficient amounts of vinegar alone (for sour pickles) or a combination of vinegar and sugar (for sweet pickles). If amounts are inadequate, fermentation usually takes place, principally by 2 groups of organisms—lactic acid-forming bacteria and yeasts. Molds and film yeasts may also grow on the surface of the liquor chiefly as the result of faulty jar closure.

Liquor of the sample should be examined for (a) total number of microorganisms, (b) acid-forming bacteria, (c) yeasts and molds, and (d) film yeasts using methods set forth in I, A. Salt-Stock Vegetables.

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 it will complicate the counts for acid formers and yeasts when the latter groups are present, if shaken up with the sample. Examination for coliform bacteria, salt-tolerant cocci, halophiles, and butyric acid bacteria is not normally required due to acidity of these products.

Significance of Observations—A total count of a few thousand organisms per ml is normally found in unspoiled pickle products. These counts are composed chiefly of resistant, aerobic spore-forming types that remain inactive in the acid medium of the pickle liquor. Active yeast fermentation in the product is usually characterized by vigorous gas production so that the liquor becomes highly charged with gas and possesses a stinging taste. Gas production may be sufficient to blow lids on jars having vacuum-type closures, to break jars having screw-type lids; or to burst sealed cans.[10] Also, whole pickles may become "bloaters" (hollow) due to the gaseous fermentation by yeasts and/or gas-producing types of acid-producing bacteria.[10]

The acid content of the liquor may be increased due to activity of acid-producing bacteria.

Extensive mold and film yeast growth usually result in a reduction in acidity of the liquor, and, in advanced stages, the vegetable may be softened due to such growth.

C. *Pasteurized Types of Pickle*

In general, pasteurization to an internal product temperature of 165° F, for 15 min, 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.[10] There are probably a dozen or more different types of cucumber pickle that fall into this classification, such as various types of fresh dills, fresh sliced cucumber pickle, and low-acid sweet pickle (from salt stock). Also, many noncucumber products are included: dilled tomatoes, sweet peppers, and fresh vegetable relishes that are prepared from uncured stock.

Spoilage occurs in these classes of products when they are improperly pasteurized, and is due chiefly to yeasts and/or acid-forming bacteria that survive faulty heat treatment. Molds and film yeasts are factors principally in cases of poor jar closure.

Examination of the liquor from the products of this class should be made as set fourth under I, B. Finished Pickle Products.

The significance of organisms found in the products is essentially the same as described under B. Finished Pickle Products. 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 improper processing be recognized and corrected.

II. Salted and Brined Vegetables for Nonpickle Use

A. *Salted Vegetables*

Most of the vegetables, after blanching, are preserved according to the dry salt method,[11] using a ratio of 1:5 with respect to salt and vegetable weight, and stored in tightly-headed wooden casks, preferably at refrigerator temperature within the range of 1.7° to 4.4° C (35–40° F). Vegetables treated in this manner are generally green peas, corn, snap beans, cut okra, and cut celery. These salted vegetables are used in preparation of soups, mixed vegetables, and strained vegetable products.

B. *Brined Vegetables*

Products such as whole okra, whole celery, and sweet red peppers are usually brined at about 20 per cent salt concentration rather than by the dry-salt method. They are used in the same manner as the above salted vegetables.

Microbiological Examination—Brine samples from both A and B types of these vegetables should be examined for: total 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 I, A Salt-Stock Vegetables and Genuine Dills.

1. *Significance of Observations*—In these products, gaseous fermentation is associated with active development of coliform bacteria, yeasts, and obligate halophiles, all of which can tolerate the high salt concentrations (15 per cent 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 may also 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.

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

Microbial Group Involved	Culture Medium	Classes of Products in Which Microbial Group Is Likely to Be Present *	Remarks Concerning Microbial Groups
Total count	Nutritive caseinate agar (46)	All classes of products IA and B, and IIA and B	For determination of general microbial populations. In pasteurized products helps to indicate the effectiveness of the treatment.
Acid-forming bacteria	Nutritive caseinate agar (46) V-8 Medium (72)	IA: Fermenting salt-stock vegetables and genuine dills. IB: Finished pickle products. IC: Pasteurized pickle products.	Acid fermentation. Salt-tolerant up to 15 per cent; not likely to be found in brined and salted vegetables above this concentration. (IIA and B)
Salt-tolerant cocci	Nutritive caseinate agar (46)	II: Brined and salted vegetables for nonpickle use; also, 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 (12), violet red bile agar (73), or desoxycholate agar (16)	IA: Fermenting salt-stock vegetables and genuine dills. IIA and B: Brined and 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.

* Refer to outline for more detailed classification of products listed under IA, B, and C, and IIA and B.

Table 1—(Continued)

Microbial Group Involved	Culture Medium	Classes of Products in Which Microbial Group Is Likely to Be Present *	Remarks Concerning Microbial Groups
Obligate halophiles	Liver broth plus salt (33)	IIA and B: Brined and salted vegetables for nonpickle use. Also, in other vegetable brines at high salt concentration.	Gaseous fermentation. Group requires 5–15 per cent salt in culture medium and reduced oxygen tension. Sensitive to acid. General information on behavior not well known.
Fermentative yeasts, film yeasts and molds	Dextrose agar (acidified) (17) Dextrose broth plus salt † (19)	All classes of products (IA, B, and C, and IIA, B), 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 (32)	Uncommon in brined and salted vegetables; examination should be made if malodorous fermentation is detected.	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 IA, B, and C, and IIA and B.
† For culturing film forming yeasts in general.

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 carefully examined, as it is likely that they will not be acid-producing bacteria of the lactic 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 where 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.

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 greatly restricted. However, at salt concentrations of approximately 10 per cent strength the cocci may grow rapidly at about 1.7° C (35° F).

SUMMARY OF PROCEDURE

A summarization 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.

DISCUSSION ON USE OF CULTURE MEDIA AND TYPES OF MICROORGANISMS

I. Nutritive Caseinate Agar (46)

This medium has been found very useful because several types of bacteria can be detected and enumerated on the same Petri plate, thus saving time, effort and glassware. It can be used for enumeration of total bacterial count, acid-forming bacteria and salt-tolerant cocci, and for determining population trends of acid-producing bacteria in dill pickles,[12] salt-stock,[9, 13] improperly pasteurized fresh cucumber pickle,[14] and in the storage of salted and brined vegetables.[15, 16]

Since this medium contains less agar than usual solid media, care should be exercised in amount poured per plate. Not over 15 ml should

be used to insure solidification and prevent dropping of agar when plates are inverted. During hot weather plates should be cooled prior to inversion and incubation.

In the presence of bromcresol purple, acid-forming bacterial colonies show a zone of precipitated casein and a yellow halo. 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 cases of doubt, stained preparations should be made. Occasionally a high percentage of tiny (0.1 mm or less) acid-forming colonies is found in certain vegetable fermentations. Growth of these poor lactose-fermenters is often greatly enhanced by addition of 0.1 per cent dextrose to the medium. The additional dextrose will likewise enhance the growth of yeasts if present in the sample. For this reason careful examination for yeasts should always be made when the dextrose supplement is added to nutritive caseinate agar.

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 usually found 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.

II. V-8 Medium (72)

For determining numbers of lactobacilli, Fabian, et al.,[17] 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 the lactose. The bromcresol green is inhibitory to most of the nonacid formers.

III. Brilliant Green Lactose Bile Agar (12)

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 preparation just prior to use is preferred. 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 (31).

IV. Dextrose Agar (Acidified) (17)

This medium is preferred over malt agar (36), for detecting yeasts in fermenting vegetable brines [18] since it is more inhibitive to the lactic acid types of bacteria. Occasionally yeasts that will not grow on this medium are found in high salt concentrations, 15 to 20 per cent.[19] By reducing the tartaric acid to 3 ml per 100 ml growth can often be obtained. However, this modification should not be used where the salt concentration is known to be below 15 per cent, since acid-forming bacteria will grow sufficiently to make counting of yeasts very difficult.

Mold colonies are readily distinguished 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, Zygosaccharomyces, Candida* and *Pichia* [20, 21]) are generally dull and very rough as contrasted to the usually round, raised, white, glistening colonies of the fermentative, subsurface yeasts (i.e. species of *Torulopsis, Brettanomyces, Hansenula, Zygosaccharomyces,* and *Torulaspora* [22-24]). However, even where distinguishing colony characteristics of the 2 yeast groups exist they are not considered sufficiently clear cut for separation. Because of this, the procedure outlined under I, A (Film Yeasts) should be used. Film yeasts rapidly form a heavy, wrinkled surface film at one or both salt concentrations. Certain species, such as *Zygosaccharomyces halomembranis*, form heavier films at 10 per cent salt than at 5 per cent.[18, 21]

V. Liver Broth Plus Salt (33)

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.

VI. Liver Broth Medium (32)

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 neglible 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 will be obtained in old brines. Positive results in this medium would indicate that these types of bacteria were responsible for the malodorous fermentation.

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CANNED FOODS

The enormous quantity of canned foods that is manufactured and consumed makes it necessary that methods be available for the purpose of plant control, spoilage detection, guide to storage life, and general condition of products as consumed. This chapter is concerned with the canned food itself and does not attempt to go into methods for raw material, establishment of processing conditions, etc. Methods in this chapter differ from those in most other chapters in that actual enumeration of microorganisms is not carried out extensively as presence or absence of spoilage contamination is the main issue. Incubation at 37° C is carried out in most cases in the interest of obtaining results quickly instead of the 32° C incubation used for most of the other types of food.

I. GENERAL CONSIDERATIONS

Microbial spoilage of canned foods is due either to underprocessing or leakage.[1, 2] Underprocessing is the failure to destroy during the heat process all bacteria capable of subsequent growth in the product. Leakage is the contamination of the product after an adequate heat process by reason of a faulty seal.

A. Underprocessing

In spoilage due to underprocessing, there is a limited number of bacterial groups of importance and the limitation exists through the nature of the canning process. Products of low acidity, of which corn and peas are examples, are processed at high temperatures in steam under pressure. This procedure has the effect of eliminating all bacteria except those having spores of exceptionally high resistance to heat. Products of high acidity, of which tomatoes and fruits are examples, are inhibitory to most of the highly heat-resistant bacteria, and, consequently, much less severe heat processes need be used. In either case, the acidity (usually expressed as pH) of the food is an important factor in determining the type and character of spoilage.

Recommended Methods for the Microbiological Examination of Foods

The Subcommittee on Methods for the Microbiological Examination of Foods has prepared this report. It has been reviewed by the Coordinating Committee on Laboratory Methods and recommended for publication. Publication has been authorized by the Committee on Evaluation and Standards of the American Public Health Association.

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