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Procedure for Bacteriological Examination of Brined, Salted, and Pickled Vegetables and Vegetable Products*

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THE tentative methods described are presented for the examination of certain brined, salted, and pickled vegetables and vegetable products. The products given consideration are broadly classified as follows:

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 vegetable)
4. Vegetables other than cucumbers (onions, peppers, tomatoes, etc.)

II. BRINED AND SALTED VEGETABLES FOR NON-PICKLE 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)

General directions for collection, storage, transportation, and preparation of samples are given for the products as a whole. Individual discussion of products (IA, B, and C; IIA, and B) is presented chiefly as to: Introductory material of descriptive nature; microbial groups involved; significance of observations; and other remarks incident to the bacteriological examination. Directions for the preparation and use of differential culture media employed in the examination of the products are combined under a separate

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section. The media as listed are: BG, Brilliant Green Lactose Bile Agar; NC, Nutritive Caseinate Agar; AD, Acidified Dextrose Agar; LBS, Liver Broth Plus Salt; and LB, Liver Broth without Salt.*

Procedure for microscopic examination and directions for determination of acidity, pH and salt are given from a general standpoint.

It should be pointed out that the bacteriological methods of examination presented herein are based essentially on methods previously used by the authors in studying the predominating microbial changes occurring in certain brined and salted vegetables undergoing natural fermentation. They have also been used to some extent in the examination of certain manufactured pickle products undergoing spoilage as evidenced by active fermentation of one or more microbial groups.[1]

For the types of material receiving consideration, examination for one or more of the following general classes of non-pathogenic microörganisms† should be given attention: Acid-forming bacteria, coliform bacteria, yeasts, molds, mycoderma,‡ salt-tolerant cocci, and obligate halophiles. Also, for any specific case involving a malodorous, butyric acid fermentation, examination for butyric acid bacteria should be included.

An incubation temperature range of 32–35° C. is suggested for the mesophilic groups dealt with in this report. However, 35° has been found satisfactory for adequate growth of the eight groups of microörganisms that might be involved in various products listed. While it may be argued that a few degrees above or below 35° C. may be slightly better for the cultivation of a certain group, it does not seem sufficient cause wholly to justify recommendation of three or more specific incubation temperatures (32°, 35°, and 37° C.) to cover the mesophiles involved. Also, the diversity of standard incubation temperatures that have been suggested for growth of microörganisms in the examination of food products (particularly mesophiles having similar temperature requirements), is such that the average laboratory, especially those in the field, finds it impracticable, if not impossible, to meet these requirements, due to limited incubator facilities. Furthermore, the use of a temperature range of 30–32° C. in certain sections of the country cannot be maintained for about 4 months of the year without an incubator equipped with a refrigerator unit.

COLLECTION, STORAGE, AND PREPARATION OF SAMPLES

Collection of Samples — Brine or pickle liquor covering the vegetable material is required for examination. The size of the 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 sev-

* When a specific medium is referred to in the text for use in determining a group of organisms, it is identified for reference purposes by the proper abbreviation (e.g., BG for Brilliant Green Lactose Bile Agar) in parenthesis.

† The possibility of food poisoning microörganisms (and other pathogens) occurring in pickled, brined, or salted vegetables and vegetable products is generally considered somewhat remote. For this reason, methods of examination for these groups are not dealt with in this report. In event such specific examinations are required, accepted procedures should be used.[2]

‡ For convenience, the term "mycoderma" is used herein to denote the film-forming yeasts responsible for the luxuriant surface growth on cucumber pickle brines exposed to the air but sheltered from direct sunlight. While the expression is misleading, it is in common usage in the pickle industry. Actually, scum formation in the products to be discussed is probably not limited to the asporogenous yeasts of the *Mycoderma* genus but may well include sporogenous yeasts of one or more of the remaining genera (*Hansenula, Debaryomyces, Pichia, Zygopichia*) of film-forming yeasts.[3, 4]

eral 1/16" holes for a distance of 6–8" from the sealed end) is inserted through an opening in the false head, down into the brine toward the center of the vegetable material. The brine sample is withdrawn through a previously attached piece of rubber tubing into a 12 oz. juice bottle. The receiving bottle is fitted 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 the siphoning action. The length of the steel sampling tube to be used will be governed by the depth of the container to be sampled.

Approximately 24 oz. are withdrawn before taking the final sample (about 10 ml.) in a sterile test tube. If microbial changes during the fermentation are to be followed, sampling should be started at the time the material is salted or brined and continued at regular intervals (one to two days) during active fermentation.

For tightly headed barrels, such as are used for genuine dills and salted vegetables for non-pickle use, the sample should be taken through the top or side bung.

For smaller containers, such as jars or cans of pickle products, the sample, after a thorough shaking, is taken directly from the center of the material by use of a sterile pipette. Tops of metal cans should first be washed with alcohol, flamed, and punctured. A "Canco" beer can opener is very useful for puncturing metal tops. If the containers show evidence of gas pressure, first release the gas carefully by puncturing the top with a flamed ice pick.

Brine samples for subsequent chemical determinations can be preserved by the addition of sodium 2,4,5 trichlorphenolate (Dow) sufficient to make a dilution of about 1–10,000 as described by Veldhuis.[5] The samples are collected in standard crown finish bottles, such as beer bottles or soft drink bottles, of convenient size (6 to 12 oz. capacity). About 10 drops of a 10 per cent aqueous solution of the chemical is added for 12 oz. samples. The bottles are capped with 26 mm. crown closures, using a hand operated beer bottle capper, and are shaken to distribute the chemical. Crown closures having cork or composition cork liners alone are not satisfactory for prolonged storage of samples. For this purpose, caps having an additional "spot" liner of the "panaseal" or "vinylite" type should be used.*

Samples preserved with the above chemical compound are unfit for human consumption.

Storage of Samples—Brine samples from actively fermenting material cannot be satisfactorily stored or held under refrigerated conditions without marked changes taking place in the microbial flora present. For this reason, samples should be examined promptly after collection. The same is true for samples of packaged pickle products.

Preparation of the Sample—Suitable decimal dilutions of the brine or pickle liquor samples are made in the usual manner. These are plated on the various differential solid media, or inoculated into liquid media described under Preparation and Use of Media.

MICROSCOPIC EXAMINATION

Microscopic examination of the samples is helpful at times, particularly when carried out in conjunction with

* The same type of bottles (either amber or clear glass) and closures are suggested as containers for bacteriological media, especially for field work. The media are bottled at 65–70° C., capped, and then sterilized in the usual manner. About 250–300 ml. are put in a 12 oz. bottle. For media which are to be used in a relatively short time, bottles with screw type finish for use with caps having rubberized liners have been found more convenient to use. A bottle of melted medium can either be transferred to a sterile flask and promptly cooled for pouring, or, it can be allowed to cool in the bottle and poured. Bottles just out of the steamer should not be plunged into cold water.

plate count observations. Direct counts can be made if desired. The following procedure is suggested: Place 0.01 ml. portions of the fermenting brine or liquor in sequence on slides at each sampling interval. Where extremely high populations of organisms are suspected, a 1:10 dilution of the sample should be used. The smears are prepared and counted according to the method of Wang,[6] a modification of the Breed[7] technic. The preparations are stained according to the Kopeloff and Cohen[8] modification of the Gram stain. The actual counts are made on the basis of the numbers and morphological types of individual Gram-positive and Gram-negative cells present per ml. of brine.

TITRATABLE ACIDITY AND pH

Determinations of titratable acidity and pH of the samples are extremely useful in providing supplementary information in bacteriological analysis. Titratable acidity is determined on 5 or 10 ml. amounts of sample. The sample is diluted with 30–50 ml. of distilled water, brought just to boiling, cooled, then titrated with 0.10 N NaOH, using phenolphthalein as the indicator. For brine samples, the values should be calculated in terms of grams lactic acid per 100 ml. of sample; for liquor samples from packaged pickle products, in terms of acetic acid. Where only a small amount of original sample is available, a 2 ml. sample can be used for titration purposes. The pH determinations are made with the glass electrode.

SALT

In the microbiological examination of brines, knowing the approximate salt content is often helpful. This can be obtained by use of a salometer providing sufficient sample is available (about 100 ml.). For small amounts of sample, a chemical test for salt[9] is required.

EXAMINATION PROCEDURE

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 types of 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*

Examine the fermenting brines by the plating technique with respect to the following: Total count (NC); coliform bacteria (BG); lactic acid bacteria (NC); and yeasts (AD). For brines of high salt concentration, 15 per cent salt and above, consideration should also be given to the salt-tolerant cocci (NC) and obligate halophiles (LBS). Under curing and storage conditions where the brine surface is exposed to the air and sheltered from direct sunlight, examine for molds and mycoderma (AD). In any instance where a malodorous fermentation is observed, examine brine samples for the butyric acid group (LB).

Significance of Observations — The acid fermentation, resulting from active growth of the lactic acid bacteria, is to be expected at brine concentrations below 15 per cent strength.[10] The developed brine acidity in combination with the salt results in preservation of the salt stock and genuine dills.

Activity by the coliforms 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 both groups of organisms are extremely salt-tolerant

(20 per cent or above), the coliform bacteria are not acid-tolerant and are not usually found in brines having appreciable acidity. The exception to this may be found in cases of highly buffered material, such as dry-salted peas.

Luxuriant growth of mycoderma scum may occur at various salt concentrations and will result in loss of brine acidity. When molds accompany scum growth, the texture of the vegetable material may be seriously affected. Heavy scum 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. With these products, preservation is dependent upon sufficient amounts of vinegar alone (for sour pickles) or a combination of vinegar and sugar (for sweet pickles) being used. If such is not the case fermentation principally by two groups of organisms—the lactic acid bacteria and the yeasts—will usually take place. Molds and mycoderma may also grow on the surface of the liquor, chiefly as the result of faulty jar closure.

Examination of the liquor sample should be made for: Total count (NC); acid-forming bacteria (NC) and yeasts, molds, and mycoderma (AD). In undisturbed sample jars, the growth of surface mold and scum growth may be obvious. It should be carefully removed after first recording the extent of growth, since if shaken up with the sample, it will only complicate the counts for acid formers and yeasts when the latter groups are present. Examination for coliform bacteria, salt-tolerant cocci, halophiles, and butyric acid bacteria is not normally required due to the acidity of these products.

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-types that remain inactive in the acid medium of the pickle liquor.

Significance of Observations—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 off jars having vacuum type closures; to break jars having screw-type lids; or burst sealed cans.[11]

The acid content of the liquor may be increased due to the activity of the acid-producing bacteria. Also, whole pickles may become "bloaters" (hollow) due to the gaseous fermentation by yeasts and/or gas-producing types of acid-producing bacteria.[11]

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

C. *Pasteurized Types of Pickle*

In general, pasteurization is required. (165° F. for 15 min.) for pickle products that do not contain sufficient amounts of added vinegar and sugar to stop fermentation by certain organisms. There are probably a dozen or more different types of cucumber pickle that fall in this classification, such as various types of fresh dills; fresh, sliced cucumber pickle; and low-acid and -sugar sweet pickle (from salt stock). Also, many non-cucumber products are included (e.g., dilled tomatoes, sweet

peppers, and fresh vegetable relishes) that are prepared from uncured stock.

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

The examination should be conducted as described under "B" (Finished Pickle Products). Also, the significance of results is essentially the same as described under that heading.

II. BRINED AND SALTED VEGETABLES FOR NON-PICKLE USE

Brined and salted vegetables such as green peas, corn, snap beans, okra, and celery are included in this classification. They differ from products previously mentioned in that they are used in the preparation of soups, mixed vegetables, and strained vegetable products.*

Most of the vegetables, after blanching, are preserved according to the dry-salting method,[12] 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.). Celery and okra in the whole state are usually brined at about 20 per cent salt concentration rather than dry-salted. The same is true for sweet red peppers.

Brine samples should be examined for: Total count (NC); acid-forming bacteria (NC); coliform bacteria (BG); salt-tolerant cocci (NC); yeasts, molds, and mycoderma (AD); and obligate halophiles (LBS). In cases where malodorous fermentation is suspected, examine for the butyric acid group (LB). However, the brine concentrations usually encountered would be considered somewhat above their usual tolerance. The same is true for the acid-forming bacteria of the lactic group.

Significance of Observations—With these products, gaseous fermentation is associated with active development of the coliform bacteria, the yeasts, and the obligate halophiles, all of which can tolerate the high salt concentrations (15 per cent and above) normally employed for preservation. Either 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 the growth of the above groups.

High populations of the salt-tolerant cocci may be found over an extended period in the 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 carefully examined. The chances are they will not be acid-producing bacteria of the lactic group, as might first be suspected, but rather acid-producing cocci types. This is particularly true in cases where the brine concentration is above 15 per cent salt.

Growth of molds and mycoderma scum is apt to be a factor where there is air above the brine surface in the container. Unrestricted growth by molds may seriously affect the texture and flavor of the vegetable material. Heavy scum growth is undesirable, principally from the flavor standpoint. Casks should be kept filled with brine at all times, irrespective of storage temperature.

* An estimated 50,000 tons of salt preserved vegetables were used in this manner yearly during 1942–1945. Under peacetime conditions, okra, celery, and sweet red peppers are probably the chief vegetables salt preserved for non-pickle use.

As mentioned earlier, refrigerated storage (about 1.7° C.) of these brined and salted products is preferred. Under such conditions, and at salt concentrations of 15 per cent and above, microbial activity of the various groups may be greatly restricted. However, at salt concentrations in the range of 10 per cent strength, the cocci may readily grow at about 1.7° C. (35° F.).

In the absence of refrigerated storage conditions, the microbial changes may continue to take place over a period of months.

PREPARATION AND USE OF MEDIA

(BG) *Brilliant Green Lactose Bile Agar*[13]

Coliform Bacteria—The above medium, Difco formula, has been found satisfactory for estimating the relative populations of the coliform bacteria during the fermentation of a number of brined and salted vegetables. The medium is prepared and used according to the directions supplied with the dehydrated product. Strict attention should be paid to the medium's sensitivity to light and preferably it should be prepared just prior to use. When this is not convenient, the prepared medium should be stored in the dark. Subsurface colonies of coliform bacteria are deep red in color against the blue background of the medium.

Incubate plates 18 hours at 32–35° C., and record the number of coliform colonies per ml. of brine or liquor examined.

Violet Red Bile Agar (Difco formula) is also suggested for use for the enumeration of the coliform organisms. It should be prepared and used according to the directions provided with the dehydrated product. For further information as to the nature of the coliform organisms present on the plating medium used, the representative colonies can be streaked on Levin's eosin-methylene-blue agar. Identification studies on isolates of coliform bacteria from cucumber fermentation[14] showed that they belonged to the genus *Aerobacter;* members of the *Escherichia* genus were not found.

(NC) *Nutritive Caseinate Agar (Difco)*

This medium has proved most successful for determining population trends of acid-producing bacteria during the fermentation of: dill pickles[15]; cucumbers for salt stock[10]; improperly pasteurized fresh cucumber pickle[1]; and, also, in connection with the fermentation of a number of other brined and salted vegetables. It is also used for enumerating total count, salt-tolerant cocci, and peptonizing bacteria.

Prepare the medium for use according to the directions provided with the dehydrated product, but add 0.04 gm. of solid brom-cresol-purple per liter as an indicator. Since Nutritive Caseinate Agar contains less agar than the usual solid media, care should be exercised in the amount of medium poured per plate, as well as in cooling the plates, to avoid solidification difficulties. Not over 15 ml. of medium should be used per plate to insure a reasonable solidification time, and, during hot weather, facilities for cooling the plates will prove helpful.

Acid-forming Bacteria—These organisms show a zone of precipitated casein and a yellow color about the colony on Nutritive Caseinate Agar. The degree of casein precipitation and change in indicator may vary, depending on the activity and type of acid-former present. Subsurface colonies range in size from about 0.5 to 2.5 mm. and are mostly elliptical in shape. Surface growth is usually poor.

As a rule, yeasts, other than lactose-fermenters, do not grow out well on Nutritive Caseinate Agar. Furthermore, yeasts tend to give a slightly alkaline reaction to the medium, as contrasted with the acid-forming bac-

teria just mentioned. In cases where doubt exists as to whether yeast counts are being confused by the presence of acid-forming bacteria, the colonies should be stained.

Occasionally a high percentage of tiny (0.1 mm. or less) acid-forming colonies is found in certain vegetable fermentations. The growth of these poor lactose-fermenters is often greatly enhanced by the 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.

Salt-tolerant Cocci—While Nutritive Caseinate Agar is not considered a differential medium for the salt-tolerant coccus forms, it may be used for estimating the numbers of these organisms present in brines of high salt concentration. Differentiation is based on the characteristics of the colonies on the agar and by stained preparations from the principal colonies present. In routine examination of brines of high salt concentration, these organisms, as a rule, are the principal types found on the above mentioned medium. Their presence is usually indicated by two predominating types of colony—a greyish white, entire, glistening colony of moderate size and a similar colony that is light orange to yellow in color. The subsurface colonies are elliptical to lenticular in shape. The cells of the white colony type are distinctly smaller than those of the pigmented variety. Due to their sensitivity to brine acidity, they are not usually encountered in fermentations where active growth of the acid-forming bacteria has taken place (i.e., brines containing up to 15 per cent salt by weight). Deep subsurface colonies of some of the cocci may give an acid reaction to the indicator in the medium, but on prolonged incubation the reaction becomes alkaline. In highly buffered salted vegetables, at salt concentrations bordering on the range of salt tolerance for the acid-forming bacteria, care should be exercised that such acid-producing colonies are not recorded as true acid-producing bacteria of the lactic group.

The plates are incubated 3 days at 32–35° C. and first counted as to total colonies, acid-forming colonies, and cocci colonies per ml. of brine or liquor examined. For an estimate of the number of peptonizing bacteria, the plates (after recording total, acid-former, and cocci counts) are then flooded with a 5 per cent solution of glacial acetic acid. The colonies surrounded by clear zones are recorded as peptonizing bacteria.

(AD) *Acidified Dextrose Agar*[16]

Yeasts—Yeast populations in fermenting vegetable brines[17] and pickle products[1, 15] can be detected by the use of Acidified Dextrose Agar. This medium consists of ordinary Dextrose Agar (Difco formula) to which 5 ml. of sterile 5 per cent tartaric acid is added per 100 ml. of the melted agar prior to pouring the plates. The addition of the tartaric acid brings the pH of the medium to the range of 3.5–3.7 and thereby inhibits active development of the other usual brine organisms.

Yeasts in High Salt Brines—Occasionally yeasts are found at high salt concentrations, 15 to 20 per cent, that will not grow well on the medium containing the amount of tartaric acid indicated above.[14] A medium containing 3 ml. rather than 5 ml. of the acid per 100 ml. of melted agar usually corrects this condition. However, the modified medium should not be used where the salt content of the sample is known to be much below 15 per cent because acid-forming colonies, if present, will grow sufficiently to confuse the yeast count. During the active fermentation

TABLE 1

Guide to the Bacteriological Examination of Certain Brined, Salted, and Pickled Vegetables and Vegetable Products

Microbial Group Involved	*Culture Medium Used (and Abbreviation)*	*Classes of Fermenting Products in Which Microbial Group Is Likely to Be Present*[2]	*Remarks Concerning Microbial Groups*
Coliform bacteria	Brilliant green lactose bile agar (BG) or violet red bile agar	IA: Fermenting salt stock vegetables and genuine dills. IIA&B: Brined and salted vegetables for non-pickle 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.
Acid-forming bacteria	Nutritive caseinate agar[1] (NC)	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&B)
Salt-tolerant cocci	Nutritive caseinate agar	IIA&B: Brined and salted vegetables for non-pickle 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.
Yeasts, mycoderma[3] and molds	Acidified dextrose agar (AD)	All classes of products (IA, B,&C, & IIA&B) for yeasts. Molds and mycoderma on liquid surface of products exposed to air and sheltered from sunlight.	Yeasts: gaseous fermentation; acid- and salt-tolerant. Molds and mycoderma: acid- and salt-tolerant; both groups utilize acid of products and require free oxygen for growth.
Obligate halophiles	Liver broth plus salt (LBS)	IIA&B: Brined and salted vegetables for non-pickle use. Also, in other vegetable brines at high salt concentration.	Gaseous fermentation. Group requires about 15 per cent salt in culture medium and reduced oxygen tension. Sensitive to acid. General fermentation behavior not well known.
Butyric acid group	Liver broth plus particles (without salt) (LB)	Uncommon in brined and salted vegetables; examination should be made if malodorous fermentation is detected.	Malodorous, gaseous fermentation. Not particularly acid- or salt-tolerant. Active fermentations rare in properly brined or salted vegetables.

[1] Also used for total plate count.
[2] Refer to outline for more detailed classification of products listed under IA, B, and C, and IIA and B.
[3] Refers to film-forming yeasts in general.

of high-acid producers of the lactic group, some tiny colonies may begin to show through, even when the full amount of tartaric acid is used. In such cases, numerous small, undeveloped acid-producing colonies form a halo effect about the yeast colonies where the acid reaction is presumably of less concentration than the surrounding medium. Acidified Dextrose Agar is definitely preferred for detecting yeasts in brines rather than Wort or Malt Agar, since the pH values (about 4.5 and 5.5, respectively) for the latter media are much less inhibitive for the lactic acid bacteria.

Mycoderma and Molds — Certain conditions, i.e., available free oxygen and absence of direct sunlight, may permit active growth of mycoderma scum and molds on the surface of brined and pickled material. These organisms will grow out on Acidified Dextrose Agar. Molds and yeasts can readily be distinguished by the difference in the appearance of their colonies, whereas routine differentiation of yeasts and mycoderma may present some difficulty. An estimate of the numbers of the scum-yeast group occurring in the brine or liquor sample, mostly as the result of surface scum development, can be made as follows: Surface colonies of yeasts indicative of mycoderma scum are normally flat, dull, irregular and spreading as contrasted with raised, round, white, glistening and entire brine yeast colonies. Subsurface scum yeast colonies appear white and fuzzy, whereas brine yeasts are mostly elliptical and entire. Mycoderma scum colonies when stained appear to be made up of rather large, irregular, elongated cells. In the absence of storage conditions suitable for scum growth, such colonies are rarely found.

Incubate plates 3 days at 32–35° C. and record number of yeast, mycoderma, and mold colonies per ml. of brine or liquor examined.

When yeast colonies are not well developed within 3 days, the incubation period is extended to 5 days.

(LBS) *Liver Broth Plus Salt*

Obligate Halophiles—This medium is intended for the detection of the gas-producing, Gram-negative, obligate halophilic bacteria that have been observed by the authors in dry-salted and brined corn at 17 and 21 per cent salt respectively. Either Liver Agar containing 15 per cent salt, or Liver Broth Plus Salt can be used. The liquid medium is probably more convenient to handle. The basic liver medium is prepared according to the following formula of Cameron [18]:

> Ground beef liver is mixed with water in the proportion of 500 gm. to 1,000 ml. This mixture is boiled slowly for one hour, adjusted to approximately pH 7.0, and boiled for an additional 10 minutes, after which the boiled material is pressed through cheese cloth and the liquid is made to 1,000 ml. To the broth are added 10 gm. of peptone and 1 gm. of dipotassium phosphate. The reaction is adjusted to pH 7.0 . . .

Fifteen per cent salt (c.p.) by weight is added to the broth which is readjusted to pH 7.0 and then put in 5/8" x 6" tubes containing about 1/2" of partially dried liver particles remaining from preparation of the broth. The tubes are autoclaved at 15 lb. pressure for 20 minutes. For Liver Agar add 1.25 per cent agar to the broth, bring to a boil to dissolve, tube, and sterilize. Tubes of the media, previously boiled and cooled, are inoculated with decimal dilution of the sample and sealed with 1–2 ml. of sterile, melted petroleum jelly. Positive tubes are indicated by raising of the petroleum seal due to gas production and absence of any distinctive odor.

Incubate tubes at 32–35° C. for 1 week and record positives daily.

No interference has been encountered to date by the growth of the coliforms or yeasts in this medium during the test

for obligate halophiles. Such might be anticipated, provided they were present in the sample, since both groups grow readily in fermenting vegetable brines at high salt concentration, and both are gas producers. The lack of interference is presumably due to their known inability to initiate satisfactory growth in laboratory media at even moderately high salt concentration without previous subculturing. Also, the reduced oxygen tension of the medium probably exerts a retarding effect. Nevertheless, it is desirable to make routine determinations for the yeasts and coliform bacteria, including stained preparations, on any brine that is being examined for obligate halophiles.

(LB) *Liver Broth Plus Particles (without salt)*

Butyric Acid Group — Liver Broth Plus Particles is recommended [18] for the detection of saccharolytic and putrefactive mesophilic anaerobes. For brine samples, neutralize the acidity with an excess of sterile calcium carbonate, and heat a 50 to 100 ml. sample in a water bath to 80° C. for 20 minutes so that only viable spores remain. Inoculate decimal dilutions of the heated sample into previously boiled and cooled tubes of Liver Broth Plus Particles, and stratify with melted sterile petroleum jelly or plain agar. Positive cultures are indicated by gas production and a strong butyric acid odor; the more putrefactive anaerobes give a putrid odor, and may decompose the liver particles.

Incubate tubes at 32–35° C. for one week, observe daily, and record positives.

A positive test merely gives presumptive evidence as to the presence of mesophilic, sport-forming, gas-producing anaerobes. For further information as to the saccharolytic or putrefactive nature of the cultures, specific bacteriological tests are required on the isolates from positive tubes.

It should also be pointed out that the test for growth of spores from boiled samples for certain members of this group of organisms is not necessarily a reliable index to their previous activity. Spore formation may be negligible prior to inhibition or death of the vegetative cells, due to acid production in the presence of a readily fermentable carbohydrate. Nevertheless, active growth would be associated with a malodorous fermentation.

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.

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