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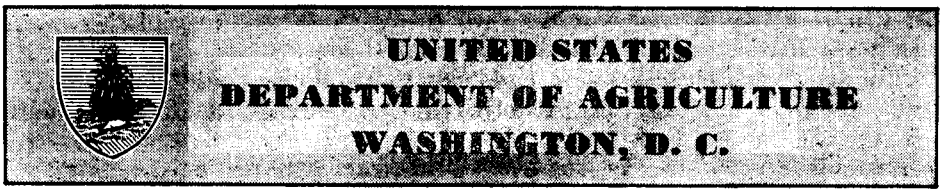

Bacteriological Changes During the Fermentation of Certain Brined and Salted Vegetables[1]

By JOHN L. ETCHELLS, *Bacteriologist, Agricultural Chemical Research Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration,* IVAN D. JONES, *Biochemist in Horticulture, North Carolina Agricultural Experiment Station,* and WILLIAM M. LEWIS, *Formerly Scientific Aide, Agricultural Chemical Research Division, Bureau of Agricultural and Industrial Chemistry*

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INTRODUCTION

The preservation of vegetable material by salting or brining is usually accompanied by fermentation that is characterized by chemical changes induced by microbial activity. The salt exerts a selective

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action on the normally occurring micro-organisms, resulting in a general fermentation that develops as the consequence of the growth of one or more surviving salt-tolerant groups. These organisms use as their nutritive material the soluble constituents that diffuse into the brine as a result of the action of salt on the vegetable tissue. As a further effect of microbial growth, various compounds, such as lactic and acetic acids, alcohols, and gases, may be formed as end products. The character of the fermentation proper, with respect both to the predominating microbial groups involved and the end products formed, is greatly influenced by the salt concentration employed in the preservation treatment, as well as by the type of vegetable being preserved.

Bacteriological investigations on vegetable fermentations during the past 40 years have dealt chiefly with the preservation of cucumbers for subsequent use as pickle products, and with the production of sauerkraut from cabbage. The principal emphasis has been placed on that phase of the fermentation brought about by the lactic acid bacteria.

The bacertiological work on sauerkraut has been adequately reviewed by Pederson in connection with rather comprehensive studies of the floral changes during the fermentation of that product (*23*, *24*, *25*).[2] Briefly, Pederson's findings showed that the developed acidity resulted from growth by gas-forming cocci of the *Leuconostoc* genus, and nongas-forming and gas-forming bacilli of the *Lactobacillus* genus. In the normal fermentation these organisms occurred in the sequence mentioned above and represented the predominating microbial changes.

Previous bacteriological research with respect to cucumber fermentation has been rather thoroughly covered by Tanner and Eagle (*27*); Fabian, Bryan, and Etchells (*14*); and Etchells, Fabian and Jones (*5*). Most of the investigations reported through 1935 dealt with the character of the acid fermentation. Not until 1940 was the gaseous fermentation by yeasts clearly indicated as a part of the fermentation proper (*4*). More recently, it has been demonstrated that a gaseous fermentation phase may also be brought about by members of the *Aerobacter* genus (*3*, *5*). Identification studies (*9*) have been made by the authors on the principal acid-producing bacteria isolated from fermenting cucumber brines at approximately 5-, 7.5-, and 10-percent strength, in connection with brining work conducted under conditions typical of the pickle industry. The 49 isolates could be readily classified as belonging to the *Lactobacillus* genus and their characteristics were typical of those described for *Lactobacillus plantarum*. Vahlteich, Haurand, and Perry (*28*) also found this species in commercial cucumber fermentations. Furthermore, the identification work of Pederson (*26*) would indicate that this species of the *Lactobacillus* genus is common in various types of fermenting vegetable material.

Thus, in general, the lactic acid bacteria of the *Lactobacillus* genus, the yeasts, and members of the *Aerobacter* genus should be considered predominating groups of organisms associated with cucumber fermentations.

During the last 3 years, the salting and brining of vegetables other than cucumbers, for subsequent table use as nonpickle products, has

[2] Italic numbers in parentheses refer to Literature Cited, p. 60.

been investigated (*7, 8, 11, 13, 20, 29*). Such vegetables as green peas, green beans, corn, okra, lima beans, carrots, and certain leafy vegetables have been given attention in the studies reported. The work was stimulated by the need for additional technical information concerning this method of preservation, in order that its application might be extended to a wider range of vegetable materials. With a clearer understanding of the physical, chemical, bacteriological, and nutritive value changes involved, the salting and brining process can be used as a supplementary method of vegetable preservation for both home and industry.[3]

The publications on the brining of vegetables have not emphasized the changes in the predominating bacterial flora associated with the various stages of the general fermentation of vegetables destined for use as nonpickle products. The investigations reported by Fabian and Blum (*13*), Wadsworth and Fabian (*29*), and Etchells, Jones, and Hoffman (*11*) were primarily concerned with the effect of the salting and brining procedures employed, on the general quality of the vegetables studied.

Fabian and coworkers (*13, 29*) discussed the fermentation changes in peas, corn, lima beans, green beans, and okra at three brine concentrations (10-, 15-, and 20-percent), essentially on the basis of total counts and acid-producing bacteria. In that investigation, little attention was given to the nature of the flora occurring when the vegetables were brined at 15- and 20-percent salt concentrations, but in the vegetables brined at 10-percent salt and gradually raised to 15-percent salt, the acid-producing bacteria were found to predominate.

While total counts may give a reasonable indication of the extent of microbial activity in brines, a break-down with respect to the types of groups of predominating brine organisms is necessary to get further insight into the different phases of the fermentation. The use of differential media for detecting lactic acid bacteria particularly is of prime importance, especially at brine strengths below 15 percent, as there is little or no growth by these organisms at or above that brine strength.

In a general report on brine preservation of vegetables by Etchells, Jones, and Hoffman (*11*), the authors pointed out the principal bacteriological changes occurring in cucumber fermentations at 5-, 10-, and 15-percent salt brines. In addition, they suggested that for other vegetables the basic fermentation changes would probably be similar in nature to the cucumber fermentation. In the following pages data are presented which substantiate this conclusion.

SALT PRESERVATION METHODS

There are two basic methods of preserving vegetables by the use of salt. These are referred to as brining and dry-salting. In brining, fresh vegetable material is first covered with brine of a given concentration; then dry salt is added, in order to maintain the initial concentration and thus prevent dilution of the brine which would otherwise

[3] Based on information gathered during the 1943 season, several million pounds of vegetables, such as corn, green peas, green beans, celery, and okra, were salt-preserved by commercial concerns for use in food products. Substantial amounts were also preserved in the home by this method.

occur with the withdrawal of water from the vegetable. In the dry-salting method, dry or solid salt is added directly to the vegetable material. The water withdrawn when salt is in contact with vegetable tissue dissolves the salt and thereby forms brine. With both methods, salt brine is directly in contact with the vegetable tissue, and therefore the same fundamental changes may be expected to take place in the tissue and in the brine. When a small amount of solid salt or weak brine is employed, an active fermentation causing the production of a decided amount of acid usually takes place. Under these conditions, the preserving effect of the brine is obtained by the combined action of the salt and the developed acidity. On the other hand, when a large amount of solid salt or a strong brine is used, usually little brine acidity is produced. In that case the preserving effect of the brine results principally from the action of the salt.

The application of brining and dry-salting methods for preservation of different vegetables has been previously summarized (*11*) as follows:

Dry salting.—Dry salting, using a small amount of salt (2½ to 5 percent by weight) is usually employed for vegetables that are readily cut or shredded, that are high in water, and that contain enough readily fermentable sugar to support a vigorous fermentation—cabbage, lettuce, and turnips are typical examples of vegetables that are salted in this manner. Certain vegetables are best preserved when a large amount of salt (20 percent by weight) is used. Corn, lima beans, and green peas are examples of vegetables considered to be in this group.

Brining.—Brining is generally used for preserving bulky or whole vegetables and those that may be low in water content. Also, brining may be used to advantage where the effect of shrinkage on the shape and structure of the vegetables, caused by the use of dry salt, would be unduly severe. For some vegetables a weak brine plus a small amount of vinegar is used. The addition of vinegar to the brine aids in bringing about a desirable fermentation and averts possible spoilage.

Commercial packers of such vegetables as green peas, cut green beans, lima beans, and cut corn generally prefer the recommended dry-salting method (*8*), where solid salt is used at the rate of 1 pound of salt for each 5 pounds of blanched vegetable. This is commonly referred to as the 1:5 dry-salting treatment. Such a procedure, besides being well suited to these vegetables, has the added advantage of permitting the packing of considerably more material in a given-sized container than is possible with the use of the brining method.

SCOPE OF PRESENT WORK

In the work presented herein, an attempt was made to obtain a clearer insight into the nature of brine flora of vegetable fermentations by differentiation of the total populations into predominating types or groups of micro-organisms. A number of vegetables, therefore, were studied with respect to their fermentation behavior under one or more brining or dry-salting treatments. In some instances, direct microscopic counts are presented in conjunction with counts obtained by the plating technique. Changes in brine acidity are given for all fermentations; in a few instances brine pH determinations were not made. The differential plate count technique was followed in obtaining the total count and the populations of lactic acid bacteria, yeasts, and coliforms in all but six cases.

In addition, examination for mycoderma [4] populations was usually carried out on lots at weak brine strengths where growth by these organisms was apt to be a factor. Also stressed were determination of the numbers of salt-tolerant cocci, particularly in lots at strong salt concentrations, and observations of the colonial types that exhibit the ability to peptonize casein. Furthermore, observations were made for molds on all lots, but for this group only summarized results are presented. Samples for analysis were taken at as frequent intervals as possible, over as long a storage period as practicable, in an effort not to miss any important phase of the fermentation proper.

According to the plan outlined previously, 87 fermentations involving the following vegetables were studied: Green beans, green peas, green lima beans, wax beans, white corn, yellow corn, butter beans, lettuce, carrots, tomatoes, and okra. Leafy vegetables such as kale, mustard greens, turnip greens, and spinach were included, but observations were restricted to microscopic counts and developed acidity. Finally, some observations were made on several lots of brined and salted celery.

In addition to the bacteriological studies, observations on the general quality of the brined and salted vegetable material were made at intervals during the fermentation and subsequent storage period. Observations were also made during this period for evidence of malodorous conditions associated with development of the butyric acid bacteria.

The major part of the experimental work was done during 1942 and 1943.

PROCEDURE

Brining and Salting Treatments

The essential details of the brining and dry-salting treatments reported in this study are given in a series of tables presenting bacteriological data for each vegetable. Other pertinent information, such as the source, variety, amount, and prebrining treatment of the different vegetables and the size and type of container is given either in the tables, or at the time each vegetable series is discussed.

For the most part, the brining treatments used can be classified according to the following three modifications: (1) Those in which the brine concentration was gradually raised to 15 percent within 4 to 5 weeks; (2) those in which the original brine concentration was maintained by the addition of salt; and (3) those in which the vegetable material was merely covered with a brine of definite strength, without the addition of more salt. In the last modification, the final concentration of the brine covering the vegetable at equilibrium would be approximately one-half that initially used. Certain of the brining treatments were further modified by the addition at the start of small

[4] 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 (*9*).

quantities of vinegar or lactic acid, or both. The amounts and strengths of these, when used, are given in the tables.

One of two types of dry-salting treatments were used. The first treatment consisted of adding an amount of salt equivalent to a certain percentage of the weight of the vegetable. The second treatment consisted of adding an amount of salt sufficient to make a definite proportion of salt and vegetable by weight, such as 1:7, 1:5, and 1:4. These ratios would be equivalent to about 12, 17, and 20 percent of salt, respectively, when calculated on a weight basis.

Storage Conditions

The vegetables brined in 20-gallon kegs were stored under outside conditions. Some of the unsheltered, outside lots were kept in open-headed kegs while others were tightly headed at the start. The average brine temperature during curing was about 80° to 90° F., and the range of temperature during curing and subsequent storage was from 95° (high) to about 15° (low). The small-scale brining and salting experiments were carried out under laboratory conditions and the lots were stored at room temperature in 32- and 64-ounce glass jars, on which two-piece metal caps were used. During active fermentation, the jars were kept partially sealed in order to control surface scum development and at the same time allow the escape of fermentation gas. During prolonged storage, they were sealed tightly.

Bacteriological Media and Methods

Brine samples were taken for bacteriological examination by means of sterile pipettes from the approximate center of the containers. Ten milliliters were taken from the 20-gallon kegs and 1 to 2 milliliters from the 32- and 64-ounce jars. Decimal dilutions of the brine samples were examined according to the plating technique with respect to total number of bacteria, coliform bacteria, lactic acid bacteria, salt-tolerant cocci, peptonizing bacteria, yeasts, molds, and mycoderma. For the obligate halophiles, a liquid medium was used (*2*). The preparation and use of the differential media employed for estimating the numbers of the above microbial groups is described in the appendix to this report.

Microscopic Counts

Microscopic counts are reported for certain of the vegetable fermentations. These were made by placing 0.01-ml. portions of the fermenting brine in sequence on slides at each sampling interval. When extremely high populations of brine organisms were present, a 1:10 dilution of the brine was used. The smears were prepared and counted according to the method of Wang (*30*), a modification of the Breed (*1*) technique. The preparations were stained according to the Kopeloff and Cohen (*22*) modification of the Gram stain. The actual counts were made on the basis of the numbers and morphological types of individual Gram-positive and Gram-negative cells present in the fields observed.

Acidity and pH

Titratable acidity and pH determinations were made on the brine samples removed from the approximate center of the containers of fermenting material. The pH determinations were made with the glass electrode. Titratable acidity was determined on either 2- or 10-ml. aliquot amounts of the brine by titrating with 0.111 N sodium hydroxide, using phenolphthalein as the indicator. The values were calculated in terms of grams of lactic acid per 100 ml. of brine. The 2-ml. sample for titration purposes was used when it was necessary to conserve the original brine covering the vegetable material. This was the case with 32- and 64-ounce containers.

RESULTS

Brined Whole Green Beans, Unshelled Lima Beans, and Unshelled Peas

Three brining treatments were used in the preservation of whole green beans (Tendergreen), unshelled lima beans (Fordhook), and unshelled peas. The lima beans and green beans were obtained from Florida and the peas were from eastern North Carolina. Approximately 60-pound lots of each vegetable were brined at 5-, 10-, and 15-percent salt concentration in 20-gallon open-headed kegs which were maintained under out-of-door conditions.

GREEN BEANS

The principal bacteriological changes during fermentation of whole green beans at three brine treatments are shown in table 1. Only the fermentation receiving 5-percent brine treatment developed what could be called a typical acid fermentation, and this was of moderate intensity. In this case, the growth of the acid-producing bacteria took place during the first 2 weeks and brought about a brine acidity of approximately 0.60 percent, and pH of 3.2. In the 10- and 15-percent brines, less than 0.20-percent acid was produced. The gradual decrease in acidity of the three lots during storage is attributed mostly to dilution by rain and brine loss resulting from both sample removal and container leakage. This was common to all brined lots exposed in unsheltered containers. The presence of relatively few acid-producing bacteria in the 15-percent brine lot was expected, since these organisms are unable to grow to any extent at that salt concentration.

TABLE 1.—*Fermentation of 60-pound lots of whole green beans in 5-, 10-, and 15-percent salt brines [1] stored under outside conditions in unsheltered open-headed 20-gallon kegs*

Age in days	K–2 (5-percent brine)						K–4 (10-percent brine)						K–6 (15-percent brine)					
	Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity					
	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*	
0	310	200	10	0	0.01	---	130	0	100	0	0.01	---	16	3	100	0	0.01	---
2	3,500	2,500	100	0	.05	5.5	80	50	1	0	.03	5.5	110	50	1	0	.03	5.4
4	42,000	42,000	10	0	.16	4.5	19	------	1	0	.09	5.1	10	------	1	1	.07	5.1
6	31,000	31,000	0	2.6	.42	3.5	13	10	1	.1	.11	5.1	7	0	1	0	.10	5.2
8	15,000	15,000	0	15	.54	3.2	60	50	1	0	.11	4.8	8	------	.1	0	.09	4.9
10	700	700	0	3	.57	3.2	13	6	1	.1	.12	4.8	4	0	.1	0	.10	5.0
12	770	710	0	2	.54	3.2	53	53	1	1.2	.13	5.1	3	3	.1	0	.09	5.4
14	60	60	0	0	.54	3.2	148	140	1	10	.14	5.0	4	1	0	2	.11	5.3
16	30	------	0	1	.52	3.2	310	200	0	71	.14	4.7	14	7	0	0	.10	4.8
18	12	0	<.01	0	.51	3.1	140	140	1	15	.15	4.5	5	0	1	0	.10	4.9
20	17	0	<.01	0	.48	3.1	47	47	<.01	0	.14	4.5	15	1	<.01	13	.11	4.9
22	20	0	<.01	0	------	---	6	6	<.01	0	------	---	140	0	<.01	140	------	---
24	5	0	------	0	.47	3.2	1	0	------	0	.13	4.6	250	0	------	230	.12	4.6
26	2	0	------	.3	------	---	1	------	------	4	------	---	90	0	------	77	------	---
30	1	0	------	0	------	---	240	0	------	300	------	---	48	0	------	58	------	---
34	4	0	------	4	.44	3.1	250	0	------	250	.11	4.5	------	------	------	111	.09	4.6
38	6	0	------	6	.42	3.0	196	1	------	185	.09	4.5	68	0	------	50	.08	4.6
42	21	0	------	5	------	---	------	------	------	197	------	---	7	0	------	25	------	---
46	4	0	------	11	------	---	136	0	------	118	------	---	10	0	------	31	------	---

50	2	0	5		110	1		119					8		
54	0	0	0	.403.0	78	0		56	.10	4.6	33	0		33	.08	4.7
58		0	0		59	0		14			26	0		15		
63	3	0	0		5	0		3			19	0		4		
70	.1	0	0	.393.1	0	0		0	.10	4.8	.1	0		0	.08	5.0
77	.1	0	0		.6	0		0			1.2	0		0		
84	5	5	0	.363.3	.3	0		0	.09	4.9	.4	0		0	.08	5.0
98	0	0	0		0	0		0			0	0		0		
112	0	0	0	.323.4	0	0		0	.08		0	0		0	.08	
126	0	0	0		0	0		0			0	0		0		
140	0	0	0	.26	2	0		0	.04		0			0	.03	
168	2	0	0	.27	4	0		0			11	0		0		

[1] In these 3 brining treatments, the first 2 (5 and 10 percent) were gradually raised to 15 percent within 4 to 5 weeks, while the 15-percent treatment was maintained at that concentration.

[2] Mycoderma counts less than 1,000 per milliliter at each plating interval.

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As shown in the table 1, rather prolonged yeast fermentations occurred in both the 10- and the 15-percent brine treatments. A few thousand yeasts per milliliter were found at several plating intervals in the 5-percent brine, but there was no definite indication of progressive populations. It is of interest to note that in the 10-percent brine the principal portion of the yeast fermentation followed the activity of the acid-producing bacteria. Apparently, therefore, fermentable carbohydrate was available at that time, and some factor other than carbohydrate concentration accounted for the lack of development of the acid-producing bacteria in that brine.

The data on the coliform bacteria indicate that these organisms were present initially in all three lots, but showed little activity during the fermentation proper.

Since at the time of this series of experiments, observations for the salt-tolerant cocci were not a part of the routine procedure, it is possible that in some cases the total count values shown in the tables include cocci. This would be particularly true in the 10- and 15-percent brines. In the 5-percent brines the amount of acidity produced was sufficient to inhibit the cocci group.

LIMA BEANS

The fermentation results for unshelled lima beans at three brine concentrations are shown in table 2. The data indicate that active growth by the acid-producing bacteria was restricted to the 5-percent brine treatment. In this case, although the populations remained in the 10 million per milliliter range for approximately 3 weeks, the developed brine acidity was only about 0.30 percent. In the 10- and 15-percent treatments very little acid was produced. The data with respect to the acid-producing bacteria in the 10-percent lot were not clear, because of interference from the coliform group, and possibly from the cocci. These groups, rendering the plates alkaline, prevented detection of the acid-producing colonies. But judging from the rather low acid content of the brine, it would appear that the acid-producing bacteria were not the predominating organisms.

The coliforms were found in considerable numbers during the early part of all three fermentations. While there was no evidence of well-defined yeast fermentation in any of the lima bean brines, yeast activity was noted on several occasions in counts of a few hundred per milliliter, particularly in the 5-percent brine lot.

GREEN PEAS

The bacteriological changes during the fermentation of unshelled green peas at three brine strengths are shown in table 3. The period of growth of the acid-producing bacteria in the 5- and 10-percent brines is reflected in an increase in brine acidity to about 0.50 and 0.30 percent, respectively, within a period of approximately 10 and 20 days. In the 15-percent brine, there was little or no growth by these organisms and the brine acidity was somewhat less for that brine than for the others. Correspondingly lower populations of the acid-producing bacteria are shown for the 10-percent brine treatment as compared to the 5-percent.

TABLE 2.—*Fermentation of 60-pound lots of unshelled lima beans in 5-, 10-, and 15-percent salt brines* [1] *stored under outside conditions in unsheltered open-headed 20-gallon kegs*

Age in days	K–8 (5-percent brine)						K–10 (10-percent brine)						K–12 (15-percent brine)					
	Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity					
	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*	
0	120	50	100	0	0.01	---	400	(3)	>100	0	0.01	---	1,000	500	100	0	0.01	---
2	1,900	1,000	>100	0	.06	5.4	940	(3)	>10	0	.04	5.4	700	10	>1	.1	.04	5.7
4	50,000	50,000	>100	0	.18	4.4	600	(3)	>10	0	.07	5.3	400	100	>10	0	.06	5.5
6	38,000	38,000	10	0	.27	3.9	260	(3)	>100	0	.09	5.3	500	100	>10	0	.07	5.5
8	26,000	26,000	10	.3	.28	3.9	90	(3)	------	0	.10	5.0	300	300	>100	.2	.06	5.5
10	15,000	15,000	1	0	.25	4.1	160	(3)	10	.1	.12	5.2	150	80	10	.2	.07	5.7
12	29,000	29,000	>1	1.2	.27	4.3	80	(3)	1	0	.11	5.2	80	80	1	0	.08	5.7
14	16,600	16,600	1	0	.27	4.3	200	<10	10	0	.11	5.4	300	<10	1	0	.09	5.8
16	11,200	11,200	1	.9	.25	4.4	110	<10	10	0	.12	5.1	0	<10	1	0	.09	5.3
18	11,100	11,100	1	1	.23	4.5	100	<10	1	0	.11	5.3	30	0	1	0	.09	4.8
20	12,600	12,600	0	0	.20	4.5	210	20	1	0	.11	5.4	75	0	0	0	.11	4.7
22	6,700	6,700	1	0	------	---	60	0	<.01	0	------	---	30	0	<.01	0	------	---
24	2,800	2,800	------	0	.22	4.6	80	0	------	0	.09	5.4	1	0	------	0	.10	5.0
26	1,400	1,400	------	0	------	---	22	0	------	0	------	---	9	0	------	0	------	---
30	440	440	------	0	------	---	------	0	------	0	------	---	------	0	------	0	------	---
34	200	200	------	1	.22	4.6	------	------	------	0	.08	5.4	------	------	------	0	.10	4.9
38	27	------	------	0	.20	4.5	180	------	------	0	.09	5.1	80	------	------	0	.10	4.8
42	10	0	------	0	------	---	100	0	------	0	------	---	49	0	------	0	------	---

See footnotes at end of table.

TABLE 2.—*Fermentation of 60-pound lots of unshelled lima beans in 5-, 10-, and 15-percent salt brines* [1] *stored under outside conditions in unsheltered open-headed 20-gallon kegs*—Continued

Age in days	K–8 (5-percent brine)						K–10 (10-percent brine)						K–12 (15-percent brine)					
	Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity					
	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*	
46	8	0		0				0		0			4	0		0		
50				0						0						0		
54	6	0		0	0.21	4.5		0		0	0.10	5.1	7	0		0	0.10	5.1
58	4	0		0			5	0		0			4	0		0		
63	4	0		0			15	0		0			120	0		0		
70	1.8	0		0	.20	4.7	6	0		0	.10	5.3	150	0		0	.09	5.3
77	3	0		0			30	0		0			40	0		0		
84	6	2		0	.18	4.8				0	.10	5.5	24	0		0	.10	5.3
98	1	0		0				0		0				0		0		
112	2	0		0	.16			0		0	.08			0		0	.08	
126	1			0						0						0		
140	4	0		0	.12			0		0	.06			0		0	.07	
168	4	0		0				0		0				0		0		

[1] For brining details, see footnote 1, table 1.
[2] Mycoderma, counts less than 1,000 per milliliter at each plating interval.
[3] Absent in 1–100,000 dilution; not detected in 1–10,000 dilution because predominance of alkali-producing organisms.

TABLE 3.—*Fermentation of 60-pound lots of unshelled green peas in 5-, 10-, and 15-percent salt brines [1] stored under outside conditions in unsheltered open-headed 20-gallon kegs*

Age in days	K–14 (5-percent brine)						K–16 (10-percent brine)						K–18 (15-percent brine)					
	Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity					
	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*	
0	130	0	10	0	0.01	---	80	<10	100	0.5	0.01	---	70	<10	1	0.3	0.01	---
1	200	200	1	0	.01	5.2	43	18	1	0	.01	5.2	10	0	1	.1	.02	5.3
3	26,000	24,000	>100	1.6	.12	4.2	13	12	1	0	.04	5.5	13	12	.1	0	.03	5.4
5	51,000	51,000	>100	1.8	.19	3.9	10	0	1	0	.06	5.5	3	0	.1	.1	.04	5.5
7	30,000	30,000	0	61	.36	3.5	28	28	1	0	.08	4.7	6	0	1	.1	.03	5.5
9	20,000	20,000	0	8.4	.45	3.5	60	40	0	2	.18	4.3	3	2	.1	0	.12	4.5
11	21,000	21,000	0	4	.37	3.5	2,800	2,800	0	9	.22	3.8	1	1	0	0	.13	4.6
13	15,000	15,000	0	2	.36	3.6	2,700	2,700	0	1	.27	3.9	1	1	.1	0	.14	4.5
15	14,400	14,400	0	0	.34	3.7	1,590	1,590	1	0	.26	3.9	0	0	1	0	.13	4.5
17	16,200	16,200	<.01	3	.32	3.7	1,260	1,260	<.01	0	.27	3.9	0	0	<.01	0	.17	4.5
19	5,300	5,300	<.01	2	.33	3.8	1,800	1,800	<.01	3	.31	3.9	1	0	<.01	0	.17	4.5
21	2,400	2,400	<.01	0	------	---	1,160	1,160	<.01	1	.27	3.9	1	0	<.01	0	.16	4.4
23	2,000	2,000	------	0	.33	3.8	150	120	------	0	.28	3.9	3	0	------	0	.17	4.5
25	112	112	------	0	------	---	6	6	------	0	------	---	0	0	------	0	------	---
29	120	120	------	0	.32	3.9	18	17	------	0	.25	4.0	1	0	------	0	.11	4.7
33	114	109	------	1	.31	3.8	1	1	------	0	.24	4.0	6	0	------	1	.12	4.7
37	42	42	------	0	.29	3.8	4	2	------	3	.22	4.0	32	0	------	23	.12	4.8
41	3	0	------	0	------	---	3	0	------	0	------	---	26	0	------	18	------	---
45	1	0	------	0	------	---	1	0	------	3	------	---	54	0	------	96	------	---

See footnotes at end of table.

TABLE 3.—*Fermentation of 60-pound lots of unshelled green peas in 5-, 10-, and 15-percent salt brines [1] stored under outside conditions in unsheltered open-headed 20-gallon kegs*—Continued

Age in days	K–14 (5-percent brine)						K–16 (10-percent brine)						K–18 (15-percent brine)					
	Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity						Micro-organisms per milliliter of brine and acidity					
	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH	Total count	Acid formers	Coliforms	Yeasts [2]	Acidity as lactic acid	pH
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*	
49	0	0	-------	2	-------	---	1	0	-------	1	-------	---	29	0	------	27	-------	---
53	0	0	-------	0	0.29	3.8	2	0	-------	0	0.21	4.1	18	0	------	11	0.12	4.7
57	0	0	-------	0	-------	---	2	0	-------	0	-------	---	7	0	------	4	-------	---
62	2	0	-------	0	-------	---	1	0	-------	1	-------	---	3	0	------	1	-------	---
69	.4	0	-------	0	.28	3.9	2	0	-------	0	.19	4.4	.2	0	------	0	.12	5.0
76	.3	0	-------	0	-------	---	.2	0	-------	0	-------	---	1	4	------	0	-------	---
83	0	0	-------	0	.26	4.0	.2	0	-------	0	.18	4.5	0	0	------	0	.08	5.8
97	4	2	-------	5	-------	---	0	0	-------	0	-------	---	1	0	------	0	-------	---
111	11	1	-------	2	.20	4.4	29	0	-------	33	.16	---	29	20	------	0	.06	6.3
125	0	0	-------	0	-------	---	1	-------	-------	0	-------	---	3	0	------	0	-------	---
139	4	0	-------	0	.19	---	1	0	-------	0	.12	---	1	-------	------	0	.06	---
167	1	0	-------	0	.22	---	2	1	-------	0	.12	---	13	0	------	0	.08	---

[1] For brining details, see footnote 1, table 1.
[2] Mycoderma counts less than 1,000 per milliliter at each plating interval.

Yeast fermentations were found in the 5- and 15-percent brine treatments. In the former, growth started during the first few days and lasted about a week; in the latter, growth began after about a month, and continued for about 20 days. Yeasts were observed in the 10-percent brine at a number of sampling dates, but no well-characterized fermentation trend was noted.

The occurrence of the coliform group in appreciable numbers was restricted principally to the 5-percent brine treatment during the first week. The counts for these organisms in the other two lots remained in the range of 100 to 1,000 per milliliter for about 2 weeks. After that time they appeared to be absent or in very low numbers.

MICROSCOPIC COUNTS

Microscopic counts were made for the predominating acid-forming bacterial cell types in the 5-percent brine treatments for the three vegetables in this series. These are shown in figure 1, together with the plate counts for the acid-forming bacteria.

The data show that similar fermentation trends for the acid-forming bacteria were obtained by the two methods used for examining the fermenting brines, particularly during the active phase of the acid fermentation. Later, however, the microscopic counts tended to decline to a certain level and remain rather constant, while the plate counts, after the peak of activity, showed a continuous decline. During the period that the microscopic observations showed relatively constant numbers of organisms present in the brine, the stains showed the individual cells intact, well stained, and with no detectable evidence of loss of definition or other signs of deterioration. However, the plate counts appear to indicate that these cells were either nonviable or incapable of reproducing when plated out on the routine culture medium.

Three principal Gram-positive cell types occurred during the acid fermentation. These were designated as large, stout, and small bacilli. The cocci were either absent or present in relatively few numbers. The large bacilli occurred either singly or in short chains composed of two or three elements and ranged in size from 1×8 to 14 microns. The stout bacilli occurred usually singly or in pairs and ranged from 1.2×2 to 4 microns in size; they were typically blunt-ended and in many cases tended toward a cocco-bacillus type. The small bacilli varied greatly in arrangement, occurring singly, in pairs, and in chains up to five or six elements, with the cell measurements ranging from 0.75×1.5 to 4 microns.

Mixed Lot of Brined Whole Vegetables

The vegetables left over from setting up the brining operations described in the previous experiment were mixed, placed in an open-headed, unsheltered 45-gallon cask, and then covered with a 5-percent brine which was maintained at that concentration by the addition of salt. Prior to brining, the vegetables were kept under refrigerator conditions for about 10 days.

The purpose of the experiment was twofold, namely, to observe the nature of the fermentation of a mixture of vegetables, and to ascertain whether a prolonged delay between harvesting and subsequent brining

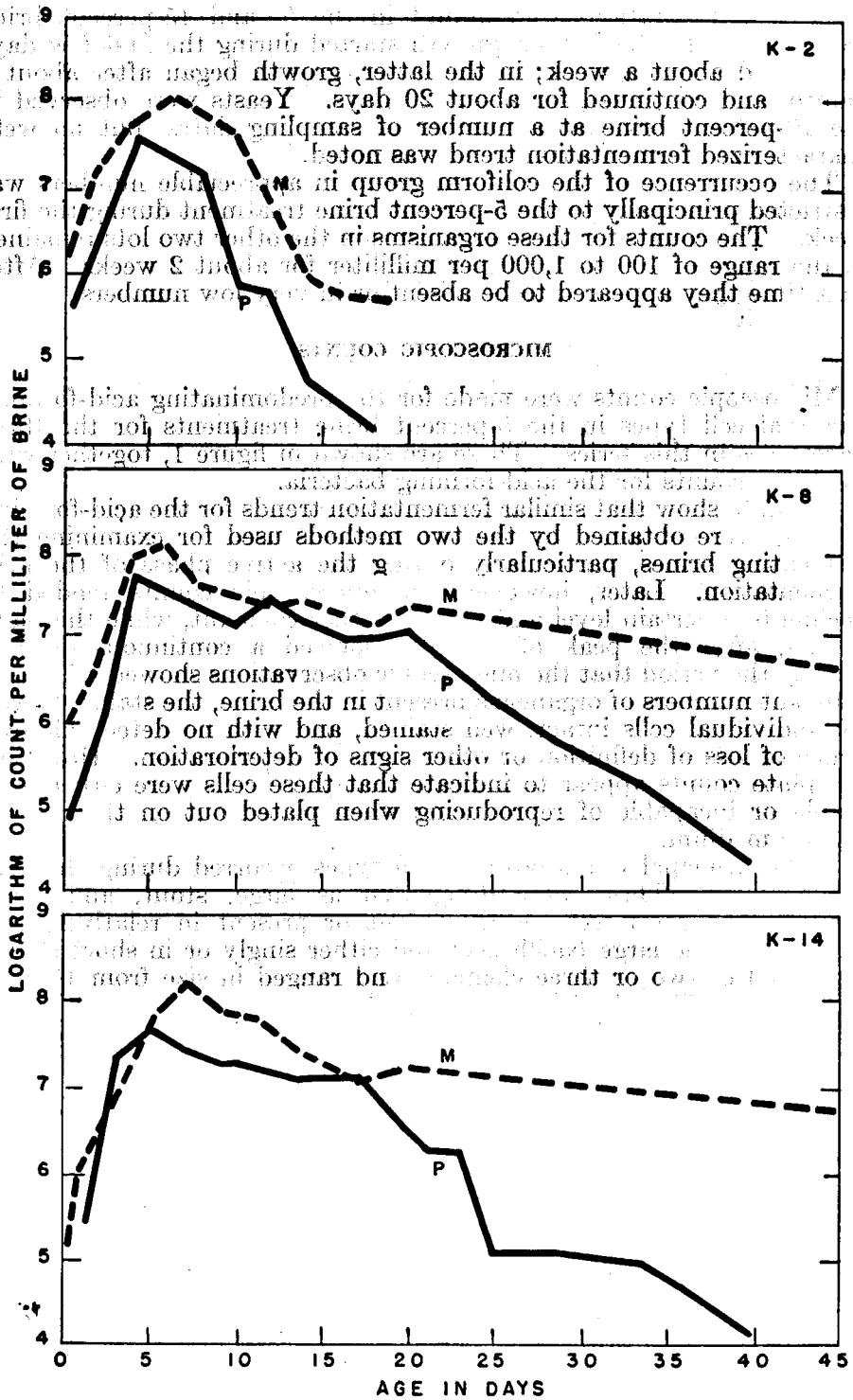

FIGURE 1.—Populations of acid-forming bacteria in brined whole green beans (K–2), unshelled lima beans (K–8), and unshelled peas (K–14), as indicated by the plate (P) and microscopic (M) count techniques.

would bring about any marked difference in the type of fermentation which would take place.

TABLE 4.—*Fermentation of a 115-pound mixed lot of whole green beans (63 pounds), unshelled lima beans (18 pounds), and unshelled green peas (34 pounds), covered with 5-percent salt brine (20° salinity) and maintained at that concentration, stored under outside conditions in an open-headed, unsheltered 45-gallon cask*

Age in days	MB–19 (5-percent brine; mixed vegetables)						
	Micro-organisms per milliliter of brine and brine acidity						
	Total count	Acid formers	Coliforms	Yeasts	Mycoderma	Acidity as lactic acid	pH
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*	
½	1, 600	600	10			0. 01	
2	340		1, 000			. 07	5. 2
3	1, 200	900	100	0	0	. 10	4. 7
4	48, 000	48, 000	0	10	0	. 18	3. 9
6	106, 000	106, 000	<. 01	0	0	. 56	3. 2
8	52, 000	52, 000	<. 01	0	8	. 67	3. 2
10	23, 000	23, 000	<. 01			. 63	3. 3
12	12, 000	12, 000		0	14	. 61	3. 3
14	4, 900	4, 900		0	7	. 61	3. 4
16	6, 200	6, 200				. 62	
18	8, 600	8, 600		0	8	. 62	
20	4, 800	4, 800				. 62	
22	4, 100	4, 100		0	6	. 65	3. 6
24	4, 200	4, 200		0	32	. 62	
26	6, 600	6, 400		0	2	. 60	
30	430	400		0	31	. 62	
32	380	360		0	24	. 60	
34	470	470		0	4	. 61	
38	350	270		0	50	. 59	3. 5
40	200	150		0	13	. 60	
42	129	114		25	20	. 60	
44	150	120				. 60	
46	95	70		0	21	. 60	3. 5
48	70	70				. 61	
51	111			17	61	. 44	
58	38	26		6	15	. 55	
65	67	44		0	17	. 53	3. 6
72	23	23		0	2	. 57	
86	37	0		1	36		
100	9			2	19	. 50	3. 8
115	8			0	2	. 50	
131	22	0				. 47	3. 8
152	132	132		0	13	. 43	
197	55	34				. 43	

The results, shown in table 4, are very similar to those obtained in the salting of the given vegetables separately at a comparable brine strength. Substantial populations of acid-forming bacteria brought about a rapid acid fermentation resulting in a maximum brine acidity

of about 0.65 percent. The pH of the brine was lowered rapidly from an initial value of about 5, to 3.2 in a few days. The acid-forming bacteria remained in the brine in considerable numbers during the first 30 days; then gradually decreased. Prior to the start of the acid fermentation, a fermentation of short duration was brought about by the coliform group, whose continued growth appears to have been restricted, and finally inhibited, by the acid fermentation and the subsequently developed brine acidity. There was little evidence of an active yeast fermentation, although the presence of mycoderma or scum yeast was noted consistently during the period of analyses.

Brined Whole Wax Beans

Four 60-pound lots of wax beans from eastern North Carolina were brined according to two treatments in 20-gallon open-headed kegs which were stored under outside conditions. All lots were covered with a 4-percent salt brine. In one treatment the brine was maintained at that concentration, while in the other it was gradually raised to 15-percent brine strength within 4 to 5 weeks by the addition of salt at weekly intervals.

The results of the bacteriological findings are shown in tables 5 and 6. An examination of the data shows that the principal microbial changes were similar for both treatments. The acid-forming bacteria were the predominating organisms and brought about a vigorous fermentation resulting in a maximum acidity of about 0.80 percent within about 10 days. According to the salting schedule followed in the lots in which the brine concentration was raised from 4 to 15 percent of salt, the brine strength was still only about 5 percent at the end of the first 10-day period. Therefore, the acid production occurred in all lots at a time when the brine strength was well within the range favoring vigorous growth by the acid-producing bacteria. The pH of the brine decreased from an initial value of about 5.7 to the 3.2 range during the same period. The acid-producing bacteria remained in the brine in considerable numbers throughout the period of analyses in the beans kept at 4-percent strength. In the two lots raised gradually to 15 percent, these organisms were reduced to relatively low numbers within a month.

TABLE 5.—*Fermentation of duplicate 60-pound lots of whole wax beans in 4-percent salt brine (15° salinity) maintained at that concentration, stored under outside conditions in unsheltered open-headed 20-gallon kegs*

Age in days	K–20 (4-percent brine)							K–21 (4-percent brine)						
	Micro-organisms per milliliter of brine and brine acidity							Micro-organisms per milliliter of brine and brine acidity						
	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid	pH	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid	pH
	Thou-sands	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Percent*		*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Percent*	
0	51	10	1			0. 01	5. 7	170	40				0. 02	5. 9
2	45, 000	45, 000	300	0	0	. 14	3. 5	61, 000	61, 000	260	0	0	. 15	3. 5
3	60, 000	60, 000	0	0	0	. 51	3. 4	81, 000	81, 000	0	0	0	. 50	3. 3
4	310, 000	310, 000	0	0	0	. 66	3. 3	123, 000	123, 000	0	0	3	. 67	3. 2
6	60, 000	58, 000		0	0	. 74	3. 3	58, 000	58, 000		0	10	. 75	3. 2
8	46, 000	46, 000		0	4	. 75	3. 3	57, 000	57, 000		0	12	. 83	3. 2
10	39, 000	39, 000		0	38	. 77	3. 3	83, 000	83, 000		0	12	. 87	3. 2
12	20, 500	20, 500		0	84	. 73	3. 3	27, 000	27, 000		0	33	. 79	3. 2
14	10, 600	10, 600		0	2	. 73	3. 3	5, 300	5, 300		0	22	. 83	3. 2
16	4, 600	4, 600		0	26	. 73	3. 3	8, 800	8, 800		0	27	. 83	3. 2
18				0	25	. 69	3. 3	3, 000	3, 000		0	20	. 79	3. 2
20	430	400		0	27	. 77		1, 700	1, 700		0	27	. 87	
22	540	540		0	9	. 72		540	540		0	9	. 80	
24	430	360		0	71	. 71		266	260		0	4	. 80	
26	540	320		0	200	. 70		47	18		0	42	. 77	
28	160	160		0	14	. 66	3. 3	230	160		0	28	. 75	3. 2
30	280	280		0	70	. 70		190	140		50	39	. 78	
32	420	20		0	400	. 57		133	96		0	41	. 73	
34	1, 150	360			790	. 57	3. 3	290	120		0	170	. 68	3. 2
36	700	650		0	62	. 55		800	800		0	52	. 75	

TABLE 5.—*Fermentation of duplicate 60-pound lots of whole wax beans in 4-percent salt brine (15° salinity) maintained at that concentration, stored under outside conditions in unsheltered open-headed 20-gallon kegs*—Continued

Age in days	K–20 (4-percent brine)							K–21 (4-percent brine)						
	Micro-organisms per milliliter of brine and brine acidity							Micro-organisms per milliliter of brine and brine acidity						
	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid	pH	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid	pH
	Thou-sands	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Percent*		*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Percent*	
39	240	220		100	80	0. 56	3. 3	370	320		8	310	0. 68	3. 2
46	280	30		3	270	. 45		230	0		0	310	. 55	
53	210	210		0	50	. 38	3. 5	36	26		0	50	. 50	3. 3
60	39	12		0	57	. 40		47	31		0	12	. 55	
74	230	200		1	21	. 35		220	190		0	25	. 45	
88	260				90	. 24	3. 8	170				38	. 36	3. 5
102	200			0	0	. 25		210	210			0	. 37	
116		0		0	52				0		0	47		
137	270	0		0	23			785	785		0	1		
184	470	470		1	4	. 20		240	240		0	2	. 30	

TABLE 6.—*Fermentation of duplicate 60-pound lots of wax beans in 4- percent salt brine (15° salinity) raised to 15 percent in 4 to 5 weeks, stored under outside conditions in unsheltered open-headed 20-gallon kegs*

Age in days	K–22 (4-percent brine raised to 15 percent)							K–23 (4-percent brine raised to 15 percent)						
	Micro-organisms per milliliter of brine and brine acidity							Micro-organisms per milliliter of brine and brine acidity						
	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid	pH	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid	pH
	Thou-sands	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Per-cent*		*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Per-cent*	
0	44	0				0. 05	5. 7	20	0	10			0. 05	5. 7
2	100, 000	100, 000	290	0	0	. 14	3. 7	122, 000	122, 000	280	0	0	. 11	4. 0
3	60, 000	60, 000	0	0	0	. 51	3. 4	88, 000	88, 000	0			. 44	3. 5
4	57, 000	57, 000	0	0	0	. 69	3. 3	137, 000	137, 000	0			. 69	3. 2
6	16, 000	16, 000		0	25	. 70	3. 3	170, 000	170, 000		0	2	. 74	3. 3
8	45, 000	45, 000		0	14	. 77	3. 3	49, 000	49, 000				. 77	3. 3
10	40, 000	40, 000		0	32	. 82	3. 3	37, 000	37, 000		0	26	. 84	3. 3
12	20, 000	20, 000			33	. 78	3. 3	21, 000	21, 000			21	. 78	3. 2
14	3, 400	3, 400		0	38	. 76	3. 3	4, 100	4, 100		0	2	. 76	3. 2
16	7, 400	7, 400		0	6	. 80	3. 3	3, 600	3, 600		0	13	. 80	3. 2
18	2, 800	2, 700		0	34	. 76	3. 3	1, 200	1, 000		0	21	. 75	3. 2
20	1, 600	1, 600		0	15	. 82		1, 200	1, 200		0	9	. 77	
22	25	25		1	3	. 80		16	16		0	3	. 80	
24	3	0		0	4	. 75		7	0		0	4	. 75	
26	0	0		0	5	. 73		3	0		0	1	. 75	
28	230	160		0	28	. 69	3. 1	15	10		0	0	. 69	3. 1
30	2	0		0	0	. 72		0	0		0	0	. 76	
32	3	0		0	4	. 76		0	0		0	0	. 75	
34	1	1		0	0	. 69	3. 0	1	0		0	0	. 70	3. 0
36	7	0		0	2	. 72		2	0		0	. 2	. 77	
39	2	1		0	1	. 72	3. 0	2	0				. 69	3. 0

TABLE 6.—*Fermentation of duplicate 60-pound lots of wax beans in 4-percent salt brine (15° salinity) raised to 15 percent in 4 to 5 weeks, stored under outside conditions in unsheltered open-headed 20-gallon kegs*—Continued

Age in days	K–22 (4-percent brine raised to 15 percent)							K–23 (4-percent brine raised to 15 percent)						
	Micro-organisms per milliliter of brine and brine acidity							Micro-organisms per milliliter of brine and brine acidity						
	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid	pH	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid	pH
	Thou-sands	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Per-cent*		*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Per-cent*	
46	2	0		0	1	0. 61		0			0	1	0. 64	
53	0	0		0	0	. 57	3. 1	1	0		0	0	. 57	3. 2
60	0	0		0	0	. 54	3. 2	0	0		0	0	. 54	3. 2
74	0	0		0	0	. 50		0	0		0	0	. 50	
88	0	0		0	0	. 39	3. 3	0	0		0	1	. 39	3. 4
102	0	0		0	0	. 42		0	0		0	0	. 42	
116	1	0		0	0			0	0		0	0		
137	0	0		0	0			0	0		0	0		
184	0	0		0	0	. 35		0	0		0	0	. 34	

No typical yeast fermentations were found in any of the four fermentations followed. The mycoderma, however, were consistently found in the brines of the two lots that were maintained at 4-percent brine strength; and in the case of those gradually raised to 15-percent strength, they were present chiefly during the period when the brine strength was below 15 percent. In this connection it should be emphasized that in fermentations at low salt concentrations, exposure of the brine surface to sunlight is not always enough to prevent the growth of mycoderma scum. Sufficient growth may occur during warm summer nights to create a surface film. Furthermore, during a few days of cloudy weather, there can occur on weak brine a luxuriant growth which, once established, will persist even in bright sunlight. This actually took place in the case of lots maintained at 4-percent brine strength; and in addition the mycoderma scum acted as a support for a vigorous mold growth which somewhat softened the original texture of the beans.

Brined Whole Green Beans

Freshly harvested western North Carolina green beans (Tendergreen) were packed in amounts ranging from 60 to 80 pounds in 20-gallon kegs and covered with a 4.5-percent brine. No further salt was added. Duplicate lots of blanched beans, unblanched beans plus vinegar, and unblanched beans plus lactic acid were brined. All kegs were tightly headed at the start and were stored under outside conditions. A small hole in each head was left unbunged during the first 10 days.

The bacteriological changes that occurred during the fermentations are presented in tables 7, 8, and 9. These fermentations, from the standpoint of the principal organisms involved, are essentially the same. The acid-forming bacteria were the predominating group. Reaching maximum numbers within the first few days, they remained in the brine at the 10,000,000-per-milliliter level for about 3 weeks, and continued to be present in lower numbers throughout the balance of the period of observation, about 7½ months. As the data show, the maximum acidity developed in the lots receiving only 4.5-percent brine was approximately 0.65 percent, reached within about the first week; while in those acidified with vinegar, and those with lactic acid, the maximum acidity values were slightly higher. The pH values of the brines in all the fermentations were reduced to about 3.5 within a week.

TABLE 7.—*Fermentation of duplicate 60-pound lots of blanched (2 minutes in boiling water) whole green beans covered with 4.5-percent salt brine (17° salinity), stored under outside conditions in tightly headed 20-gallon kegs*

Age in days	K–31 (4.5-percent brine only)						K–32 (4.5-percent brine only)					
	Micro-organisms per milliliter of brine and brine acidity						Micro-organisms per milliliter of brine and brine acidity					
	Total count	Acid formers	Yeasts	Myco-derma	Acidity as lactic acid	pH	Total count	Acid formers	Yeasts	Myco-derma	Acidity as lactic acid	pH
	Thousands	*Thousands*	*Thou-sands*	*Thou-sands*	*Percent*		*Thousands*	*Thousands*	*Thou-sands*	*Thou-sands*	*Percent*	
0	2	0	1	0	0. 19	------	10	0	0	0	0. 19	------
2	330, 000	330, 000	14	20	. 27	3. 7	260, 000	260, 000	------	------	. 36	3. 6
4	110, 000	110, 000	1	0	. 63	3. 4	160, 000	160, 000	0	0	. 61	3. 5
6	82, 000	82, 000	1	22	. 63	3. 5	87, 000	81, 000	0	4	. 63	3. 5
8	35, 000	35, 000	0	30	. 59	3. 5	67, 000	62, 000	0	34	. 63	3. 5
12	10, 300	10, 300	0	130	. 60	------	19, 000	19, 000	0	120	. 65	------
14	6, 900	6, 900	0	170	. 58	------	14, 000	14, 000	0	31	. 64	------
16	2, 600	2, 600	0	140	. 57	------	15, 000	15, 000	0	150	. 65	------
18	2, 400	2, 000	0	300	. 58	------	9, 000	9, 000	0	21	. 67	------
20	1, 700	1, 700	0	70	. 57	------	15, 000	15, 000	0	60	. 66	------
22	1, 020	1, 020	0	30	. 59	------	2, 600	2, 600	0	90	. 66	------
24	1, 900	1, 900	0	30	. 57	------	1, 900	1, 600	0	90	. 69	------
26	1, 900	1, 900	0	40	. 57	------	1, 040	1, 040	0	20	. 65	------
28	2, 300	2, 300	0	60	. 58	------	2, 000	2, 000	0	80	. 68	------
30	2, 600	2, 300	0	240	. 56	------	2, 300	1, 800	0	280	. 66	------
32	1, 450	1, 200	0	140	. 50	3. 5	200	200	0	<10	. 62	3. 5
34	2, 100	1, 800	0	260	. 53	------	610	580	0	60	. 65	------
36	1, 300	1, 000	0	360	. 50	------	3, 800	3, 800	0	170	. 64	------
39	3, 200	3, 200	0	360	. 50	------	360	360	0	10	. 64	------
41	3, 600	3, 600	------	170	. 43	------	350	260	------	50	. 65	------

44	2,800	2,800	0	190	.52		1,020	1,020		190	.63								
46	3,400	3,000	0	230	.50		430	340	0	170	.67								
48	3,900	3,900	0	400	.47		1,200	1,200	0	100	.63								
53	3,000	2,400	0	300			800	800	0	120									
60	2,900	2,400					1,200	1,200	0	50	.62								
71	1,000	1,000	0	170	.43		60	<100	0	<10	.63								
85	1,470	1,410	0	220	.40		970	970	0	<10	.62								
101	750	750	0	280	.37		<100	<100	0	<10	.63								
131	930	830	0	30	.38		120	120	0	90	.60								
232	2,200	2,200	0	350	.34		120	70											

TABLE 8.—*Fermentation of duplicate 83-pound lots of unblanched whole green beans covered with 4.5-percent salt brine plus 600 milliliters of 116-grain vinegar (11.6 percent), stored under outside conditions in tightly headed 20-gallon kegs*

Age in days	K–33 (4.5-percent brine+vinegar)						K–34 (4.5-percent brine+vinegar)					
	Micro-organisms per milliliter of brine and brine acidity						Micro-organisms per milliliter of brine and brine acidity					
	Total count	Acid formers	Yeasts	Mycoderma	Acidity as lactic acid	pH	Total count	Acid formers	Yeasts	Mycoderma	Acidity as lactic acid	pH
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Percent*		*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*	
0	1	1	0	0	0. 20	------	0	0	0	0	0. 28	------
2	240, 000	240, 000	0	0	. 48	3. 6	116, 000	116, 000	0	8	. 37	3. 8
4	60, 000	60, 000	0	0	. 92	3. 4	80, 000	80, 000	5	10	. 92	3. 3
6	27, 000	24, 000	30	110	. 86	3. 5	46, 000	46, 000	110	280	. 89	3. 4
8	5, 500	5, 500	0	79	. 82	3. 5	11, 100	11, 100	12	32	. 89	3. 4
12	23, 000	23, 000	1	32	. 87	------	13, 000	13, 000	0	130	. 90	------
14	14, 100	14, 100	0	17	. 87	------	17, 000	17, 000	2	130	. 91	------
16	26, 000	26, 000	0	120	. 90	------	11, 000	11, 000	0	200	. 92	------
18	22, 000	22, 000	0	10	. 92	------	14, 000	14, 000	0	12	. 94	------
20	14, 000	14, 000	0	20	. 93	------	20, 000	20, 000	0	40	. 94	------
22	9, 100	9, 100	0	<10	. 95	------	1, 700	1, 700	0	10	. 97	------
24	7, 900	7, 900	0	40	. 93	------	8, 700	8, 700	0	40	. 95	------
26	7, 500	7, 500	0	120	. 88	------	9, 100	9, 100	0	<10	. 93	------
28	4, 800	4, 800	0	10	. 92	------	4, 700	4, 700	0	40	. 93	------
30	2, 600	2, 600	0	20	. 82	------	4, 800	4, 300	0	260	. 93	------
32	4, 100	4, 100	0	20	. 80	3. 5	1, 800	2, 000	0	90	. 82	3. 5
34	3, 800	3, 700	0	80	. 90	------	8, 500	8, 500	0	70	. 92	------
36	5, 400	5, 400	0	20	. 86	------	3, 300	3, 300	0	390	. 82	------
39	2, 100	2, 100	0	30	. 85	------	2, 400	2, 400	0	50	. 84	------
41	1, 200	1, 200	--------	<10	. 82	------	2, 500	2, 100	--------	70	. 88	------
44	1, 260	1, 260	--------	10	. 85	------	2, 700	2, 600	--------	160	. 85	------
46	140	140	0	80	. 80	------	1, 400	1, 400	0	130	. 85	------

48	940	940	0	20	.80		2,300	2,300	0	100	.82			
53	710	710	0	30			1,350	1,280	0	40				
60	1,200	1,200					4,500	4,300						
71	400	400	10	<10	.61		2,600	2,600	30	20	.79			
85	240	240	0	20	.60		1,600	1,600	0	80	.67			
101	360	360	0	10	.55		180	180	0	90	.77			
131	270	270	0	20	.58		150	150	0	20	.75			
232	670	670	0	140	.45		660	450	0	110	.65			

TABLE 9.—*Fermentation of duplicate 83-pound lots of unblanched green beans covered with 4.5-percent salt brine plus 183 milliliters of 50-percent lactic acid, stored under outside conditions in tightly headed 20-gallon kegs*

Age in days	K–35 (4.5-percent brine+lactic acid)						K–36 (4.5-percent brine+lactic acid)					
	Micro-organisms per milliliter of brine and brine acidity						Micro-organisms per milliliter of brine and brine acidity					
	Total count	Acid formers	Yeasts	Myco-derma	Acidity as lactic acid	pH	Total count	Acid formers	Yeasts	Myco-derma	Acidity as lactic acid	pH
	Thousands	*Thousands*	*Thou-sands*	*Thou-sands*	*Percent*		*Thousands*	*Thousands*	*Thou-sands*	*Thou-sands*	*Percent*	
0	1	1	0	2	0. 18	------	0	0	0	0	0. 20	------
2	280, 000	280, 000	4	0	. 34	3. 6	180, 000	180, 000	--------	--------	. 26	3. 9
4	62, 000	62, 000	12	0	. 85	3. 3	40, 000	40, 000	2	0	. 85	3. 4
6	78, 000	78, 000	920	210	. 88	3. 4	61, 000	61, 000	30	240	. 77	3. 5
8	14, 000	13, 000	46	33	. 88	3. 4	9, 300	9, 300	0	46	. 77	3. 5
12	23, 000	23, 000	18	37	. 90	------	27, 000	27, 000	0	58	. 82	------
14	13, 000	13, 000	6	48	. 90	------	24, 000	24, 000	0	37	. 84	------
16	38, 000	38, 000	2	100	. 90	------	13, 000	13, 000	0	80	. 82	------
18	17, 000	17, 000	0	26	. 92	------	3, 000	3, 000	2	0	. 85	------
20	22, 000	22, 000	0	20	. 95	------	6, 200	6, 200	0	10	. 82	------
22	7, 000	6, 900	0	140	. 95	------	3, 900	3, 900	0	20	. 84	------
24	4, 600	4, 600	0	90	. 94	------	5, 300	5, 300	0	10	. 84	------
26	4, 400	4, 400	0	10	. 90	------	3, 500	3, 430	0	10	. 80	------
28	4, 200	4, 200	0	60	. 95	------	1, 700	1, 700	0	20	. 82	------
30	3, 400	2, 400	0	200	. 80	------	2, 500	2, 200	0	180	. 90	------
32	2, 100	2, 100	0	100	. 82	3. 4	1, 500	1, 300	0	120	. 80	3. 5
34	5, 000	5, 000	0	30	. 95	------	4, 600	4, 600	0	30	. 75	------
36	2, 000	2, 000	0	30	. 89	------	3, 600	3, 600	0	<10	. 72	------
39	2, 600	2, 600	0	10	. 90	------	3, 700	3, 700	0	<10	. 71	------

41	2,200	2,100		10	.85		1,800	1,800		20	.63	
44	1,400	1,400		1,100	.87		500	500		80	.54	
46	1,300	1,300	0	190	.85		4,500	3,300	0	130	.52	
48	900	900	0	420	.87		6,900	6,900	0	80	.48	
53	1,000	1,000	0	130			8,300	8,300	0	50		
60	1,800	1,600		30	.84		9,000	9,000		<10	.44	
71	2,200	2,000	10	100	.82		6,900	6,900	0	<10	.40	
85	1,850	1,850	0	370	.76		9,500	8,700	0	20	.42	
101	200	200	0	70	.81		4,100	3,100	0	<10	.40	
131	300		0	70	.60		220	220	0	210	.34	
232	840	690	0				5,000	5,000	0			

Only one fermentation of the series (K–35) gave a well-defined yeast fermentation. In the other fermentations the yeasts found were not sufficiently consistent or numerous to be considered typical in behavior.

The results of this series show, therefore, that there was no appreciable difference in the general fermentation behavior of weak-brined blanched green beans, and weak-brined unblanched beans acidified with vinegar or lactic acid. The populations of the acid-forming bacteria followed the same general pattern in all fermentations and the maximum acidities developed were in the same range. Furthermore, duplicate fermentations of the three individual treatments can be considered similar from the standpoint of population trends among acid-producing bacteria and developed brine acidity.

Brined Whole Carrots

The experiment with brined whole carrots was the first in a series of small-scale brining and salting studies carried out under laboratory conditions and stored at prevailing room temperature. Approximately 2-pound lots of the blanched and unblanched whole carrots were packed in 64-ounce glass jars and covered with 6.2-percent salt brine. In addition to the lot treated with brine alone, lots were made up with small amounts of vinegar, of lactic acid, and of mixtures of vinegar and lactic acid. In a fifth lot only blanched carrots were used.

The fermentation characteristics for the five lots of carrots are shown in table 10. In general, the first four listed behaved in a similar manner with respect to the fermentation caused by the acid-producing bacteria, both as to total populations and developed acidity. The acidity developed by fermentation for the lot receiving brine only, after about 1 month, was 0.75 percent as compared to about 0.70 percent for those acidified with vinegar, lactic acid, and a mixture of vinegar and lactic acid. The populations of acid-producing bacteria were somewhat lower (about one-tenth), for the blanched, acidified carrots, as compared to the above lots, and the developed acidity was slightly lower.

An active fermentation by the coliform group took place in the nonacidified brine, whereas it was not present in the remaining four which were acidfied at the start. An active yeast fermentation was found in three of the fermentations (CR–3, CR–4, and CR–5) but was not present in the remaining two (CR–1 and CR–2). The growth of mycoderma scum was satisfactorily controlled in four of the lots; a poor jar seal was partly responsible for the heavy growth in the fifth lot.

The fact that the start of the usual acid fermentation was retarded somewhat in all five lots can be attributed in part to the low room temperature (about 60° F.) which prevailed at night during the early period of the experiment.

The microscopic observations for this series showed that the Gram-positive bacilli were the principal cell types present during the acid fermentation. These occurred as large and small bacilli. The former were about 1×2.5 to 7 microns and were present singly, in pairs, and in short chains of three or four elements. The latter appeared as two distinct groups with respect to size: Bacilli about

TABLE 10.—*Fermentation of 2-pound lots of whole carrots covered with 6.2 percent (24° salinity) brine with and without added organic acids* [1] *stored at room temperature (60–70° F.) in 64-ounce jars*

CR–1 (6.2-PERCENT BRINE ONLY)

Age in days	Micro-organisms per milliliter of brine and brine acidity					
	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid
	Thou-sands	*Thou-sands*	*Hun-dreds*	*Thou-sands*	*Thou-sands*	*Percent*
2	3	2	60	0	0	0. 06
4	140	140	800	0	0	. 10
7	52, 000	52, 000	1, 700	0	0	. 20
9	98, 000	98, 000	480	0	0	. 15
11	133, 000	133, 000	24	10	0	. 27
15	123, 000	123, 000	0	0	500	. 55
29	3, 200	3, 200	--------	28	0	. 75
185	74	30	--------	0	0	--------
CR–2 (BRINE+VINEGAR)						
2	0	0	0	0	0	0. 47
4	0	0	0	0	0	. 47
7	147	147	0	0	0	. 47
9	180, 000	180, 000	0	0	0	. 67
11	580, 000	580, 000	0	6	0	. 86
15	210, 000	210, 000	0	0	14	1. 06
29	16, 000	16, 000	--------	1	0	1. 15
185	16	9	--------	1	0	--------
CR–3 BRINE+LACTIC ACID)						
2	0	0	0	0. 5	0	0. 27
4	2	2	0	. 2	0	. 27
7	2, 100	2, 100	0	30	0	. 29
9	360, 000	360, 000	0	1, 200	20	. 43
11	490, 000	490, 000	0	860	0	. 53
15	280, 000	280, 000	0	30	0	. 79
29	112, 000	112, 000	--------	40	0	. 95
185	8	5	--------	0	0	--------
CR–4 (BRINE+VINEGAR AND LACTIC ACID)						
2	0	0	0	0	0	0. 39
4	52	52	0	1	0	. 38
7	3, 200	3, 200	0	46	3	. 37
9	190, 000	190, 000	0	440	10	. 47
11	440, 000	440, 000	0	620	8	. 60
15	380, 000	380, 000	0	23	14	. 90
29	141, 000	141, 000	--------	4	1	1. 08
185	10	10	--------	0	0	--------

See footnote at end of table.

TABLE 10.—*Fermentation of 2-pound lots of whole carrots covered with 6.2 percent (24° salinity) brine with and without added organic acids* [1] *stored at room temperature (60–70° F.) in 64-ounce jars*—Continued

CR–5 (SAME AS CR–4 WITH BLANCHED CARROTS)

Age in days	Micro-organisms per milliliter of brine and brine acidity					
	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid
	Thou-sands	*Thou-sands*	*Hun-dreds*	*Thou-sands*	*Thou-sands*	*Percent*
2	0	0	0	0. 1	0	0. 36
4	0	0	0	. 1	. 5	. 36
7	1, 400	0	0	20	1, 400	. 38
9	10, 000	10, 000	0	20	800	. 38
11	26, 000	26, 000	0	80	2, 400	. 37
15	51, 000	51, 000	0	440	280	. 54
29	27, 000	27, 000	--------	8	75	. 80
185	15	15	--------	0	0	--------

[1] CR–1; 6.2 percent brine only.
CR–2; brine plus 50 milliliters of 102-grain (10.2 percent) vinegar.
CR–3; brine plus 5 milliliters of 85-percent lactic acid.
CR–4; brine plus 25 milliliters of 102-grain vinegar and 2.5 milliliters of lactic acid.
CR–5; same as CR–4 except 1 pound of blanched (2 minutes in boiling water) carrots used plus 0.005 to 0.01 gram of d-iso-ascorbic acid.

0.8×1.2 microns arranged almost always in chains or pairs, and bacilli somewhat larger, 1×1.5 to 3 microns, arranged singly or in pairs but seldom in chains.

The microscopic counts of the Gram-positive cells, as well as the plate counts, for the five carrot fermentations, are presented graphically in figure 2. (The results for one of the lettuce fermentations, also shown, will be discussed in the next vegetable series.)

The general similarity of the population curves, showing comparable trends for all the lots, was indicated by both count techniques used.

LETTUCE KRAUT

Eastern North Carolina market-type lettuce was used for the study of lettuce kraut. The heads were trimmed of outer leaves, halved, and the cores cut out. The lettuce was then chopped and mixed with dry salt. Two 75-pound lots were mixed with 2 percent of salt by weight and packed in 10-gallon crocks, and one was put down at 2.5 percent. These were stored at room temperature, about 70° F.

The bacteriological changes during the fermentations are shown in table 11. The first lot underwent a vigorous and rapid acid fermentation which resulted in an acidity of 1.8 percent after about 1 week. A short period of activity by the coliform group preceded the acid fermentation, and after about 1 week, a yeast fermentation occurred. Mycoderma were found continually after the first week.

The characteristics of the acid fermentation in the lots at 2 percent and at 2.5 percent salt (LK–2 and LK–3), although somewhat similar

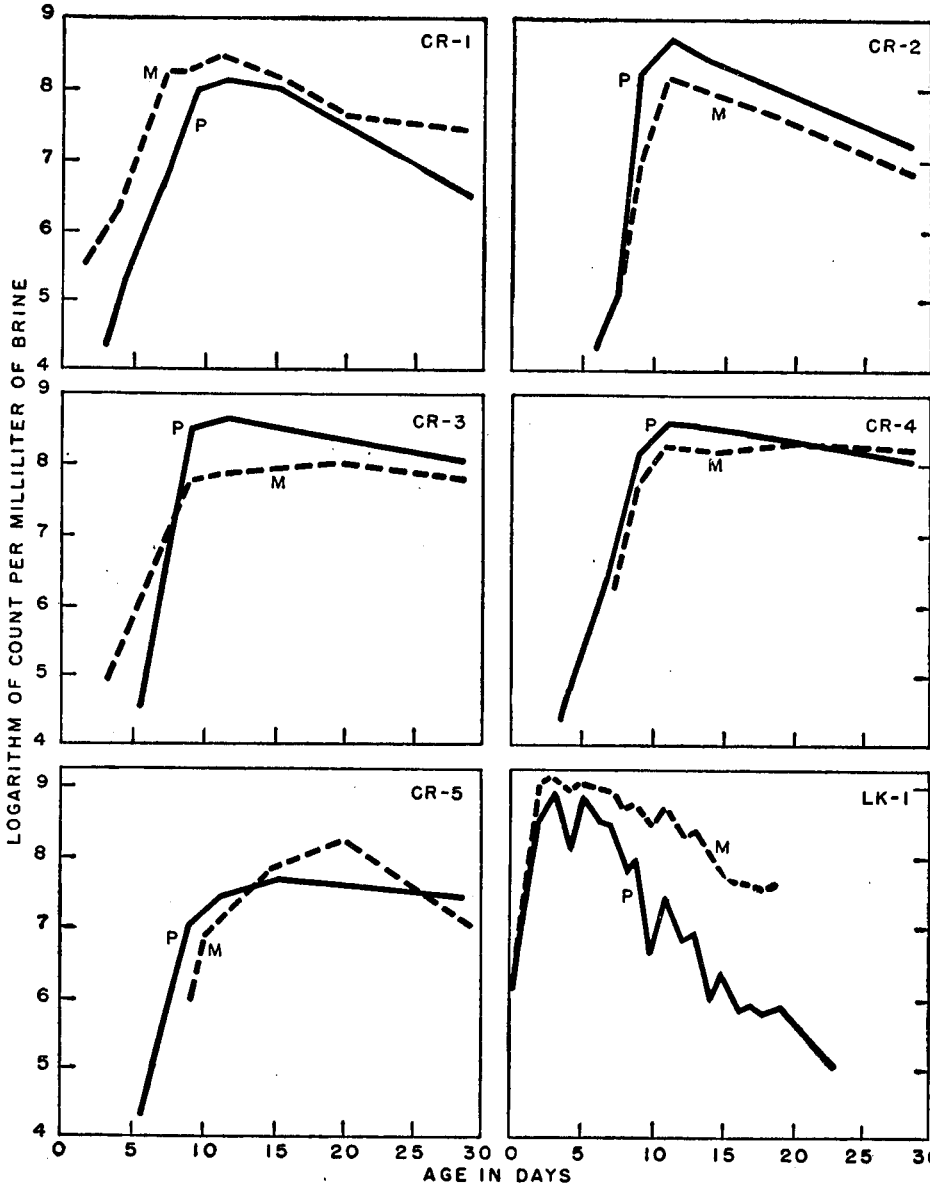

FIGURE 2.—Populations of acid-forming bacteria in brined carrots (CR–1 to CR–5) and dry-salted lettuce (LK–1), as indicated by the plate (P) and microscopic (M) count techniques.

to the first, were of less intensity, indicated by the production of only about half as much acid. There was no evidence of active yeast fermentations in these two lots. The growth by the coliform group appeared comparable in the two fermentations when determinations for these organisms were made (LK–2 and LK–3). In general, the results are in good agreement with those of Hohl and Cruess (*17*), particularly with respect to the activity of the acid-producing bacteria and resultant brine acidity.

One of the fermentations in this series (LK–1) was followed by the microscopic and plate count. The results are shown in the lower part of figure 2. As shown in this illustration the two curves are similar, particularly during the active phase of the acid fermentation. During the period when the counts decline, however, there is considerable divergence; and near the end of the fermentation, the curve based on microscopic observations appears to level off. This relationship between microscopic and plate counts has been discussed earlier.

TABLE 11.—*Fermentation of 75-pound lots of chopped lettuce for kraut at 2- and 2.5-percent salt by weight, stored in 10-gallon crocks at room temperature (70° F.)*

LK–1 (2-PERCENT SALT)

Age in days	Micro-organisms per milliliter of brine and brine acidity						
	Total count	Acid formers	Coliforms [1]	Coliforms [2, 3]	Yeasts	Mycoderma	Acidity as lactic acid
	Millions	*Millions*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*
0	7	2	-------	10	3	0	0. 03
1	50	25	-------	1, 000	3	0	. 18
2	520	520	-------	100	0	0	. 60
3	1, 010	1, 010	-------	10	2	0	. 85
4	113	113	-------	<. 01	0	0	1. 23
5	880	880	-------	<. 01	0	0	1. 60
6	380	380	-------	<. 01	7	0	1. 67
7	330	330	-------	-------	200	80	1. 83
8	71	71	-------	<. 01	230	40	1. 80
9	101	101	-------	<. 01	220	170	1. 80
10	3	3	-------	-------	70	490	-------
11	44	44	-------	-------	120	100	1. 72
12	6	6	-------	-------	16	40	1. 70
13	10	10	-------	-------	30	160	1. 52
14	1	1	-------	<. 01	10	-------	1. 42
15	2	2	-------	<. 01	0	120	1. 40
16	. 7	. 7	-------	-------	0	0	1. 35
17	. 8	. 8	-------	-------	0	17	1. 36
18	. 7	. 7	-------	-------	4	12	1. 40
19	. 8	. 8	-------	-------	1	6	1. 40
23	. 1	. 1	-------	-------	1	0	1. 38

LK-2 (2-PERCENT SALT)

0	5	---------	3, 300	[4]>100	0. 6	2	0. 03
1	22	---------	8, 500	1, 000	0	-------	. 05
2	170	170	<10	0	-------	6	. 35
3	58	58	0	0	0	. 2	. 40
4	50	50	-------	. 01	0	2. 4	. 65
5	0	0	-------	-------	0	280	. 75
8	2	1	-------	-------	10	66	. 80
9	2	2	-------	-------	0	48	. 80
11	. 2	. 1	-------	-------	. 2	15	. 80
13	1	1	-------	-------	-------	3	. 81

See footnotes at end of table.

TABLE 11.—*Fermentation of 75-pound lots of chopped lettuce for kraut at 2- and 2.5-percent salt by weight, stored in 10-gallon crocks at room temperature (70° F.)*—Continued

LK–3 (2.5-PERCENT SALT)

Age in days	Micro-organisms per milliliter of brine and brine acidity						
	Total count	Acid formers	Coliforms [1]	Coliforms [2,3]	Yeasts	Mycoderma	Acidity as lactic acid
	Millions	*Millions*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Percent*
0	18	----------	6, 600	[4] >100	0. 8	--------	0. 03
1	22	----------	23, 000	1, 000	. 1	--------	. 05
2	220	220	< 10	0	0	--------	. 45
3	61	61	0	0	0	2. 4	. 45
4	110	110	--------	>. 01	0	. 9	. 77
5	3	0	--------	----------	0	800	. 75
8	0	0	--------	----------	0	300	. 73
9	15	15	--------	----------	0	73	. 85
11	. 5	. 4	--------	----------	0	1	. 75
13	. 1	. 1	--------	----------	0	10	. 75

[1] Using brilliant green lactose bile agar (Difco).
[2] Using lauryl sulfate tryptose broth (Difco).
[3] Values shown represent highest dilution of brine where a positive (growth + gas) was obtained.
[4] No higher dilutions made.

The predominating cell types found during the acid fermentation were the large and small Gram-positive bacilli. The former were approximately 1×2 to 6 microns and were arranged singly, in pairs, and in short chains. The latter, about 0.75×1.5 microns, occurred mostly in pairs, with a few occurring singly.

DRY-SALTED CUT GREEN BEANS

Blanched, fresh, green beans, in 2-pound lots, were dry-salted at four different concentrations, 2.5, 5, 10, and 15 percent by weight. Each salting treatment was carried out in duplicate in 32-ounce glass jars which were partially sealed. One set was stored at room temperature (70° F.) and the other at refrigerator temperature (35.6°). The bacteriological observations made during storage at both temperatures are given in tables 12 and 13.

ROOM TEMPERATURE LOTS.—In the dry-salted green beans stored at room temperature (DSB–1, DSB–3, DSB–5, and DSB–7) as shown in table 12, vigorous acid fermentations took place within 3 days in those at 2.5 and 5 percent salt, resulting in a maximum developed acidity of approximately 1.0 percent for both fermentations. In the 10-percent lot the acid-forming bacteria, in general, occurred in lower numbers, and the maximum acidity produced was somewhat less. In this instance a secondary acid fermentation, coinciding with the end of yeast activity, resulted in an appreciable increase in acidity. In the 15-percent salt treatment there was no indication of acid fermentation.

TABLE 12.—*Fermentation of 2-pound lots of blanched (3 minutes in steam), cut green beans, dry-salted at 2.5-, 5-, 10-, and 15-percent salt by weight, stored at room temperature (60°–70° F.) in 32-ounce jars*

DSB–1 (2.5-PERCENT SALT)

Age in days	Micro-organisms per milliliter of brine and brine acidity					
	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid
	Thou-sands	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Per-cent*
3	6, 500	6, 500	500	0	0	0. 37
5	8, 000	8, 000	0	<. 1	0	. 35
18	8, 100	8, 100	0	0	0	. 96
24	27, 000	27, 000	0	0	0	. 73
31	81, 000	81, 000	0	. 1	0	. 88
38	133, 000	133, 000	0	. 4	0	. 84
45	18, 600	18, 600	0	----------	----------	. 99
54	8, 200	7, 200	0	0	300	. 70
61	27, 000	20, 000	0	0	800	. 90
68	17, 000	17, 000	0	20	130	. 50
75	8, 900	8, 900	0	0	200	. 55
82	9, 400	8, 600	0	2	200	. 65
89	3, 100	1, 600	0	0	210	. 68
101	10, 400	8, 700	0	100	1, 000	. 71
109	4, 800	4, 200	0	40	280	. 72
116	5, 700	5, 700	0	8	160	. 77
123	4, 000	4, 000	----------	0	77	. 66
130	11, 000	10, 000	----------	0	140	. 68
144	12, 800	12, 800	----------	180	0	. 71

DSB–3 (5-PERCENT SALT)

3	13, 400	0	17, 800	1	0	0. 22
5	13, 700	13, 700	140	<. 1	0	. 25
18	4, 000	4, 000	0	0	0	. 66
24	5, 000	5, 000	0	0	0	. 65
31	41, 500	41, 500	0	15	0	. 71
38	14, 100	14, 100	0	331	0	. 62
45	3, 800	3, 800	0	170	0	. 94
54	2, 800	2, 800	0	40	0	. 90
61	2, 800	2, 100	0	670	210	. 88
68	1, 500	1, 200	0	20	230	. 50
75	800	600	0	<10	170	. 55
82	800	800	0	<10	200	. 59
89	380	280	0	<10	140	. 65
101	2, 300	240	0	30	1, 200	. 60
109	340	260	0	70	70	. 66
116	440	440	0	0	110	. 63
123	550	270	----------	27	150	. 57
130	1, 200	1, 200	----------	5	110	. 66
144	20, 000	----------	----------	0	2, 500	. 66

TABLE 12.—*Fermentation of 2-pound lots of blanched (3 minutes in steam), cut green beans, dry-salted at 2.5-, 5-, 10-, and 15-percent salt by weight, stored at room temperature (60°–70° F.) in 32-ounce jars*—Continued

DSB–5 (10-PERCENT SALT)

Age in days	Micro-organisms per milliliter of brine and brine acidity					
	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Acidity as lactic acid
	Thou-sands	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Per-cent*
3	8, 800	----------	59	5	0	0. 11
5	4, 600	3, 800	37	. 5	3. 5	. 18
18	9, 200	9, 200	0	1	0	. 22
24	3, 800	3, 800	0	10	0	. 32
31	260	260	0	59	0	. 27
38	8, 800	8, 800	0	2, 500	0	. 24
45	3, 000	<10	0	2, 200	0	. 26
54	4, 400	<10	0	7, 400	0	. 20
61	14, 000	2, 000	0	11, 000	0	. 19
68	9, 000	<10	0	2, 800	0	. 16
75	2, 700	<100	0	2, 200	0	. 15
82	1, 300	<10	0	3, 800	0	. 17
89	3, 000	<10	0	2, 300	0	. 18
101	40, 000	<10	0	65, 000	0	-------
109	60, 000	<10	0	60, 000	0	. 31
116	61, 600	61, 600	0	300	0	. 48
123	15, 000	15, 000	----------	80	0	. 45
130	6, 500	3, 000	----------	3, 400	0	. 49
144	900	600	----------	10	260	. 53

DSB–7 (15-PERCENT SALT)

3	84	74	1. 2	0	0	0. 11
5	330	180	. 4	0	0	. 12
18	440	0	0	36	0	. 13
24	70	0	0	0	0	. 11
31	156	----------	0	. 1	0	. 12
38	1, 800	0	0	12	0	. 08
45	850	0	0	. 7	0	. 15
54	290	0	0	22	0	. 12
61	2, 500	10	0	1, 900	0	. 14
68	1, 500	0	0	1, 600	0	. 08
75	2, 000	<10	0	700	0	. 08
82	40	<10	0	40	0	. 06
89	20	<10	0	20	0	. 10
101	61	0	0	61	0	. 09
109	470	<10	0	600	0	. 07
116	68	3	0	. 4	0	. 09
123	40	20	----------	. 2	0	. 07
130	42	10	----------	3	0	. 07
144	18	1	----------	3	1	. 06

TABLE 13.—*Fermentation of 2-pound lots of blanched (3 minutes in steam), cut green beans dry-salted at 2.5-, 5-, 10-, and 15-percent salt by weight, stored at refrigerator temperature (35.6° F.) in 32-ounce jars*

DSB–2 (2.5-PERCENT SALT)

Age in days	Micro-organisms per milliliter of brine and brine acidity						
	Total count	Acid formers	Coli-forms	Yeasts	Myco-derma	Cocci	Acidity as lactic acid
	Thou-sands	*Thou-sands*	*Thou-sands*	*Hun-dreds*	*Hun-dreds*	*Thou-sands*	*Per-cent*
3	52	30	13	1	0	0	0. 14
5	32	22	13	4	0	0	. 15
18	1, 500	<10	1, 700	0	0	0	. 13
24	3, 200	<10	3, 200	0	0	0	. 18
31	6, 800	6, 800	440	0	0	0	. 17
38	12, 100	12, 100	-------	0	0	0	. 20
45	11, 200	11, 200	3. 6	0	0	0	. 20
54	11, 800	11, 800	0	0	0	0	. 27
61	15, 700	15, 700	0	2	0	0	. 25
68	16, 000	16, 000	0	2	0	0	. 25
75	8, 000	8, 000	0	0	0	0	. 20
82	15, 000	15, 000	0	0	0	0	. 25
89	-------	-------	0	0	0	0	. 28
101	4, 700	4, 700	0	1	0	0	. 28
109	3, 500	3, 500	0	0	0	0	. 29
116	5, 400	5, 400	0	0	0	0	. 30
123	1, 700	-------	-------	0	0	0	. 29
130	1, 900	1, 900	-------	0	0	0	. 30
144	1, 100	1, 100	-------	15	4	0	. 28

DSB–4 (5-PERCENT SALT)

3	24	12	4	0	0	0	0. 12
5	15	7	. 8	0	0	0	. 15
18	5	0	0	0	0	0	. 12
24	5	4	0	0	0	0	. 14
31	30	20	0	6	0	0	. 15
38	22	21	-------	1	0	0	. 14
45	155	155	-------	0	0	0	. 15
54	2, 600	2, 600	0	0	0	0	. 16
61	15, 700	15, 700	0	2	0	0	. 20
68	7, 800	7, 800	0	3	0	0	. 15
75	20, 000	20, 000	0	0	0	0	. 14
82	22, 000	22, 000	0	0	0	0	. 16
89	10, 000	10, 000	0	0	0	0	. 19
101	6, 700	6, 700	0	0	0	0	. 22
109	7, 100	7, 100	0	7	0	0	. 23
116	13, 300	13, 300	0	0	0	0	. 23
123	12, 200	12, 200	-------	0	0	0	. 24
130	5, 000	5, 000	-------	0	0	0	. 22
144	4, 900	4, 900	-------	1	0	0	. 26

TABLE 13.—*Fermentation of 2-pound lots of blanched (3 minutes in steam), cut green beans dry-salted at 2.5-, 5-, 10-, and 15-percent salt by weight, stored at refrigerator temperature (35.6°F.) in 32-ounce jars*—Continued

DSB–6 (10-PERCENT SALT)

Age in days	Micro-organisms per milliliter of brine and brine acidity						
	Total count	Acid formers	Coliforms	Yeasts	Mycoderma	Cocci	Acidity as lactic acid
	Thousands	*Thousands*	*Thousands*	*Hundreds*	*Hundreds*	*Thousands*	*Percent*
3	55	42	11	2	0	-------	0. 13
5	48	18	7. 6	0	0	0	. 12
18	11	2	2	0	0	0	. 12
24	6	4. 4	2	0	0	0	. 15
31	19	10	1	0	0	9	. 13
38	34	26	-------	0	0	8	. 05
45	23	0	. 2	0	0	23	. 15
54	250	0	0	1	0	250	. 17
61	930	<10	. 1	1	0	900	. 15
68	4, 500	<100	0	3	0	4, 500	. 14
75	2, 900	<10	0	0	0	2, 900	. 08
82	510	<10	0	0	0	510	. 07
89	700	<10	0	3	0	700	. 11
101	830	<10	0	1	0	830	. 10
109	750	<10	0	4	0	750	. 10
116	1, 800	<10	0	1	0	1, 800	. 09
123	610	<10	-------	4	0	610	. 10
130	900	<10	-------	0	0	900	. 10
144	430	<10	-------	1	0	430	. 06

DSB–8 (15-PERCENT SALT)

3	44	33	5	0	0	-------	0. 11
5	42	21	3	0	0	10	. 13
18	21	8	. 8	0	0	16	. 14
24	8	4	1	0	0	3	. 14
31	5. 4	3. 3	. 2	0	0	2. 1	. 15
38	5. 2	. 6	-------	0	0	. 2	. 11
45	4	2	0	0	0	2	. 14
54	1. 7	. 9	. 3	0	0	6	. 15
61	3	. 8	. 1	0	0	2	. 13
68	7	4	. 3	0	0	3	. 10
75	1	1	. 1	0	0	-------	. 07
82	2. 2	. 8	0	0	0	10	. 09
89	3	0	0	0	0	2	. 05
101	2	0	0	0	0	-------	. 08
109	2. 5	2	0	0	0	-------	. 07
116	3	1	0	0	0	-------	. 07
123	1. 4	. 4	-------	0	0	-------	. 05
130	6. 3	. 5	-------	0	0	40	. 08
144	. 5	-------	-------	0	0	8	. 06

Typical gaseous fermentations by yeasts were common to all treatments except the 2.5-percent lot. In the latter case sporadic counts were observed, but no clearly defined yeast fermentation trend was noted. Mycoderma, or scum yeasts, were present continuously in the beans at 2.5 and 5 percent salt during the last part of the observation period but were not found at the two higher salt concentrations. This was partly because of the rather long period of carbon dioxide production by the yeasts, which by lowering the oxygen tension above the brine surface controlled surface scum growth which requires oxygen.

Observations as to the presence of the coliform group were not started soon enough to get the typical picture in the 2.5- and 5.0-percent treatments. In those at 10 and 15 percent, as the data indicate, the coliforms present at the third and fifth day sampling periods did not develop further.

Refrigerated Lots.—In the beans stored at refrigerator temperature (DSB–2, DSB–4, DSB–6, and DSB–8) as shown in table 13, the active acid fermentation was arrested in the 2.5- and 5.0-percent salted beans for about 1 month and 2 months, respectively. After that time a rather slow fermentation by the acid-producing bacteria took place, resulting in about 0.30 percent of acid. There was no evidence of active growth by these organisms in the beans at 10 and 15 percent salt, although populations of a few thousands per milliliter were observed during the period of analysis.

An active gaseous fermentation by the coliform group took place in the 2.5-percent lot after about 1 week and continued until the start of the acid fermentation. There was no evidence of their activity in any of the other lots, although low counts were observed at a number of the sampling intervals. Essentially the same results were obtained for the yeasts. Growth of mycoderma scum was absent in all cases.

An active fermentation by the salt-tolerant cocci took place only in the 10-percent treatment after about 45 days, and considerable numbers of these organisms continued to be present in the brine throughout the balance of the analysis period. There was no evidence of their growth above 1,000 per milliliter in either the 2.5- or 5.0-percent salt treatments, the influencing factor for controlling their growth appearing to have been the brine acidity resulting from the acid fermentation.

The results of this experiment strongly indicate that for dry-salted beans, stored at refrigerator temperature, a salt concentration of approximately 15 percent is required to arrest active fermentation by the usual predominating brine organisms.

Brined and Dry-Salted White Corn

In the next series of experiments, white corn was blanched on the cob for 10 minutes in flowing steam and promptly air-cooled. It was then cut from the cob and approximately 3.5-pound lots in 64-ounce jars were brined at 3.7- and 21-percent strengths, and dry-salted at the rate of 1 pound of salt to 7 pounds of vegetable (1:7), and 1 pound of salt to 4 pounds of vegetable (1:4). To both brined lots a small amount of vinegar was added. The dry-salted treatments were done in duplicate and one set was stored at refrigerator temperature; the remaining set and the brined lots were kept at room temperature.

The results for this series are shown in table 14. In the corn receiving the weak brine, a rapid and vigorous fermentation was brought about by the acid-forming bacteria, resulting in a developed acidity

TABLE 14.—*Fermentation of 3.5-pound lots of blanched (10 minutes in steam) white corn according to different brining and dry-salting treatments,*[1] *stored at room (70°–80° F.) and refrigerator (35.6° F.) temperatures in 64-ounce jars*

Age in days	Micro-organisms per milliliter of brine and brine acidity							
	Total count	Acid formers	Coliforms	Peptonizers	Cocci	Yeasts	Acidity as lactic acid	pH[2]
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Hundreds*	*Percent*	
CO–1 (3.7-PERCENT BRINE+VINEGAR)								
0	240, 000	-------	62, 000	-------	-------	0	0. 37	4
2	365, 000	202, 000	800	2, 000	-------	0	. 65	4
5	1, 040, 000	1, 040, 000	0	<100	<100	40	1. 25	3
6	208, 000	208, 000	0	<10	<10	<10	1. 38	3. 72
11	63, 000	63, 000	0	<10	<10	<100	1. 20	3. 56
14	58, 000	58, 000	0	<100	<100	<100	1. 38	3. 60
17	37, 000	37, 000	-------	<100	<100	<100	1. 48	3. 58
20	22, 000	22, 000	-------	<100	<100	<100	1. 57	3. 58
23	23, 000	23, 000	-------	<100	<100	-------	1. 41	3. 56
29	3, 600	3, 600	-------	<100	<100	0	1. 59	3. 52
33	2, 400	2, 100	-------	<10	<10	<10	1. 65	3. 57
39	270	270	-------	<10	<10	<10	1. 55	3. 52
46	140	140	-------	<10	<10	<10	1. 39	3. 48
53	40	-------	-------	-------	-------	<10	1. 39	3. 52
60	1, 200	1, 200	-------	<10	<10	-------	1. 37	3. 45
70	2, 600	2, 600	0	0	0	<10	1. 65	3. 50
78	7, 800	7, 800	-------	<10	<10	<100	------	------
99	90	90	0	0	-------	<10	------	------
CO–2 (21-PERCENT BRINE+VINEGAR)								
0	6, 200	<10	220	5	-------	0	0. 14	5
2	25, 000	<10	5, 500	-------	-------	2	. 17	+4
5	5, 800	<100	30	<100	5, 800	<10	. 20	+4
8	2, 900	<10	0	-------	2, 900	<10	. 16	5. 18
11	2, 800	<10	0	2, 800	2, 800	<10	. 23	5. 13
14	3, 100	<10	0	3, 100	3, 100	0	. 19	5. 08
17	4, 000	<10	-------	4, 000	4, 000	0	. 20	5. 15
20	3, 400	<10	-------	3, 400	3, 400	0	. 30	5. 10
23	2, 600	<10	0	2, 600	2, 600	0	. 20	5. 12
29	890	<10	0	900	900	0	. 20	5. 05
33	450	<10	-------	420	450	0	. 22	5. 08
39	270	<10	-------	270	270	0	. 21	5. 12
46	120	<10	0	100	-------	0	. 24	5. 10
53	200	<10	-------	10	20	0	. 20	5. 12
60	40	0	-------	-------	40	0	. 22	5. 15
70	2	0	0	0	1	0	. 27	5. 15
78	20	0	0	-------	20	0	------	------
99	13	0	0	-------	13	0	------	------

See footnotes at end of table.

TABLE 14.—*Fermentation of 3.5-pound lots of blanched (10 minutes in steam) white corn according to different brining and dry-salting treatments,*[1] *stored at room (70°–80° F.) and refrigerator (35.6° F.) temperatures in 64-ounce jars*—Continued

CO–3 (1:7 DRY SALT)

Age in days	Micro-organisms per milliliter of brine and brine acidity							
	Total count	Acid formers	Coli-forms	Pepto-nizers	Cocci	Yeasts	Acidity as lactic acid	pH [2]
	Thousands	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Hundreds*	*Percent*	
0	44	<10	0	30	-------	0	0. 08	5
2	1, 650	<10	0	300	-------	0	. 10	5
5	5, 700	<100	0	<100	5, 700	<10	. 20	5
8	4, 000	<10	0	-------	4, 000	<10	. 24	5. 35
11	4, 000	<10	0	3, 000	4, 000	0	. 21	5. 04
14	3, 000	<10	0	900	3, 000	0	. 27	5. 15
17	2, 300	<10	-------	400	2, 300	0	. 26	5. 22
20	870	<10	-------	300	870	0	. 28	5. 16
23	240	<10	0	200	240	0	. 30	5. 12
29	140	<10	0	130	140	0	. 31	5. 08
33	230	0	-------	230	230	0	. 31	5. 05
39	47	0	-------	7	47	0	. 30	5. 02
46	21	0	0	8	21	0	. 34	5. 02
53	160	0	-------	0	160	0	. 30	5. 08
60	7, 200	<10	-------	600	7, 200	0	. 25	5. 45
70	118	0	0	<10	118	0	. 27	5. 10
78	60	0	-------	-------	60	0	------	------
99	10	0	0	-------	10	0	------	------

CO–4 (1:4 DRY SALT)

0	31	0	0	30	-------	0	0. 06	5
2	71	10	0	20	-------	0	. 07	5
5	91	<10	0	<10	100	0	. 07	5
8	80	0	0	10	80	<10	. 10	5. 92
11	510	<10	0	210	510	<10	. 08	5. 77
14	12	0	0	3	12	0	. 11	5. 88
17	100	0	-------	30	100	0	. 10	5. 80
20	27	0	-------	<10	27	0	. 09	5. 76
23	69	0	0	20	69	0	. 08	5. 78
29	69	0	0	1	69	0	. 09	5. 72
33	160	0	-------	<10	160	0	. 12	5. 72
39	1, 820	0	-------	1	1, 820	0	. 12	5. 70
46	570	<10	0	<10	570	0	. 13	5. 66
53	1, 500	<10	-------	<10	1, 500	0	. 10	5. 65
60	280	<10	-------	<10	280	0	. 12	5. 38
70	80	0	0	<10	80	0	. 12	5. 70
78	170	0	-------	-------	170	0	------	------
99	5	0	0	-------	5	0	------	------

See footnotes at end of table.

TABLE 14.—*Fermentation of 3.5-pound lots of blanched (10 minntes in steam) white corn according to different brining and dry-salting treatments,*[1] *stored at room (70°–80° F.) and refrigerator (35.6° F.) tempera tures in 64-onnce jars*—Continued

CO–3 Re (CO–3 Refrigerated, 35.6° F.)

Age in days	Micro-organisms per milliliter of brine and brine acidity							
	Total count	Acid formers	Coli-forms	Pepto-nizers	Cocci	Yeasts	Acidity as lac-tic acid	pH [2]
	Thou-sands	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Thou-sands*	*Hun-dreds*	*Per-cent*	
0	44	<10	0	30	-------	0	0. 08	5+
8	29	0	. 6	6	20	<10	. 10	6. 26
14	38	0	. 4	4	38	0	. 10	6. 20
23	58	0	0	15	58	0	. 12	6. 21
33	260	0	-------	30	260	0	-------	-------
39	100	0	-------	6	53	0	. 07	6. 04
60	84	0	-------	<10	84	0	. 10	6. 10
70	46	0	0	8	44	0	. 12	6. 10
CO–4 Re (CO–4 Refrigerated, 35.6° F.)								
0	31	0	0	30	-------	0	0. 06	5+
8	23	0	0	4	40	<10	. 06	6. 15
14	38	0	. 2	18	38	0	. 08	6. 10
23	35	0	0	5	35	1	. 09	6. 08
33	51	0	-------	25	51	0	-------	-------
39	53	0	-------	10	53	0	. 07	6. 04
60	47	0	-------	7	47	0	. 07	6. 30
70	24	1	0	10	23	0	. 10	6. 05

[1] CO–1; covered with 3.7-percent brine (14° salinity) containing 6 ounces of household vinegar per gallon.
CO–2; held at 21-percent brine strength (80° salinity); 2 ounces of vinegar per quart of brine.
CO–3; dry-salted at the rate of 1:7, salt and vegetable, by weight.
CO–4; dry-salted at the rate of 1:4, salt and vegetable, by weight.
CO–3 Re, and CO–4 Re; same as CO–3 and CO–4, but refrigerated.
[2] Whole numbers shown represent values estimated by test paper.

of about 1.6 percent and lowering the pH to approximately 3.5. These bacteria were the predominating organisms encountered. Just prior to the start of the acid fermentation, there had been a short period of activity on the part of the coliform group. Their growth ended promptly with the onset of the acid fermentation.

The three treatments at considerably higher salt concentration showed little or no growth of acid-forming bacteria, and as a consequence the brine acidity was relatively low as compared to the lot receiving weak brine. The principal organisms noted in these fermentations were the coccus forms. The character of the fermentation by these organisms appeared similar for the corn at 21-percent brine and for that dry-salted at 1:7, while for that dry-salted at 1:4

the populations of the cocci were somewhat restricted during the first month of the fermentation.

The dry-salted corn stored at refrigerator temperature showed little change with respect to microbial count, acidity, and pH during the storage period. The cocci predominated and remained in relatively constant numbers during the period of analysis.

Brined and Dry-Salted Yellow Corn

The yellow corn was handled in essentially the same manner as that described for white corn. The material was obtained from the North Carolina Agricultural Experiment Station and consisted of a mixture of Seneca Giant and an experimental yellow corn variety. Two brining treatments consisting of 21-percent brine with and without vinegar and one dry-salting treatment consisting of 1 pound of dry salt to 5 pounds of corn were used. Approximately 3.5 pounds of cut corn in 64-ounce jars were used in each treatment.

The results are presented in table 15. The usual plate counts showed relatively little activity by organisms other than the cocci group, whose growth was most active during about the first 3 weeks. The maximum acidity of the three lots was from about 0.20 to 0.30 percent. The pH values of those without added vinegar showed a decrease from the range of 6.5 to 6.7 to about 5.3 to 4.8 during a 2-month period; the pH values of the corn with added vinegar were lower during the early part of the fermentation than those of the other two lots.

In the treatments without added vinegar, the corn underwent a gaseous fermentation by organisms whose identity was not revealed by the routine plate counts used in this study. This was the first time during the experimental work that a gaseous fermentation was encountered at high salt concentrations that could not definitely be associated with activity by either the coliforms or yeasts. The new fermentation started in the corn at 21-percent brine strength after 1 week and continued moderately for about 25 days. It was characterized by a moderate gas evolution, gas pockets being noted in tightly packed corn. The gas was explosive and was presumed to be a mixture of carbon dioxide and hydrogen. No distinctive odor was detected. Later, the 1:5 dry-salted corn developed a similar type of fermentation, which started about the twenty-eighth day and continued for about a month.[5]

After rather intensive cultural observations, the responsible organism was isolated and studied, All attempts to cultivate it on ordinary media proved futile. The first growth was obtained in 1-percent yellow corn broth, provided 15 percent of salt was added, and the broth sealed with petroleum. However, even in the corn medium, growth could not be initiated from the actively fermenting material when a dilution of above 1:100 was used. But by use of liver broth plus liver particles, containing 15 percent salt, or liver agar plus salt, growth was obtained with dilutions of the fermenting material as high as 1:10,000,000. The liver media, therefore, was used exclu-

[5] Earlier preliminary work with dry-salted peas indicated the possibility of such a fermentation. In that instance, however, fermentation activity ceased before any definite information concerning the nature of the responsible organism could be established.

sively for the cultivation. It was found that during the gaseous fermentation in the salted corn the number of organisms was about 10 million per milliliter, and that the total count of Gram-negative cells was about 100,000,000 per milliliter. Gram-positive cells were

TABLE 15.—*Fermentation of 3.5-pound lots of blanched (10 minutes in steam) yellow corn in 21-percent brine (80° salinity) with and without added vinegar, and dry-salted at the rate of 1:5, salt and vegetable by weight, stored at room temperature (70°–80° F.) in 64-ounce jars*

YCO–1 (21-PERCENT BRINE)

Age in days	Micro-organisms per milliliter of brine and brine acidity							
	Total count	Acid formers	Coliforms	Peptonizers	Cocci	Yeasts	Acidity as lactic acid	pH
	Thousands	*Hundreds*	*Hundreds*	*Thousands*	*Thousands*	*Hundreds*	*Percent*	
0	6	0	0	1	6	2	0.02	6.75
3	10	<10	0	5	10	0	.02	6.63
6	140	<10	0	40	140	0	.02	6.35
7	360	0	-------	40	-------	0	.04	6.02
9	10	-------	-------	5	10	0	.15	5.16
12	4	<10	-------	1	-------	0	.21	4.76
15	.1	0	0	0	-------	0	.28	4.78
18	.2	0	-------	1	.2	0	.21	4.82
21	.2	0	0	0	-------	0	.23	4.70
25	.3	-------	-------	0	0	0	.22	4.82
31	.1	0	-------	-------	-------	0	.22	4.82
38	0	0	0	0	0	0	.22	4.83
45	1	0	-------	-------	-------	0	.28	4.85
52	0	0	-------	0	0	0	.20	4.80
62	0	0	-------	0	0	0	------	------
70	.3	0	-------	-------	0	0	------	------
91	.1	0	0	-------	-------	0	------	------

YCO–2 (21-PERCENT BRINE+VINEGAR) [1]

0	3	0	1	0	3	0	0.10	5.08
3	830	0	0	440	830	0	.08	5.74
6	880	0	0	650	880	0	.08	5.62
9	150	-------	-------	80	150	0	.09	5.68
12	44	<10	-------	20	44	0	.09	5.62
15	100	<10	0	80	100	0	.11	5.42
18	700	0	-------	.2	.5	0	.20	4.58
21	0	0	0	0	-------	0	.22	4.88
25	0	0	-------	0	0	0	.26	4.72
31	.1	0	-------	-------	-------	0	.25	4.82
38	.5	0	1	0	.5	0	.24	4.75
45	0	0	-------	-------	-------	0	.23	4.79
52	1	0	-------	.3	.8	0	.25	4.75
62	.7	0	0	0	.7	0	.27	4.78
70	.3	0	-------	-------	.2	0	------	------
91	0	0	0	-------	-------	0	------	------

[1] 1 ounce of household vinegar added.

TABLE 15.—*Fermentation of 3.5-pound lots of blanched (10 minutes in steam) yellow corn in 21-percent brine (80° salinity) with and without added vinegar, and dry-salted at the rate of 1:5, salt and vegetable by weight, stored at room temperature (70°–80° F.) in 64-ounce jars*—Con.

YCO–3 (1:5 DRY SALT)

Age in days	Micro-organisms per milliliter of brine and brine acidity							
	Total count	Acid formers	Coliforms	Peptonizers	Cocci	Yeasts	Acidity as lactic acid	pH
	Thousands	*Hundreds*	*Hundreds*	*Thousands*	*Thousands*	*Hundreds*	*Percent*	
0	20	<10	2	6	20	2	0.03	6.45
3	11	<10	0	4	11	0	.05	6.50
6	130	<10	0	10	130	0	.04	6.30
9	22	-------	0	7	22	0	.05	6.34
12	49	<10	-------	0	49	0	.06	6.32
15	90	<10	0	50	90	0	.05	6.14
18	22	0	-------	5	22	0	.08	6.08
21	7	0	0	0	7	0	.07	6.10
25	7	0	-------	1	7	0	.05	6.02
31	5	0	-------	.2	5	0	.08	6.06
38	2	0	0	.2	2	0	.15	5.42
45	2	0	-------	0	2	0	.14	5.22
52	.5	0	-------	.1	-------	0	.12	5.95
62	.1	0	0	0	0	0	.20	5.35
70	.1	0	-------	-------	0	0	------	------
91	0	0	0	-------	-------	0	------	------

not noted. The period of active fermentation in the salted corn (YCO–3), starting after about 38 days, coincided with a slight increase in acidity and a sharp decrease in brine pH. In the brined corn (YCO–1), a similar behavior as to acidity and pH was noted, which likewise coincided with the start of activity of the gaseous fermentation (after about 1 week).

Thus a study of the isolates made with the liver media showed that the organisms were gas-producing, Gram-negative rods, requiring reduced oxygen tension and at least 15 percent salt in the medium for growth. In addition it was found that the optimum pH range for growth was 6.5 to 7.0. Growth at pH 5.25 was somewhat slower than at the optimum and at pH 4.0 growth was absent. The optimum temperature for growth was about 35° F. The fact that none of the isolates could be made to initiate growth in the presence of less than 15 percent of salt by weight in either liquid or solid media indicates strongly that the organisms can be classified as obligate halophiles. Spores were not present in any of the strains examined microscopically, and subcultures from broth samples boiled 2 minutes failed to grow, indicating further that spores were not present.

To the author's knowledge, this type of gaseous fermentation resulting from the presence of halophilic organisms has not been reported previously in connection with brined or salted vegetable fermentations.

Brined and Dry-Salted Butter Beans

In another series of experiments, two brining and two dry-salting treatments of butter beans (Carolina Sieva) were used. In brief, these treatments consisted of: (1) Treating blanched beans with 21-percent brine strength plus vinegar; (2) and (3) giving blanched and unblanched beans the 1:5 dry-salting treatment; and (4) treating unshelled beans with 15-percent brine concentration. The lots salted and brined in 64-ounce jars ranged from 1.5 pounds for the unshelled beans to 3.5 pounds for the shelled beans.

The bacteriological changes that occurred during the fermentations are presented in table 16. The first three treatments show fairly similar plate count values which indicate relatively little microbial activity throughout the period of observation. The maximum acidity was in the range of 0.20 percent and pH was in the range of 5.0 to 5.5. The beans with added vinegar showed slightly lower pH values. Limited microbial activity was also indicated by the fact that the brine of the first three lots remained practically clear. In striking contrast to other vegetables, such as white corn, which received very similar salting and brining treatments, the salt-tolerant cocci appeared inhibited in the butter bean treatments. This suggests the presence of an inhibiting agent in the blanched beans and the unshelled beans. In dry-salted unblanched butter beans (BB–4), the cocci were the predominating organisms found; but their populations, except at one observation, did not exceed 40,000 per milliliter. This lot shows acidity values consistently higher than those previously discussed for the butter beans, or for any of the vegetables dry-salted in this range of salt content; however, the brine acidity at brine equilibrium (achieved after about 3 days) was likewise higher.

Dry-Salted Ripe Tomatoes

Ripe tomatoes (Marglobe) were dry-salted at the ratio 1:5, salt and vegetable by weight, and at 5 percent salt by weight. Approximately 2 pounds of quartered tomatoes in 32-ounce jars were used for both experiments. The results for the two fermentations are shown in table 17.

In the tomatoes that received the 1:5 salting treatment the combined effect of salt and the initial acid content of the tomatoes appears to have been sufficient to inhibit the development of the usual brine organisms within the period of observation. The acid content alone would be sufficient to inhibit active growth by the cocci and coliforms, and the salt content (17 percent) high enough to restrict the growth of the acid-forming bacteria. In the tomatoes salted at 5 percent by weight, except for a vigorous yeast fermentation, and a few thousand acid-producing bacteria per milliliter, there was little evidence of the presence of organisms.

Brined and Dry-Salted Okra

Three 1-pound lots of whole okra and one of cut okra were handled as follows: The whole vegetable received three brining treatments: 15-percent brine with and without added vinegar, and 5-percent brine with added vinegar. The cut vegetable was dry-salted at the

rate of 1:5 by weight of salt and vegetable. All the lots were stored in 32-ounce glass jars.

The results of this series, presented in table 18, show that the fermentations at the three 15-percent concentrations behaved in a similar manner, particularly with respect to the acid fermentation. Little growth by the acid-producing bacteria was found; consequently, the brine acidity was low. A small amount of vinegar added at the

TABLE 16.—*Fermentation of 1.5- to 3.5-pound lots of raw and blanched (5 minutes in steam) butter beans under different brining and salting treatments* [1] *stored at room temperature (70°–80° F.) in 64-ounce jars.*

BB–1 (21-PERCENT BRINE+VINEGAR; BLANCHED BEANS)

Age in days	Micro-organisms per milliliter of brine and brine acidity						Acidity as lactic acid	pH [2]
	Total count	Acid-formers	Coliforms	Peptonizers	Cocci	Yeasts		
	Hundreds	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Percent*	
0	30	0	0	0	0	7	0.12	5
3	50	0	0	10	-------	0	.18	5
7	2	0	0	-------	2	0	.21	5.15
10	15	0	0	1	2	0	.21	5.12
13	8	0	0	0	8	1	.20	5.12
16	3	0	-------	0	2	0	.19	5.05
19	1	0	-------	0	-------	0	.19	5.06
22	2	0	0	1	-------	0	.21	5.06
28	1	0	0	1	-------	0	.23	5.05
32	3	0	-------	0	3	0	.22	4.98
38	0	0	-------	0	0	0	.21	5.06
45	0	0	0	0	0	0	.23	5.04
52	0	---------	-------	-------	-------	0	.20	5.08
59	0	0	-------	0	0	0	.22	5.55
69	4	0	0	2	4	0	.27	5.15
77	0	0	-------	-------	0	0	------	------
98	0	---------	0	-------	-------	0	------	------

BB–2 (1:5 DRY SALT; BLANCHED BEANS)

0	200	0	0	60	-------	1	0.12	4 +
3	30	0	0	-------	-------	0	.17	4 +
7	80	0	0	-------	80	0	.18	5.58
10	5	0	0	4	5	0	.18	5.53
13	2	0	0	3	20	0	.15	5.54
16	3	0	-------	0	2	0	.19	5.50
19	1	0	-------	0	-------	0	.18	5.56
22	1	0	0	1	-------	0	.20	5.52
28	0	0	0	0	-------	0	.22	5.48
32	0	0	-------	0	0	0	.18	5.32
38	1	0	-------	0	0	0	.19	5.48
45	110	0	3	100	110	0	.16	5.42
52	90	0	-------	30	90	0	.15	5.49
59	9	0	-------	1	7	0	.22	5.55
69	3	1	0	1	2	0	.27	5.52
77	20	0	-------	-------	-------	0	------	------
98	6	0	0	-------	5	0	------	------

See footnotes at end of table.

TABLE 16.—*Fermentation of 1.5- to 3.5-pound lots of raw and blanched (5 minutes in steam) butter beans under different brining and salting treatments* [1] *stored at room temperature (70°–80° F.) in 64-ounce jars*—Continued

BB–3 (15-PERCENT BRINE; UNSHELLED BEANS)

Age in days	Micro-organisms per milliliter of brine and brine acidity						Acidity as lactic acid	pH [2]
	Total count	Acid formers	Coliforms	Peptonizers	Cocci	Yeasts		
	Hundreds	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Percent*	
0	420	0	30	110	-------	0	0. 015	5
3	10	<10	0	-------	-------	0	. 05	5
7	2	0	0	-------	-------	0	. 08	5. 34
10	3	0	0	1	3	0	. 10	5. 28
13	0	0	0	0	0	0	. 12	5. 14
16	1	0	-------	0	-------	0	. 11	5. 22
19	3	0	-------	2	-------	0	. 14	5. 28
22	0	0	0	0	-------	0	. 14	5. 12
28	0	0	0	0	-------	0	. 18	5. 02
32	4	0	-------	0	4	0	. 18	4. 98
38	130	0	-------	60	130	0	. 16	5. 04
45	110	0	0	50	110	0	. 17	4. 98
52	100	0	-------	30	100	0	. 20	5. 02
59	140	0	-------	5	140	0	. 17	5. 05
69	140	0	0	20	140	0	. 20	5. 02
77	58	0	-------	-------	58	0	------	------
98	8	0	0	-------	8	0	------	------

BB–4 (1:5 DRY SALT; UNBLANCHED BEANS)

0	4, 100	0	580	200	-------	1	0. 04	5
3	<10	<10	2	-------	-------	1	. 25	5
7	<10	<10	<10	-------	0	0	. 37	5. 44
10	10	0	0	1	1	0	. 38	5. 40
13	360	0	0	260	360	0	. 46	5. 34
16	80	0	-------	7	7	0	. 43	5. 32
19	130	0	-------	10	130	0	. 46	5. 38
22	340	0	0	40	340	0	. 46	5. 26
28	320	0	0	1	320	0	. 54	5. 24
32	1, 170	<10	-------	20	1, 170	0	. 45	5. 12
38	280	<10	-------	10	280	0	. 44	5. 20
45	0	<10	0	-------	0	0	. 58	5. 16
52	2	0	-------	0	2	0	. 46	5. 21
59	20	<10	-------	<10	20	0	. 50	5. 55
69	290	0	0	10	-------	0	. 47	5. 20
77	10	<10	-------	-------	-------	0	------	------
98	70	0	0	0	-------	0	------	------

[1] BB–1; blanched shelled beans (2.75 pounds) held at 21-percent brine (80° salinity) concentration + 1.5-ounce vinegar.
BB–2; blanched shelled beans (3.4 pounds) dry-salted at the rate of 1:5, salt and vegetable.
BB–3; unshelled beans (1.75 pounds) held at 15-percent brine (60° salinity) concentration.
BB–4; unblanched shelled beans (1.2 pounds) dry-salted at the rate of 1:5, salt and vegetable (24-ounce jar).

[2] Whole numbers shown represent values estimated by test paper.

TABLE 17.—*Fermentation of 2-pound lots of ripe tomatoes dry-salted at the rate of 1:5 by weight and 5 percent by weight, stored at room temperature (70° F.) in 32-ounce jars*

T–1 (1:5 DRY SALT)

Age in days	Micro-organisms per milliliter of brine and brine acidity							
	Total count	Acid formers	Coliforms	Peptonizers	Cocci	Yeasts	Acidity as lactic acid	pH
	Hundreds	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Percent*	
0	10	0	0	0	10	0	0. 52	3. 64
3	0	0	0	0	0	0	------	------
6	0	0	-------	0	0	0	. 75	3. 55
9	0	0	0	0	0	0	. 75	3. 58
11	14	3	0	0	11	0	. 79	3. 55
14	0	0	0	0	0	0	. 75	3. 56
17	1	0	-------	0	4	0	. 66	3. 55
20	2	0	-------	-------	1	0	. 70	3. 53
23	0	0	-------	0	0	0	. 70	3. 60
27	2	0	-------	0	1	0	. 72	3. 60
34	3	0	-------	-------	2	0	. 67	3. 57
39	0	0	0	0	0	0	------	------
46	4	0	-------	-------	4	7	------	------
71	2	0	0	-------	-------	-------	------	------

T–2 (5-PERCENT SALT)

	Thousands					*Thousands*		
0	0. 5	0	0	-------	5	1	0. 62	3. 72
3	75	-------	0	-------	9	4	------	------
6	140	<100	-------	<10	<10	5, 400	. 82	3. 68
9	230	<100	0	0	<10	31, 000	. 98	3. 74
11	140	-------	0	<10	<10	14, 200	. 93	3. 74
14	44	0	0	<10	<10	16, 800	. 94	3. 74
17	180	100	-------	<10	<10	7, 100	. 81	3. 70
20	430	20	-------	<10	<10	3, 700	. 86	3. 72
23	550	40	-------	0	0	2, 800	. 82	------
27	250	100	-------	<10	<10	1, 400	. 80	3. 65
34	500	100	-------	0	<10	230	. 57	3. 75
39	200	220	0	<10	<10	160	------	------
46	520	300	-------	-------	<10	70	------	------
71	30	-------	0	-------	-------	25	------	------

start (OK–1) resulted in slightly higher acidity values and lower pH values as compared with two lots to which vinegar was not added. Furthermore, this addition of vinegar appeared to exert a retarding influence on the growth of the coccus forms, the predominating types found. As the data show, the use of a lower salt-content brine (OK–4) resulted in a vigorous acid fermentation. A vigorous yeast fermentation as well developed after about 1 week and continued active for over a month. There was no evidence to show that the coliform group was active in any of the four fermentations followed.

TABLE 18.—*Fermentation of 1-pound lots of whole and cut okra at different brining and dry-salting treatments* [1] *stored at room temperature (60°–70° F.) in 32-ounce jars*

OK–1 (15-PERCENT BRINE+VINEGAR)

Age in days	Micro-organisms per milliliter of brine and brine acidity							
	Total count	Acid formers	Coli-forms	Pepto-nizers	Cocci	Yeasts	Acid-ity as lactic acid	pH
	Thou-sands	*Hun-dreds*	*Hun-dreds*	*Hun-dreds*	*Thou-sands*	*Hun-dreds*	*Per-cent*	
1	0.2	0	0	0	0	0	0.16	4.48
3	<.1	0	0	0	<.1	0	.21	4.62
6	<.1	0	0	0	<.1	0	.24	4.76
9	<.1	0	-------	0	<.1	0	.20	4.71
12	<.1.	0	-------	0	<.1	0	.22	4.76
15	1	0	0	4	.1	0	.19	4.75
19	.5	10	-------	1	.3	0	.17	4.78
26	9	0	-------	0	9	0	.20	4.75
31	6	0	0	0	6	0	------	------
38	16	0	-------	-------	16	0	------	------
63	.6	---------	0	-------	-------	-------	------	------

OK–2 (1:5 DRY SALT)

1	31	0	14	50	31	0	0.07	5.52
3	14	10	1	40	14	0	.09	5.25
6	6	0	0	6	6	0	.18	5.46
9	2	0	-------	2	2	0	.13	5.46
12	2	0	-------	0	2	0	.17	5.42
15	5	0	-------	5	5	0	.13	5.60
19	42	0	-------	<10	42	0	.12	5.55
26	.2	0	-------	0	.1	0	.20	5.40
31	16	0	0	1	16	0	------	------
38	.8	0	-------	-------	8	0	------	------
63	---------	---------	0	-------	-------	-------	------	------

OK–3 (15-PERCENT BRINE ONLY)

1	14	0	2	20	14	0	0.08	5.50
4	34	20	0	17	30	0	.15	5.56
7	36	30	-------	.7	36	-------	.09	5.81
10	62	10	-------	4	60	-------	.05	5.58
13	130	0	-------	3	130	0	.10	6.08
17	7	0	-------	<10	7	0	.10	5.75
24	1	0	-------	.3	1	0	.12	5.78
29	15	0	0	0	15	0	------	------
36	250	---------	-------	-------	250	-------	------	------
61	4	---------	0	-------	-------	-------	------	------

See footnotes at end of table.

TABLE 18.—*Fermentation of 1-pound lots of whole and cut okra at different brining and dry-salting treatments* [1] *stored at room temperature (60°–70° F.) in 32-ounce jars*—Continued

OK–4 (5-PERCENT BRINE+VINEGAR)

Age in days	Micro-organisms per milliliter of brine and brine acidity							
	Total count	Acid formers	Coliforms	Peptonizers	Cocci	Yeasts	Acidity as lactic acid	pH
	Thousands	*Hundreds*	*Hundreds*	*Hundreds*	*Thousands*	*Hundreds*	*Percent*	
1	1,000	1,000	36	[2] 0	[2] 0	10	0.32	4.40
2	520,000	520,000	0	0	0	10	.79	3.52
3	720,000	720,000	0	0	0	10	1.03	3.38
4	500,000	500,000	0	0	0	10	1.08	3.52
7	96,000	96,000	-------	0	0	470	.88	3.50
10	50,000	50,000	-------	0	0	29,000	.95	3.48
11	-------	-------	-------	0	0	11,000	------	------
13	20,000	20,000	-------	0	0	5,900	.92	3.45
17	18,000	18,000	-------	0	0	8,500	.95	3.45
24	4,600	4,600	-------	0	0	810	.97	3.50
29	3,000	3,000	0	0	0	10,200	------	------
36	1,500	1,500	-------	0	0	900	------	------
61	160	---------	0	0	0	-------	------	------

[1] OK–1; Whole okra held at 15-percent brine strength (60° salinity) plus 2 ounces household vinegar.
OK–2; Cut okra dry-salted at the rate of 1:5, salt and vegetable by weight.
OK–3; Whole okra held at 15-percent brine strength.
OK–4; Whole okra covered with 5-percent (20° salinity) brine plus 1 ounce of vinegar.

[2] Zeros in column indicate that these micro-organisms were not detected on the 1–10,000 dilution plates.

BRINED AND DRY-SALTED SHELLED GREEN PEAS

In addition to the experiments previously described on the brining of unshelled green peas, a number of small-scale experiments were conducted with shelled green peas. These involved brining and dry-salting treatments of 12-ounce lots of both blanched and unblanched peas stored in 16-ounce glass containers at room temperature. The various fermentations were followed with respect to the predominating organisms occurring in the brine and to developed brine acidity. The results of this study may be summarized as follows:

In the brining treatments, four initial brine concentrations were used for blanched peas, namely, 5-, 10-, 15-, and 20-percent salt, and were maintained at these strengths by the addition of salt to the brined material. The bacteriological results showed that at brine strengths above 5 percent, correspondingly lower populations of acid-

producing bacteria occurred. This was reflected in progressively lower amounts of developed brine acidity. The maximum acidities resulting from fermentations in 5-, 10-, 15-, and 20-percent brines were about 1.0, 0.40, 0.25, and 0.15 percent, respectively. The maximum populations reached by the acid-forming bacteria were about 800,000,000 per milliliter in the 5-percent brine treatment as compared to 40,000,000 and 300,000 for the 10- and 15-percent treatments, respectively. Only a few thousands of these organisms per milliliter were noted in the 20-percent lot, and they were present only during the first few days.

The coliform group, present in all four brining treatments, was most active in the 5- and 10-percent brines where they reached populations of about 7 million per milliliter after about 1 week, and had a rather prolonged period of activity as compared to previously discussed fermentations by these organisms. This prolonged period is possibly explained by the buffering effect of the blanched peas, because the usual effect of appreciable brine acidity toward inhibition of this group appears to have been lessened. At the point where the maximum populations were observed in both brines, the brine acidities appeared sufficiently high to bring about pH conditions usually considered inhibitory for growth of the coliforms in brined vegetable fermentations. In these instances, however, the pH level had not been reduced below 5, a level still within the range found satisfactory for growth. The coliform group, while present also in the 15- and 20-percent brine treatments during the first 10 days, occurred in lower numbers at those strengths than in the 5- and 10-percent brines. The maximum numbers observed in the two strong brines were at about the 1,000,000 per milliliter level.

Observations revealed that the salt-tolerant coccus forms were the predominating organisms found in the 15- and 20-percent brines and that they were continually present throughout the 36-day sampling period. The populations noted were higher in the 15-percent treatment than in the 20-percent. The maximum numbers in the former were within the 20,000,000 per milliliter level, while in the latter the populations were about one-tenth as numerous. In the peas brined at 5-percent strength the salt-tolerant cocci were either absent or in very low numbers, a fact apparently due to the inhibitory effect of the acid developed in this fermentation. In the 10-percent brined peas, however, they were present in appreciable numbers, although the developed brine acidity had reached about 0.40 percent, which ordinarily would be considered sufficient to bring about a pH range unfavorable for growth, particularly in a medium not highly buffered. Actually, the pH of the brine was about 5.6, which is well within the range for the development of these organisms.

The buffering effect of the peas was further demonstrated by the fact that the brine of the 5-percent peas developed a titratable acidity of approximately 1.0 percent, whereas the pH of the brine was 4.5, a pH value considered relatively high compared to that for other vegetables at this salt concentration. Ordinarily, the brine pH level is about one unit lower for a comparable amount of titratable acidity.

Neither yeasts nor mycoderma were found in any of the brines in this series during the period of analyses.

In the dry-salting experiments, duplicate lots of blanched and unblanched peas, with and without added vinegar, were salted according to the 1:5 treatment. Fifteen milliliters of 110-grain vinegar was used in the acidified lots. The results were similar to those previously described for the peas brined at 15- and 20-percent strengths. The cocci were the principal organisms found throughout the observation period. When vinegar was added to either blanched or unblanched salted peas, the maximum populations of the cocci found were reduced about tenfold. Consistently higher numbers of these organisms were present throughout the fermentation period in the blanched peas as compared to those present in unblanched peas. For example, during a period extending from the sixth to twenty-first day, the counts ranged from 122,000,000 to 690,000,000 per milliliter for the dry-salted blanched peas, as compared to 900,000 to 10,000,000 for the unblanched lots during the same period. The period chosen represented the active phase of growth activity.

The fermentation of both blanched and unblanched salted peas resulted in very little developed brine acidity, the range being from about 0.10 to 0.20 percent. The corresponding brine pH range was from 5.7 to 5.2. In the lots acidified with vinegar, the titratable acidity was about 0.50 percent for both blanched and unblanched peas. This indicates that very little acidity was produced by the fermentation. Although the quantity of vinegar added to these lots was appreciable, the brine pH was not depressed below 5.0. This shows again the effect of the buffering action of the peas.

Brined Leafy Vegetables

Leafy vegetables, such as kale, mustard greens, spinach, and turnip greens, were brined in ½-pound lots in 32-ounce glass jars according to five treatments for each vegetable. The treatments used were essentially the same as those previously outlined for the carrot series, except that only half the quantities of lactic acid and vinegar were used. All jars were stored at room temperature and were examined at intervals of 4, 7, 10, 13, and 82 days, according to the microscopic-count technique. Plate counts were not made.

Since the microscopic counts showed all lots similar with respect to total cell populations during the fermentation period, only the most outstanding individual variations will be discussed. For the most part, the brine fermentations of this vegetable series started within 1 or 2 days and reached populations of about 100,000,000 cells per milliliter 5 to 7 days after brining. Then the counts declined to 1,000,000 to 10,000,000 per milliliter and remained relatively constant. Fermentation activity appeared to cover the first 2 weeks. The lots with added lactic acid were retarded a day or so in starting, as compared to the others, but later showed similar total cell populations.

The predominating cell types in the fermentations were the acid-producing, Gram-positive bacilli, divided into two principal morphological groups, the large and the small bacilli. The large bacilli were about 1×2.5 to 8 microns in size, usually occurred singly, but in some instances were in short chains of two to four elements. The small bacilli were approximately 0.75 to 1×1.5 to 2.5 microns in size and occurred for the most part singly or in pairs. The cocci were either absent or in rather low numbers in the majority of the fermentations. But in

the mustard greens that received 5-percent brine only, the cocci predominated and ranged in size from 1.2 to 1.5 microns in diameter, occurring singly, in pairs, or in tetrads.

The only instance in which the Gram-negative bacteria predominated during the active fermentation period in the leafy vegetable series, was in the case of the spinach that received 5-percent brine. The total cell counts at the sampling intervals in this lot were substantially lower (less than one-half) than for the acidified lots in the spinach series as a whole. Furthermore, this was the only lot of the 20 leafy vegetable fermentations recorded in which the material disintegrated and was considered spoiled. Less than 0.10 percent brine acidity was developed in this lot as compared to about 0.50, 0.35, and 0.40 percent in the kale, mustard greens, and turnip greens, respectively, that had received the same treatment.

Brined and Dry-Salted Celery

Several lots of cut and whole celery in the raw, steamed, or cooked condition were salted and brined. The six treatments used were as follows: One lot of raw cut celery was dry-salted at 2.5 percent by weight; the second was salted according to the 1:5 procedure; the third was steamed 5 minutes and salted with the 1:5 treatment; the fourth was cooked 10 minutes at 5 pounds' steam pressure and also salted with the 1:5 treatment; the fifth lot consisting of raw bunch celery was brined at 15-percent concentration; and the sixth lot consisting of cooked bunch celery was salted with the 1:5 treatment. In the first four of the treatments listed, 2-pound lots of cut celery in 32-ounce glass jars were used; while in the remaining two approximately 20 pounds of celery in 10-gallon crocks were used. All were stored at room temperature.

On the basis of observations covering a 2-month storage period, there was little evidence of growth of micro-organisms in any of the five high-salt-content lots. This conclusion is based on the low plate counts, the absence of gas evoluation, and the fact that the brines remained practically clear. The possible exception was in the raw bunch celery at 15-percent brine strength which showed populations of salt-tolerant cocci to the extent of about 50,000 per milliliter after about 40 days. The acidity for all the brined and salted lots after about 2 months was within the range of 0.28 to 0.35 percent, the major part of which was present initially. The pH range of the brines was from 4.90 to 4.52 after the same storage interval.

In the celery salted at 2.5 percent by weight an active fermentation by the acid-producing bacteria resulted in the formation of a considerable amount of acid. The fermentation was very similar to that previously discussed for lettuce kraut.

DISCUSSION

With only one or two exceptions, the salt concentration used in the preservation treatment rather than the type of vegetable exerted the greatest influence on microbial flora, in the various vegetable fermentations described in this publication. The discussion, therefore, will be presented from the standpoint of the general relationships which appear to exist between the predominating types of organisms found and the nature of the salting and brining treatment.

ACID-FORMING BACTERIA.—Active fermentation was developed by acid-forming bacteria with all the vegetables studied when the amount of salt used was within their growth range. A direct relationship was observed between the bacterial populations found and the salt concentration employed. In fermentations at low salt content, 5 percent or lower (such as used for lettuce, wax beans, carrots, green beans, white corn, and okra), large populations usually occurred. Correspondingly smaller populations were found when increasingly higher salt concentrations were employed. At 15 percent salt or above, little if any growth by the acid-producing bacteria was observed, since this appeared to be above their range of salt-tolerance. These relationships were similar to those reported for cucumber fermentations (*6*). The influence of salt on the fermentation activity of this group of organisms was also reflected in the amount of developed brine acidity. Fermentations at increasing salt concentrations resulted in the production of lower amounts of brine acidity. This relationship was likewise in agreement with that reported for brined cucumbers (*18*, *19*).

COLIFORM GROUP.—Growth of coliform organisms in the vegetable fermentations studied occurred over a rather wide range with respect to salt concentrations. Typical fermentations were observed in lettuce at 2.5 percent salt, and in brined shelled peas at 20 percent. The fermentation was gaseous in nature and behaved in a way similar to that reported for brined cucumbers, where this type of fermentation yielded substantial amounts of carbon dioxide and hydrogen (*5*, *19*). In brines of low salt content, 2.5 to 5 percent, such as used for green beans, carrots, peas, and white corn, the development of these bacteria was found to be almost immediate and seldom lasted over a few days. The limiting factor for their continued growth in weak brines is attributed to the inhibiting effect of the brine acidity resulting from a simultaneous development of the acid-forming bacteria. Because of apparent sensitivity to acid, the coliform group was usually inactive in fermentations such as encountered with brined carrots that were acidified at the start by the addition of small amounts of vinegar or lactic acid. However, in a highly buffered medium such as salted shelled peas, the fermentation may be considerably prolonged in the presence of a decided amount of developed acidity.

Identification studies were not made in the present investigation; however, in cultures isolated from both cucumber (*5*) and olive fermentations (*3*), the genus *Aerobacter* predominated among the coliform bacteria found. Members of the *Escherichia* genus were not isolated in either case.

YEASTS.—Active growth of yeasts was found in a variety of the brined and salted vegetables studied and was characterized by a gaseous fermentation which resulted in the evolution of rather large amounts of carbon dioxide. In general, once yeast fermentation was started, there appeared to be little correlation between the salt concentration used and maximum yeast populations, but as a rule, the salt content of the brine appeared to influence the time at which yeast activity began, as well as the total period during which significant populations were present in the brine. Usually, fermentations at low salt content—about 5 percent—started earlier, and were of

shorter duration than those at higher concentration—10 to 15 percent. The developed brine acidity appeared to exert no significant inhibitory effect on the general yeast fermentation, nor did acidification of brines at the start with small amounts of vinegar or lactic acid appear to inhibit the general fermentation behavior of the yeasts. Thus, yeast development in vegetable fermentations was encountered over a rather wide range with respect to both salt and acid contents of the brine. These results are in agreement with those reported for the yeast activity in cucumber fermentations (*4*, *5*, *6*, *19*, *21*).

Cocci.—The occurrence of certain salt-tolerant cocci in vegetable fermentations at high salt concentration was mentioned recently in connection with fermentation of salted green beans and peas (*11*). In the present study this type of fermentation has been found common also to other vegetables, such as corn and butter beans, at high salt concentrations. These organisms are apparently sensitive to small amounts of added organic acids (such as lactic and acetic), or appreciable brine acidity. They are not, however, particularly restricted by high salt concentrations, such as brines of upwards of 20-percent strength. Furthermore, the results for dry-salted green beans indicated that the cocci were able to develop under refrigerated conditions (35° F.) in the presence of 10 percent salt and in the absence of appreciable brine acidity. But in the case of both green beans and white corn, storage under refrigerated conditions at 15- to 20-percent salt content appeared sufficient to restrict the active growth of the cocci. The fermentation by these organisms was not found to be gaseous in nature, nor did they seem to contribute appreciably in acid production, although detectable amounts might have been found in instances where growth took place under reduced oxygen tension. The cocci's lack of tolerance toward acid is a reasonable explanation for their usual absence in fermentations at concentrations well within the growth range for the acid-forming bacteria. A portion of the cocci that appeared in high-salt-content brines had the ability to peptonize casein.

Peptonizing Bacteria.—The peptonizing bacteria were not considered a distinct microbial group such as the lactic acid bacteria, or the coliforms, nor necessarily a predominating type as far as the brine organisms found in this study were concerned; but rather, as the term implies, distinction was based on the ability of certain organisms to peptonize a protein such as casein when grown on nutritive caseinate agar or a similar medium. In low-salt-content brines, the peptonizing bacteria occurred chiefly at the start of fermentation and then disappeared promptly when an appreciable amount of acid was produced by the lactic acid bacteria. In vegetable fermentations at high salt content (corn, butter beans, peas, and okra), and in the absence of appreciable brine acidity, the peptonizing bacteria found usually were the salt-tolerant cocci.

Mycoderma (Film-Forming Yeasts).—For the purpose of this discussion, the mycoderma group refers to the genera of film-forming yeasts responsible for the luxuriant surface scum commonly associated with pickle brines exposed to the air but sheltered from direct sunlight. In general, they are differentiated from most other brine yeasts in that their surface growth is chiefly oxidative and an alcoholic fermentation is not ordinarily produced. In the presence of air, the

film-forming yeasts usually appear able to oxidize readily alcohol and organic acids such as lactic and acetic (*16*). This affords a reasonable explanation for the decrease in brine acidity during storage in those lots of vegetables in which active scum growth appeared.

In vegetable fermentations, the salt content of the brine may retard growth and yet may not effectively control scum yeast development on the exposed brine surface, particularly if the latter is sheltered from direct sunlight. Under such sheltered conditions, surface growth may occur at salt concentrations upwards of 20 percent. However, in the present study, there was little evidence that these organisms grew underneath the surface of strong brines as did the brine yeasts; but in weak brines of 5 percent or less, growth was found in several instances that was not associated with a surface film. This is in keeping with previous work which has shown that rather slow scum yeast growth results in utilization of the brine acids and sugars, even under a layer of heavy mineral oil (*12*).

In the strictest sense, conditions that permit the growth of scum can hardly be considered typical of those favoring a fermentation on which the best observations as to the predominating microbial brine flora may be made. A luxuriant growth of scum, making sampling difficult, may result in an exaggerated total bacterial count and confuse the subsurface brine yeast count. Furthermore, the unrestricted surface growth may destroy all or a portion of the brine acidity, and in certain instances helps provide a foundation for mold growth such as was noted in brined wax beans, which may seriously affect the texture of the vegetable material.

MOLDS.—Observations were made for molds at each brine sampling interval for all the vegetable fermentations studied. Because they were frequently present on the vegetables before they were brined, some molds were generally found at the time the material was salted or brined, but gradually decreased and were entirely absent after a few days. In the few instances where mold populations appeared in the brines in significant numbers after the first week or so, their presence could be directly associated with surface rather than subsurface mold growth. For this reason molds are not considered among the predominating groups of organisms associated with microbial changes in brine, and in this respect can be put in the same category with the film-yeast group. However, their possible influence on the preserved vegetable material cannot be overlooked. Heavy mold growth occurring on the brine surface of vegetable material, as found on wax beans, can be extremely important from the standpoint of loss of texture.

HALOPHILES.—The possibility of a gaseous fermentation by members of the halophilic group of bacteria has not been given attention in connection with the brine vegetable fermentation work previously reported. The principal difference between these organisms, found in the yellow corn fermentations, and the other predominating groups discussed in this paper with relation to the effect of salt on them, appears to be as follows: The groups of bacteria mentioned previously are considered to have facultative salt-tolerance characteristics, because they can grow in salt brines of different strengths and also can be cultivated with ease in the absence of such amounts of salt; whereas, gas-producing, halophilic organisms were encountered that

displayed an obligate relationship with respect to salt requirements, since at least 15 percent salt was necessary in the medium before isolates could be cultivated.

CONDITION OF VEGETABLES.—Results for the observations on condition of vegetables made during the fermentation and subsequent storage period for green beans, green peas (unshelled), lima beans (unshelled), carrots, and certain leafy vegetables have recently been published (*7*, *11*). They showed that after a storage period of 6 to 12 months, all lots, except one spinach treatment, were well preserved and had good color and odor and firm texture. Similar results have been observed for lettuce, corn, butter beans, wax beans, tomatoes, shelled peas, okra, and celery after about the same length of storage.

BUTYRIC FERMENTATION.—In this study, none of the 87 lots of vegetable material observed during active fermentation and storage gave evidence of the malodorus condition associated with the growth of anaerobic, saccharolytic, butyric acid bacteria of the *Clostridium* genus. Favorable growth conditions, with respect to both brine pH and salt content, were generally absent. For example, in fermentations at low salt concentration, used for readily fermentable vegetables, such as lettuce, carrots, green beans, and okra, a vigorous lactic acid fermentation resulted which rapidly lowered the brine pH to well below the usual tolerance of the butyric acid bacteria. With vegetables such as corn, butter beans, peas, and celery, brined and salted above 10-percent concentration, the salt alone would be considered sufficient to inhibit the reproduction of these organisms. There is the possibility too of interfering action of other microbial groups present.

Apprehension, therefore, regarding the public health aspect of salt-preserved vegetables that have been brined or salted according to approved practices (*8*) is unwarranted. Nevertheless, it should be pointed out that the growth of this group of organisms (as well as the more dangerous proteolytic types, i. e., *Cl. botulinum*) might take place in brines having pH values above 4.5 and containing less than 10 percent salt. In view, also, of the buffering action noted for blanched, shelled green peas, the practice of brining or salting this vegetable, or any other with similar buffering capacity, below 10-percent salt concentration, might be questionable.

According to Gililland and Vaughn (*15*), the butyric fermentation is commonly found in certain types of green olive pickling processes. Of the 50 cultures of nonproteolytic butyric acid bacteria isolated and identified by these workers, none was capable of developing at pH 4.3, and only one strain was able to grow with 6.0 percent salt in the cultural medium.

SUMMARY AND CONCLUSIONS

Results of a bacteriological investigation dealing with the preservation of a number of vegetables by use of one or more brining and dry-salting treatments have been presented. With the vegetables used, and under the conditions that existed for this study, the following general conclusions are indicated:

In most instances, microbial activity of varied intensity accompanied vegetable preservation by salting or brining and was present over a wide range with respect to both the type of vegetable and the

amount of salt used. Fermentation resulting from the development of one or more salt-tolerant groups of micro-organisms took place in preservation treatments ranging from 2.5 percent to 20 percent salt concentration for the vegetables studied.

The predominating groups of micro-organisms found were: The acid-forming bacteria, the coliform group, the yeasts, and the coccus forms. The development of the acid-forming bacteria in the various vegetable fermentations was restricted principally to salt concentrations below 15 percent. Growth by the yeasts and the coliform bacteria was found over an extended range with respect to the amounts of salt employed in the salting and brining treatments (2.5 to 20 percent). Salt-tolerant cocci, in most instances, were the predominating organisms found in fermentations at high salt concentrations.

The organism responsible for the new type of gaseous fermentation encountered in brined and in dry-salted yellow corn at 21 and 15 percent salt content, respectively, was considered to have an obligate relationship toward salt, in that it could not be cultivated in the absence of less than 15 percent salt by weight.

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APPENDIX

Bacteriological Media and Methods [6]

COLIFORM BACTERIA

Brilliant Green Lactose Bile Agar.—Brilliant green lactose bile agar (Difco formula) was found to be a satisfactory medium for estimating the relative populations of the coliform bacteria group. It was prepared and used according to the directions supplied with the dehydrated product. Strict attention was paid to the medium's sensitivity to light; when convenient, it was prepared just prior to use; otherwise, it was stored in the refrigerator. On this medium, subsurface colonies of the coliform bacteria are deep red in color against a blue background.

The plates were incubated 18 hours at 35° C. and the number of coliform colonies per milliliter of brine counted.

ACID-FORMING BACTERIA

Nutritive Caseinate Agar (Difco).—Nutritive caseinate agar, which has proved successful for determining population trends of acid-producing bacteria during the fermentation of cucumber pickles (*6*, *21*), proved to be just as successful in connection with the fermentation of the brined and salted vegetables reported herein, and in enumerating total count, salt-tolerant cocci, and peptonizing bacteria.

The medium was prepared according to the directions provided with the dehydrated product except that 0.04 grams of dry brom-cresol-purple was added per liter of dissolved medium 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, in order to avoid solidification difficulties. Not over 15 ml. of medium was used per plate to insure a reasonable solidification time. During hot weather, facilities for cooling the plates were provided.

The acid-forming bacteria show on this medium a precipitated zone of casein and a yellow color about the colony. In the study discussed in this publication, the degree of casein precipitation and change in color of the indicator from purple to yellow varied, depending on the activity and type of acid-former present. Subsurface colonies ranged in size from about 2.5–3 mm. to 0.1 mm. or less and were mostly elliptical in shape. Surface growth was usually scant. Occasionally, a high percentage of tiny colonies (0.1 mm. or less) was found in certain vegetable fermentations. Growth of the acid-forming bacteria could often be greatly enhanced by the addition of 0.1 percent dextrose to the medium. Since the addition of dextrose likewise enhances the growth of yeasts present, careful examination of yeasts becomes necessary to separate them from the acid-producing bacteria. This differentiation is facilitated by the fact that the acid-forming bacteria usually occur in much greater numbers than the yeasts during the simultaneous activity of the two groups. Furthermore, the acid-forming bacteria give an acid reaction and casein precipitation, whereas yeasts tend to give a slightly alkaline reaction. Only the lactose fermenting yeasts, as a rule, grow out well on this medium. In this investigation, when it was doubtful whether yeasts were confusing the count of acid-forming bacteria, the colonies were stained.

SALT-TOLERANT COCCI

Nutritive Caseinate Agar.—Although nutritive caseinate agar is not considered a differential medium for the salt-tolerant coccus forms, it was satisfactorily used for estimating the numbers of these organisms present in brines of high salt concentration. Differentiation was based on colonial characteristics on the agar and by stained preparations made from the principal colonies present. In routine examination of brines of high salt concentration, these organisms, as

[6] The media and methods described herein recently have been published (*10*) in revised and enlarged form to cover various types of brined, salted, and pickled vegetables and vegetable products.

a rule, are the principal types found on the above plating medium. Their presence is usually indicated by two predominating colonial types: A grayish-white, entire, glistening colony of moderate size, and a similar colony that is light orange to yellow in color. The subsurface colonies of both types appear elliptical to lenticular in shape. The cells of the white colonial type are distinctly smaller than those of the pigmented variety.

The majority of the cocci encountered were alkali-producers, as shown by their reaction when grown aerobically on nutritive caseinate agar with indicator added. Under reduced oxygen-tension, however, as encountered in deep subsurface colonies, some types gave an acid reaction, often sufficient to precipitate zones of casein about the individual colonies within 72 hours; but, upon prolonged incubation, the reaction usually became alkaline. Thus, care must be exercised to prevent the recording of such acid-producing cocci colonies as true acid-forming bacteria of the lactic group, particularly when these colonies are found in brines that border on the range of salt concentration tolerated by the lactic acid bacteria. This precaution need not apply when brines are well within the range for active growth of the lactic acid bacteria, since then the developed acidity is normally sufficient to inhibit growth of these coccus forms.

For this medium, the plates were incubated 72 hours at 35° C. and first counted as to total colonies, acid-forming colonies, and cocci colonies per milliliter of brine. For a record of the number of peptonizing bacteria the plates were then flooded with a 5-percent solution of glacial acetic acid. The colonies surrounded by clear zones were recorded as peptonizing bacteria.

YEASTS

ACIDIFIED DEXTROSE AGAR.—Yeast populations in the fermenting vegetable table brines were determined by the use of acidified dextrose agar. This medium, which has been used previously in cucumber fermentation studies (*4*, *5*, *6*), consists of ordinary dextrose agar (Difco formula) to which 5 ml. of sterile 5-percent tartaric acid is added to 100-ml. amounts of the melted agar prior to pouring the plates. The addition of the tartaric acid, bringing the pH of the medium to 3.5–3.7, inhibits active development of the other usual brine organisms.

Occasionally, yeasts are found at concentrations of 15 to 20 percent salt that do not grow out well on the medium containing the usual amount of tartaric acid (*5*). If the amount of tartaric acid is lowered to 3 ml. per 100 ml. of medium this condition is usually corrected. The modified medium should not be used, however, when the known salt content of the brine sample is much below 15 percent; otherwise the acid-forming bacteria will grow out sufficiently to confuse the yeast count. Occasionally, during an active acid fermentation by high acid-tolerant strains of the lactic group, some tiny colonies begin to show through even when the full amount of tartaric acid is used. These numerous, small undeveloped acid-producing bacteria colonies form a halo effect about the yeast or mycoderma colonies where the acid reaction is presumably of less concentration than the surrounding medium. Acidified dextrose agar is definitely preferred for detecting yeasts in brine fermentation over Wort or Malt agar since the pH range (about 4.5 to 5.5, respectively) for the latter media is much less inhibitive for the lactic acid bacteria.

MYCODERMA (FILM-FORMING YEASTS) AND MOLDS

ACIDIFIED DEXTROSE AGAR.—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 nonfilm-forming yeasts and mycoderma scum yeasts may present some difficulty. Surface colonies of yeasts indicative of mycoderma scum are normally flat, dull, irregular, and spreading as contrasted with raised, round, white, glistening, and entire for 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.

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: Incubate plates 3 days at 32–35° C. and record number of yeast, mycoderma scum, and mold colonies per milliliter of brine or liquor examined. When yeast colonies are not well developed within 3 days, the incubation period should be extended to 5 days.

OBLIGATE HALOPHILES

LIVER BROTH PLUS SALT.—Liver broth plus salt was used as the medium for the detection of the gas-producing, Gram-negative, obligate halophilic bacteria reported in dry-salted and brined yellow corn at 17 and 21 percent salt respectively. Either liver agar containing 15 percent salt or liver broth plus salt can be used. The liquid medium is probably more convenient to handle. The basic liver broth was prepared according to the following formula of Cameron (*2*) except that peptone was replaced with tryptone:

> Ground beef liver is mixed with water in the proportion of 500 grams to 1,000 cc. 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 cc. To the broth are added 10 grams of peptone and 1 gram of dipotassium phosphate. The reaction is adjusted to pH 7.0 * * *

Fifteen percent C. P. salt by weight was added to the broth which was then put into ⅝ x 6 inch tubes containing about one-half inch of partially dried liver particles remaining from the broth preparation. The tubes were autoclaved at 15-pound pressure for 20 minutes. Tubes of the media, previously heated in a water bath and cooled, were inoculated with decimal dilutions of the sample and sealed with 1 to 2 milliliters of sterile, melted petroleum jelly. Positive tubes were indicated by the raising of the petroleum seal as a result of gas production, and the absence of any distinctive odor. The tubes were incubated at 35° C. for 4 days and observed daily for positives. Usually, growth was prompt and vigorous, often sufficient to raise the seal to the cotton plug in 24 hours. Tubes showing turbidity but no gas usually indicated the absence of halophiles and the presence of cocci.

Halophiles may be found in certain brines that grow better with less than 15 percent salt in the cultural media. For such types, the liver medium should be adjusted to the approximate concentration of the fermenting brine.

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 the known inability of yeasts and coliforms to initiate satisfactory early growth in laboratory media at even moderatly high salt concentration without previous subculturing. The reduced oxygen tension of the medium probably also exerts a retarding effect. Nevertheless, routine determinations are desirable for the yeasts and coliform bacteria, including stained preparations, on any brine that is being examined for obligate halophiles.