

BACTERIOLOGICAL CHANGES DURING THE FERMENTATION OF STEAMED POTATOES FOR SILAGE

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BACTERIOLOGICAL CHANGES DURING THE FERMENTATION OF STEAMED POTATOES FOR SILAGE¹

By JOHN L. ETHELLES, *bacteriologist, Agricultural Chemical Research Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture*, and IVAN D. JONES, *research professor of horticulture, North Carolina Agricultural Experiment Station*²

INTRODUCTION

During the summer of 1944 the War Food Administration sponsored experimental work at a number of State agricultural experiment stations on the utilization of surplus potatoes (*Solanum tuberosum* L.) for stock feed. The use of the surplus in the form of silage for livestock was stressed, and general recommendations as to the procedures to be followed were given.³ It was stated that the best method was that of steaming and ensiling in pits.

The experimental studies at the North Carolina station were based on three carloads of potatoes and involved the cooperative efforts of several departments (Agricultural Engineering, Animal Industry, and Horticulture) as well as the Bureau of Agricultural and Industrial Chemistry of the United States Department of Agriculture. The joint work was planned to cover two general phases, namely, bacteriological and chemical changes during the fermentation and feeding trials on the ensiled material.

This report deals with the principal bacteriological changes occurring during the fermentation of the steamed potatoes. Such studies are of considerable interest in view of the nature of the preparation of this type of silage which calls for steaming the potatoes until cooked and promptly ensiling (while hot) relatively large tonnages in pits. Such a procedure would naturally introduce the question of the effect of prolonged high temperature upon the micro-organisms associated with normal silage fermentations, especially the lactic acid bacteria (of the *Lactobacillus* genus) and other non-heat-resistant types, such as the yeasts and coliforms. While the preparation and use of steamed potato silage has been the subject of investigation for many years, principally abroad, the bacteriological changes have not been clearly indicated.

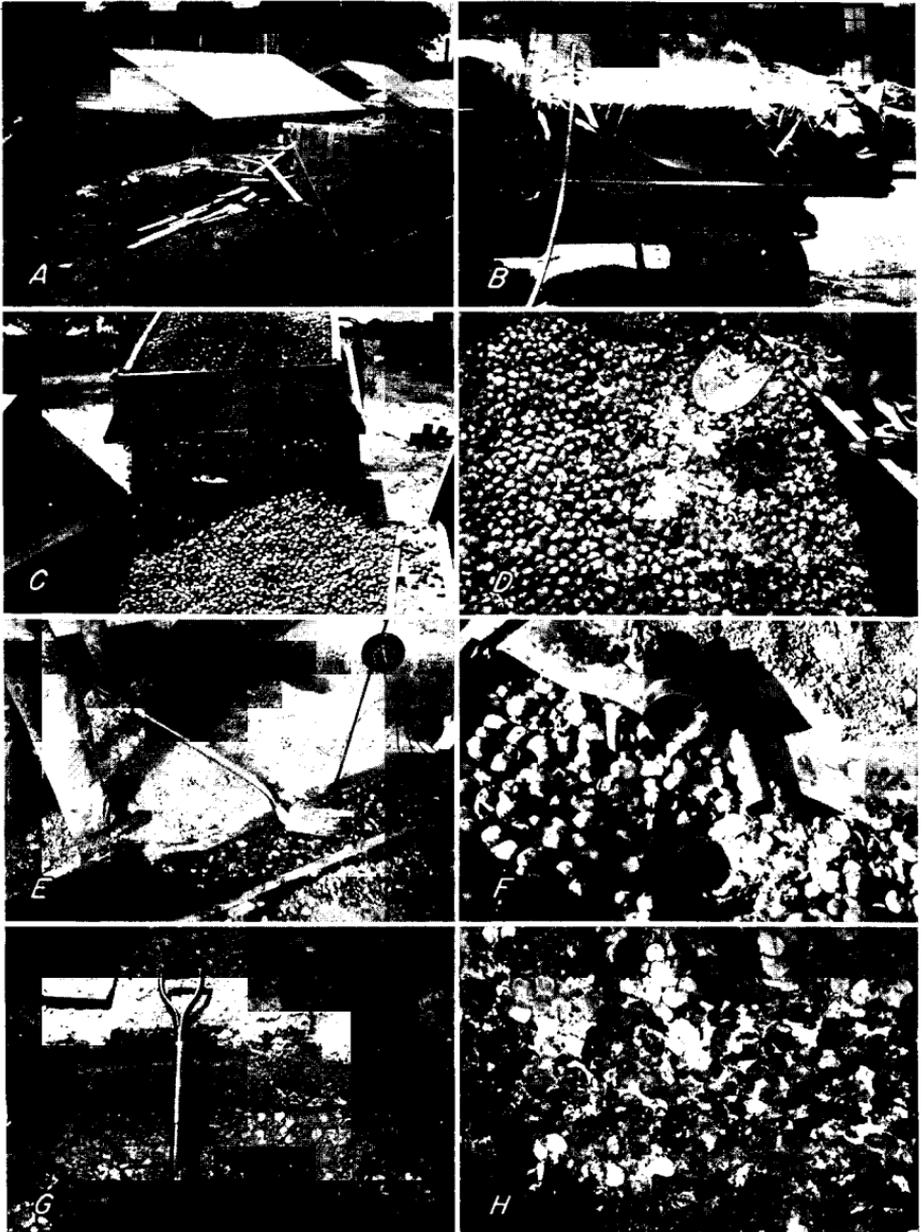
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² The writers wish to express their thanks to the War Food Administration for furnishing the potatoes used in the experiments, and to C. Hillman Moody, administrator of the War Food Administration for North Carolina, for his cooperation and interest in the work.

³ BRANDT, K., and KRAEMER, J. RECOMMENDATIONS FOR UTILIZATION OF SURPLUS POTATOES BY STEAMING AND ENSILING. 10 pp., 1944. [Mimeographed.]

PROCEDURE

Forty-five tons of U. S. No. 1, size B, potatoes were available for the work. The potatoes were shipped in carload lots from the eastern part of North Carolina and arrived in good condition. No attempt was made to remove the few rotten potatoes occasionally found. Thirty tons were used for the steamed-silage study and 15 tons were ensiled raw. Only the work on the steamed silage is discussed in this paper.



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EXPERIMENTAL SILO

The experimental trench silo used was one of two constructed at the Central Experiment Station farm near the college. These silos had approximately the following dimensions: 6 feet deep; 8 feet wide at the bottom, 11½ feet wide at the top; and 25 feet long. They had concrete floors and plastered concrete walls 3 to 4 inches thick, reinforced with wire fencing. The ends were closed by the use of 2- × 6-inch planks, supported by 4- × 4-inch posts. Both silos were provided with covers and were constructed in a hillside to provide natural drainage (fig. 1, *A*).

STEAMING OPERATION

The potatoes were steamed on July 10 and 11 at the college, two steel-bodied dump trucks being used. These trucks had a capacity of from 1½ to 2 tons of raw potatoes. On the bottom of each truck body was fastened a steam rake consisting of four parallel lengths of perforated ½-inch pipe connected to a horizontal cross member to which a vertical inlet was attached. The rake was connected to the steam line by means of a steam hose and pipe. The steam pressure was 120 pounds per square inch.

The potatoes were dumped into the trucks directly from the railroad cars, covered with heavy tarpaulins, and steamed until cooked throughout (fig. 1; *B* and *D*). This required from ¾ to 1 hour with the equipment used. The potatoes were kept covered and taken immediately to the trench silo (about a 10-minute trip) and dumped (fig. 1, *C*). In this manner, the 30 tons of potatoes were handled in about 12 working hours, using two trucks and two sources of steam.

FILLING THE SILO

The potatoes were dumped into the silo at three locations along one side. When all the steamed potatoes were in the trench, they were leveled off and covered with lapped strips of heavy tar-paper roofing (fig. 1, *E*). The mass was packed by carefully tamping the surface of the paper. A scant 4-inch layer of soil was then added for weighting and sealing. The 30 tons of steamed potatoes filled the trench to a depth of 3½ to 4 feet. About 30 hours after the first load had been steamed and dumped and about 6 hours after the last load had been put in the silo the temperature at the approximate center of the mass was in the range of 160°-164° F. Subsequent temperature readings were taken at two

FIGURE 1.—Ensiling steamed potatoes. *A*, Experimental trench silo with three-piece cover, one of which (background) has been removed preparatory to dumping in the potatoes. *B*, Steaming the covered potatoes in a metal-bodied dump truck. *C*, Dumping the steamed potatoes in the front (north) section of the trench. *D*, Steamed potatoes in the trench before leveling and covering; consistency of potatoes at this stage shown in area struck by the shovel. *E*, The filled trench at the time of the leveling and covering operation; the dial thermometer (right foreground) read approximately 160° F. at the conclusion of filling and covering. *F*, Sampling: A core of the top layer of surface potatoes removed with a petri-dish can (left) prior to taking the bacteriological sample with the smaller pipette can (right). *G*, Part of the end (south) section of the ensiled potatoes opened after 8 months' storage; the potatoes form a tightly packed mass beneath the light-colored soil layer. *H*, Close-up of ensiled potatoes after cutting through a small area near the top; cavities represent areas where individual potatoes fell away.

locations at about mid-depth in the mass. A copper-constantan thermocouple was placed at one location and the bulb of a vapor-actuated dial thermometer at the other.

SAMPLING TECHNIQUE

Samples were taken for bacteriological and chemical examination in the following manner: First, the soil was carefully removed from an area of tar-paper roofing (about 18 inches square) with a garden hoe, and the surface was brushed clean with a whisk broom. Then a U-shaped cut (8–10 inches on a side) was made in the paper and the flap was turned back. A sterile, copper petri-dish can (4½ inches in diameter and 8¼ inches long) was inverted and pushed vertically into the mass, and an 8-inch core of the top layer of potatoes was thus removed and discarded (fig. 1, *F*). The actual sample was obtained by removing a second core of potatoes with a sterile, copper pipette can (2 inches in diameter and 12 inches long) starting from the center of the surface left by the first cut. This gave about a 1½-pound sample from the central layer about 1 to 2 feet beneath the surface. The temperature of each sample area was taken by means of a maximum-indicating thermometer inserted into the wall of the cut at the time the sample was removed. After the sample was taken, the potatoes from the discarded core were tamped back into the hole. The tar-paper flap was then folded back in place and a new square of tar-paper larger than the opening was placed over the surface and covered with soil, after which the location was recorded. The sampling was restricted to about one-half of the total silo (fig. 2). This section was found to be the hottest

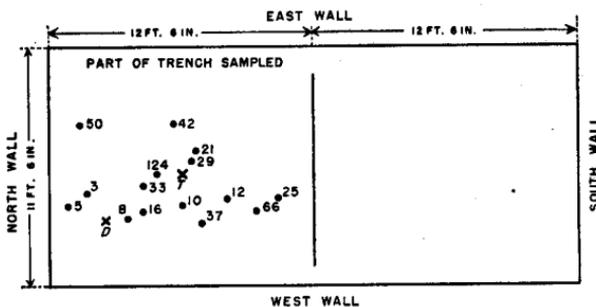


FIGURE 2.—Diagram representing top surface of the trench silo and indicating the comparative location of points at which core samples were taken for analysis. *D* marks location of the dial-type thermometer and *T*, the location of the thermocouple; the numbers indicate the age of the fermentation in days at each sampling.

after the filling operation was complete. Difference in temperature in opposite end portions of the ensiled mass was to be expected because of the method of filling the silo. This resulted in the space at the lower end of the silo being filled with cooler material, particularly after leveling.

MEDIA AND METHODS

After the sample was collected, it was taken promptly to the laboratory for analysis. Fifty grams of the potato mass was finely cut with a sterile spatula into a glass-top mason jar containing 450 grams of

sterile saline. The 1:10 mixture was shaken 100 times by hand prior to making suitable dilutions for use with solid and liquid media. Duplicate tests on 50-gram subsamples were run at each sampling interval. The remainder of the potato sample was reserved for chemical analysis by sealing it in glass jars and storing in a freezing cabinet. The major chemical changes occurring during the fermentation are not given in this report, but the titratable acidity and pH values, based on examination of the 1:10 dilution used for bacteriological analysis, are included.

Bacteriological examination of the silage was made for various mesophilic and thermophilic groups of micro-organisms. These are listed in table 1 together with the liquid or solid medium used and reference to the general procedure followed. For the most part, the procedures for the mesophiles have been successfully used by the authors for a number of years in connection with brined vegetable and sweetpotato-vine-silage fermentations (5, 6, 7, 8, 9, 10).⁴ The methods of examination for the thermophilic groups are essentially those developed in the laboratories of the National Canners Association (3, 4). It should be mentioned that in examinations for viable aerobic (13) and anaerobic spore-forms (3) by use of the media indicated, a boiled sample (2 minutes) of the 1:10 silage dilution was used which had been previously neutralized with sterile calcium carbonate. The same was true for the H₂S-producing thermophilic anaerobes. The counts for facultative thermophilic anaerobes are based on colonies resulting both from viable cells and spores from unboiled samples.

TABLE 1.—*Estimates of micro-organisms on potatoes (U. S. No. 1, B size) before and after steaming for silage*

Microbial group	Culture medium used and procedure reference	Count per milliliter of wash water ¹	
		Raw potatoes	Steamed potatoes ²
Mesophiles (35° C. incubation):			
Total count.....	Nutritive caseinate agar+BCP ³ ; plates (10, 7).	86,000,000	200
Lactic acid bacteria.....	Nutritive caseinate agar+BCP; plates (10, 7).	0	0
Coliform group.....	Brilliant green agar; plates (8, 9, 6).....	25,000,000	0
Yeasts.....	Acidified dextrose agar; plates (5, 10).....	150,000	0
Molds.....	Acidified dextrose agar; plates (5, 10).....	160,000	0
Aerobic spore count.....	Nutritive caseinate agar+BCP; plates (13).	22,000	100
Anaerobic spore count ⁴	Liver broth+particles; tubes (3).....	100	10
Thermophiles (55° incubation):			
Facultative anaerobes.....	Dextrose tryptone agar+BCP; plates (3).	3,600	40
Non-H ₂ S-producing anaerobes.....	Liver broth+particles; tubes (3).....	10	10
H ₂ S-producing anaerobes.....	Sulfite agar; tubes (4).....	10	10

¹ 450 grams of whole potatoes plus 450 grams of sterile saline shaken 100 times.

² Taken from a 1- to 1½-ton lot steamed 45 minutes to 1 hour in a steel-bodied dump truck covered with a heavy tarpaulin; steam pressure at the source was 120 pounds per square inch.

³ Bromocresol purple; 0.04 gram per liter.

⁴ Not detected because of predominance of and overgrowth by alkaline and coliform types.

⁵ Includes putrefactive types as well as true butyric types of gas-forming, obligate anaerobes.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 31.

MICROSCOPIC COUNTS

At each sampling interval, 0.01-milliliter portions of the original 1:10 silage dilutions were placed in sequence on duplicate slides for microscopic counting. The smears were prepared and counted according to the method of Wang (14), a modification of the Breed (2) technique. One set of slides was stained with the Kopeloff and Cohen (11) modification of the Gram stain, and the number of individual Gram-positive cells in 100 fields per smear was determined. For the other set, the Schaeffer and Fulton modification (12) of the Wirtz (15) spore stain was used, and the number of individual spores in 100 fields per smear was noted. The microscopic counts for both cells and spores are reported in terms of millions per gram of silage.

TITRATABLE ACIDITY AND pH

As mentioned previously, titratable acidity and pH determinations were made on the original 1:10 dilution of the silage used for bacteriological purposes. The pH determinations were made with the glass electrode. Titratable acidity was run on 10-milliliter aliquots of the 1:10 dilution (after allowing it to stand 1 to 2 hours) by titrating with 0.111 N NaOH using phenolphthalein as the indicator. The values were calculated in terms of grams lactic acid per 100 grams of potato silage. These determinations were made on the 1:10 dilution for the purpose of having available, at the time of plating, a reasonably clear picture of the progress of acid production during the fermentation.

RESULTS

EFFECT OF STEAMING ON SURFACE ORGANISMS

At the time the steaming operation was going on, an attempt was made to obtain an estimate of the relative number of different groups of organisms occurring on the surface of the uncut potatoes both before and after steaming. The results are given in table 1. It is evident that the steaming procedure greatly reduced the large number of surface organisms. The relatively few that survived were the heat-resistant, spore-forming types. As far as could be determined, the usual types of organisms associated with normal silage fermentations (i. e., lactic acid bacteria, yeasts, and coliforms) did not survive the heating. Although the initial count of the facultative anaerobic thermophiles was small in comparison with the total number of organisms present on the raw potatoes, the proportion of the thermophiles surviving the steaming operation was much greater. This is of considerable importance in view of their role in the subsequent fermentation of the ensiled potatoes.

TEMPERATURE CHANGES DURING FERMENTATION OF SILAGE

After the initial steaming operation, the surviving micro-organisms on the potatoes were subjected to further prolonged exposure to rather high temperatures during the time required for the hot mass to cool in the silo. Progressive temperature changes based on readings made repeatedly at two locations (fig. 2) in the silo are shown

in table 2. The location of each individual area sampled and the temperature of that particular area at the time of sampling are also presented. In general, the temperature of the mass 2 days after ensiling was in the range of 160° F. Furthermore, it remained above 120° for about the first 12 days and above 100° for the first 3 weeks. The dial thermometer location shows higher temperature readings during the first few days and lower readings after 12 days than the thermocouple location. This is accounted for by the fact that the thermometer was located where the steamed potatoes were dumped, which was the hottest area. However, it was also closer to the front and side walls, and therefore cooled faster than the more central location occupied by the thermocouple.

TABLE 2.—*Temperature changes during the fermentation of steamed potato silage*

Date (1944)	Age	Distance of area sampled from—		Temperature record			
		North wall	West wall	Sample area	Dial thermometer ¹	Thermocouple ²	Atmosphere
	Days	Inches	Inches	° F.	° F.	° F.	° F.
July 11, 8 p. m.-----	1					160	82
12, 2:30 p. m.-----	2				164	158	91
13, 8 a. m.-----	3	24	52	162	159	152	81
15, 2:30 p. m.-----	5	12	46	136	145	140	86
18, 2:30 p. m.-----	8	46	38	132	131	132	77
20, 1:30 p. m.-----	10	76	46	126	124	127	90
22, 2:30 p. m.-----	12	102	51	122	118	122	82
26, 1:30 p. m.-----	16	52	42	108	108	114	96
31, 2 p. m.-----	21	82	76	104	101	108	87
Aug. 4, 1:30 p. m.-----	25	130	51	100	95	103	94
8, 1:30 p. m.-----	29	80	72	100	92	100	76
12, 10 a. m.-----	33	54	58	96	90	97	85
16, 10 a. m.-----	37	87	36	92	89	95	84
21, 10 a. m.-----	42	70	92	90	85	92	81
29, 11 a. m.-----	50	16	92	89	85	88	78
Sept. 14, 11 a. m.-----	66	118	42	84	82	84	80
Nov. 11, 2 p. m.-----	124	60	64		65	67	64

¹ Located 33 inches from the north wall and 33 inches from the west wall.

² Located 76 inches from the north wall and 66 inches from the west wall.

POPULATION OF MICRO-ORGANISMS DURING FERMENTATION OF SILAGE

The results of the bacteriological examination for the presence of various types of organisms during the silage fermentation are shown in table 3. Changes in acidity and pH of the ensiled potatoes as well as the approximate temperature of the core sample at each sampling interval are also given. The data indicate that the coliforms, yeasts, and lactic acid bacteria, groups usually associated with normal silage fermentation, did not contribute to the fermentation of this type of silage. This would seem reasonable as these groups are not heat-resistant and there was no evidence that they survived the steaming operation. Even if some few individual cells from these groups had survived the cooking, and some had been added during the filling operation, it is doubtful whether they would have withstood the elevated temperatures of the silage during the first 12 days.

TABLE 3.—Populations of micro-organisms occurring during the fermentation of steamed potatoes for silage

Sample date, 1944	Fermentation age	Temperature of sample	Thermophiles (56° C.), count per gram			Mesophiles (38° C.), count per gram				Spore forms		Microscopic count—individual cell and spore count per gram		pH	Titratable acidity as lactic acid
			Total count	Acid-forming colonies	Anaerobes	Total count	Acid-forming colonies	Coll. forms, yeasts, lactic acid bacteria, and molds ¹	Aerobes	Anaerobes ¹	Gram+ cells	Spores			
July 13	3	162	Millions	Millions		Millions	Millions	Hundreds	Hundreds	Millions	Millions	Percent			
15	5	136	0.083	0.083	<10	<0.001	0	0.95	0	2.8	1.8	5.50	0.40		
18	8	132	1.9	1.9	<10	<0.001	0	3.5	0	6.6	4.8	5.30	0.50		
20	10	126	2.0	2.0	<10	<0.001	0	2.0	0	6.6	6.6	4.70	0.70		
22	12	122	5.1	5.1	<10	2.13	0	<1	0	15.2	9.0	4.78	0.58		
25	16	108	2.8	2.8	100	22.5	0	13.0	0	11.6	25.2	4.90	0.48		
21	104	7.6	7.6	1,000	<10	12.5	0	4.5	10	19.2	6.4	4.20	0.87		
Aug. 4	25	100	11.5	11.5	<10	10.2	0	2.3	0	32.0	70.8	4.11	1.18		
8	29	100	5.11	5.11	<10	6.7	0	6	0	14.2	45.6	4.60	1.60		
12	33	96	0.01	0.01	<10	10.9	0	18.0	0	33.8	45.6	4.00	1.35		
16	37	92	0.25	0.25	<10	1.4	0	1.1	0	182.4	6.8	4.17	1.15		
21	42	90	11.5	11.5	100	9.0	0	13.5	1	50.8	37.6	4.06	1.15		
29	50	89	4.1	4.1	<10	4.6	0	11.5	0	52.0	101.2	4.03	1.83		
Sept. 14	66	84	2.7	2.7	<10	1.6	0	505.0	0	210.0	64.6	3.96	1.36		
Nov. 11	124	67	1.0	1.0	<10	>.27	0	750.0	0	210.4	23.4	3.90	1.50		
					<10		0	19.0	1	210.4	63.8	3.95	3.40		

¹ Zeros in column indicate less than 10 per gram.² Not detected because of predominance of and overgrowth by alkaline types.³ Spore count by plating boiled sample was 7.3 millions per gram.⁴ At this interval a count of 750 yeasts and 2,200 molds per gram was obtained, indicating probable contamination from the surface layer during sampling.⁵ Total count at 55° C. was 24 million per gram, using nutritive caseinate agar.

Examination of the silage with respect to certain of the anaerobic spoilage groups of bacteria, namely, the non- H_2S - and the H_2S -producing thermophiles, showed the former group to be present only in relatively small numbers on several occasions. The latter group appeared to be absent or was present to the extent of less than 10 per gram at the six times when examinations were made. Active growth by these two groups, if present, would have been expected during the 12-day interval when the potatoes were at or near the optimum range for thermophilic growth. Essentially the same results were obtained for the putrefactive anaerobes (mesophiles) including the true butyric and butylic types of gas-forming, obligate anaerobes. Here positive indication of the presence of spores was noted in only 5 of the 15 periodic samples and then only in relatively low numbers. The temperature of the potatoes during the first 12 days would be considered too high for growth of this group of anaerobes. After that time the reaction of the silage (about pH 4.5) would be unsuitable for growth. However, the test for growth of spore forms from boiled samples for certain members of this group of organisms is not necessarily a reliable index to their previous activity. Spore formation may be negligible prior to inhibition or death of the vegetative cells because of acid production in the presence of a readily fermentable carbohydrate. Nevertheless, active growth by these organisms in the silage would be associated with a malodorous fermentation. This condition was not found.

PREDOMINATING GROUPS OF MICRO-ORGANISMS

The previous discussion has dealt with several of the groups of micro-organisms, both thermophilic and mesophilic, that appeared either inactive or absent during the fermentation proper. Further consideration of the bacteriological data in table 3 indicates that the thermophilic, facultative anaerobes were the predominating organisms during the fermentation and were responsible for the developed acidity and resultant decrease in pH of the ensiled mass. It will be noted that this group started activity rather soon after the potatoes were ensiled and for the most part showed populations in the millions per gram not only during the period when the mass was at elevated temperatures (120°-160° F.) but also when it was below the optimum temperature range for obligate thermophiles. The wide range of temperature tolerance accounts for the presence of rather similar plate counts obtained for this group listed in table 3 as mesophiles incubated at 35° C. (using nutritive caseinate agar). However, the 55° C. plate counts for the first three sampling periods, during the very early phase of the fermentation, indicate principally obligate thermophiles involved since comparatively few colonies were noted on the 35° C. plates at the same period. The facultative relationship toward temperature of the predominating types, after active fermentation was under way, was further demonstrated by the fact that, when the principal colonies from the 55° C. routine plates were transferred into liver broth and incubated at 35° C., growth occurred. The same was true for those picked from the 35° plates and incubated at 55°. Hence, so far as could be determined from the limited number of samples taken from the trench during the active acid fermentation, the predominating groups that grew out at either incubation temperature (55° or 35°) were essentially the

same. There was one exception to this. In certain instances during the routine examinations, one specific colony type grew well at 35° on nutritive caseinate agar, but very poorly, if at all, at 55° on dextrose tryptone agar. However, this was found to be a problem of nutritive requirements concerning the two media used rather than temperature, as will be discussed later.



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The routine plate counts during the fermentation period further showed that the major portion of the total count in most instances was composed of acid-forming colonies of the thermophilic, facultative anaerobic group. This was true for the plates incubated at either 35° or 55° C. These organisms brought about an acid fermentation that resulted in acidification of the ensiled mass and consequent preservation. The general course of acid development is shown by the lowering of the pH from about 5.5 at 3 days to about 3.95 at 50 days and thereafter until the conclusion of the sampling. During this period the titratable acidity increased from about 0.4 percent (calculated as lactic acid) to about 3.0 percent. The intensity of fermentation activity was not the same in all locations in the silo, as was indicated by the pH and titratable acidity values as well as by the irregular nature of some of the plate and spore counts. These counts did not always show typical, progressive populations. A greater lack of uniformity among samples would be expected in a more or less solid medium like the silage than in a fluid medium.

MICROSCOPIC COUNTS

The microscopic counts of individual Gram-positive cells give a reasonably good picture of the progress of the fermentation with respect to bacterial populations. In some ways it appears to give a better insight into the nature of this type of fermentation than do the cultural methods. Since fermentation activity did not always progress at the same rate in all locations in the ensiled mass, the sequence of microscopic fields was selected principally on the basis of the stage of fermentation of individual silage samples and not with strict regard to fermentation age of the particular sample (fig. 3).

The microscopic study revealed that the predominating flora was wholly Gram-positive and divided more or less into two morphological types; a short thick rod and a rather long rod of medium thickness (fig. 3, *C* and *D*). There is a possibility that a third type occurred, a much smaller rod than the others. Both principal types tended to elongate and form long chains and filaments in the more acid samples taken during the latter part of the fermentation (fig. 3, *G*). Predominating colonies picked from the routine platings likewise fell into the above morphological groups and showed the same tendency toward elongation in old cultures of broth media that had reached maximum acid production.

FIGURE 3.—Photomicrographs of the predominating micro-organisms present during the acid fermentation of steamed potato silage. All $\times 1700$. Slides used were those prepared and stained for the microscopic cell counts of Gram-positive cells. *A, B*, Vegetative cells from samples taken during the early stage (10 and 16 days) of fermentation; dark area in background of *B* is a particle of steamed potato tissue. *C, D*, Samples taken at 33 and 50 days; both showing active acid fermentation with large populations of short, thick vegetative rods and a few of the more slender types. *E, F*, Samples taken at 21 and 42 days showing more advanced stage of fermentation and evidence of active spore formation; note clostridial form (*E, a*) and free, oval type spores (*F, a*). The background in each case (*E, F*) is composed of indistinct dead cells. *G*, Elongated cell type that predominated during the latter part of the fermentation; note definite swollen condition of one end (*a*) of the cell. Such cells gave rise to small spherical spores rather than to the large oval types shown in *F, a*. *H*, Mass of entangled vegetative cells at the edge of a particle of potato tissue; growth appears to channel into the tissue (at right); silage sample 42 days old.

The spore counts reveal that active spore formation took place during the fermentation, which would suggest that the principal organisms involved were sporulating types. This view gains further support from the fact that all principal colonies isolated from routine plates proved to be Gram-positive spore formers. This is not surprising in view of the degree of heat resistance required by any group surviving both the steaming operation and the period of elevated temperature of the mass during the first 12 days in the silo. The counts further showed that the individual spores fell into two general classes, those that were spherical, and those that were oval (fig. 3, *F*). There seemed to be at least two distinct sizes of oval spores. The majority of the spores counted were free from the cells.

CLASSIFICATION OF THE PREDOMINATING GROUP

During the active fermentation period, a number of isolates representative of each predominating colonial type were made from the dextrose tryptone agar and nutritive caseinate agar routine plates. Thus far sufficient work has not been done to assure identification other than classification within the genus *Bacillus* (Cohn) as thermophiles in group X, as listed by Bergey et al. (1). Furthermore, they are nongas-producing, spore-bearing, acid-producing rods, which are facultative with respect to oxygen and temperature requirements. One culture in particular is of interest in that it does not appear to ferment dextrose broth or give visible growth on dextrose or plain agar slants at either 35° or 55° C. However, excellent growth and acid production are obtained in liver broth and in liver agar stabs at both of these temperatures.

In general, liver broth and liver agar were more satisfactory than other media for the cultivation of the cultures isolated from the fermentation. Also, during the latter part of the routine platings, plain liver agar was used in conjunction with the routine media for 55° and 35° C. counts with superior results. With the few trials that were made, liver agar plus bromocresol purple gave higher total counts, larger colonies, and faster growth than the routine media used in this study.

QUALITY OF THE SILAGE

At each sampling period the potatoes were examined to determine the general silage quality. The core samples were all judged good and had a characteristic aromatic odor, and acid taste, and firm texture. The texture resembled that of a boiled potato when cold. The ensiled material formed a more or less solid mass of potatoes pressed tightly together and was free from channels and gas pockets (fig. 1, *G* and *H*). It was not mushy, and no free liquid was present. The aromatic nature of the silage appeared to become stronger with the age of the sample and suggested the presence of a mixture of alcohols, organic acids, and esters. Furthermore, the older samples gave some indication of starch hydrolysis or other structural changes not present in those taken earlier. There was no appreciable color change of the potatoes resulting from the fermentation. The surface layer of potatoes, directly underneath the paper covering was considered spoiled since it had an unpleasant odor and soft texture.

SUMMARY AND CONCLUSIONS

The results of a bacteriological examination of the fermentation of hot-ensiled steamed potatoes (*Solanum tuberosum*) have been presented. The bacteriological findings indicated that the thermophilic, facultative anaerobes were the predominating micro-organisms during the fermentation and were responsible for the developed acidity and resultant preservation of the silage. They are considered to be non-gas-producing, acid-forming, spore-bearing rods, which are facultative with respect to oxygen and temperature requirements. These organisms may be classified according to Bergey et al. as thermophiles belonging in group X of the genus *Bacillus*.

LITERATURE CITED

- (1) BERGEY, D. H., BREED, R. S., MURRAY, E. G. D., and HITCHENS, A. P. 1939. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY . . . Ed. 5, 1032 pp. Baltimore.
- (2) BREED, R. S. 1911. THE DETERMINATION OF THE NUMBER OF BACTERIA IN MILK BY DIRECT MICROSCOPICAL EXAMINATION. *Zentbl. f. Bakt. [etc.]* (II) 30: [337]-340, illus.
- (3) CAMERON, E. J. 1936. REPORT ON CULTURE MEDIA FOR NON-ACID PRODUCTS. *Assoc. Off. Agr. Chem. Jour.* 19: 433-438.
- (4) ———. 1938. REPORT ON MICROBIOLOGICAL METHODS FOR THE EXAMINATION OF CANNED VEGETABLES. *Assoc. Off. Agr. Chem. Jour.* 21: 452-454.
- (5) ETCHELLS, J. L. 1941. INCIDENCE OF YEASTS IN CUCUMBER FERMENTATIONS. *Food Res.* 6: 95-104, illus.
- (6) ———, FABIAN, F. W., and JONES, I. D. 1945. THE AEROBACTER FERMENTATION OF CUCUMBERS DURING SALTING. *Mich. Agr. Expt. Sta. Tech. Bul.* 200, 56 pp., illus.
- (7) ——— and GORESLINE, H. E. 1940. METHODS OF EXAMINATION OF FRESH CUCUMBER PICKLE. *Fruit Prod. Jour. and Amer. Vinegar Indus.* 19: 331-335.
- (8) ———, and JONES, I. D. 1942. PASTEURIZATION OF PICKLE PRODUCTS. *Fruit Prod. Jour. and Amer. Vinegar Indus.* 21: 330-332.
- (9) ———, and JONES, I. D. 1943. BACTERIOLOGICAL CHANGES IN CUCUMBER FERMENTATION. *Food Indus.* (2) 15: 54-56, illus.
- (10) JONES, I. D., VELDHUIS, M. K., ETCHELLS, J. L., and VEERHOFF, O. 1940. CHEMICAL AND BACTERIOLOGICAL CHANGES IN DILL-PICKLE BRINES DURING FERMENTATION. *Food Res.* 5: 533-547, illus.
- (11) KOPELOFF, N., and COHEN, P. 1928. FURTHER STUDIES ON A MODIFICATION OF THE GRAM STAIN. *Stain Technol.* 3: 64-69.
- (12) SCHAEFFER, A. B., and FULTON, M. 1933. A SIMPLIFIED METHOD OF STAINING ENDOSPORES. *Science* 77: 194.
- (13) SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE ON BACTERIOLOGICAL TECHNIC. 1937. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA. THE STUDY OF OBLIGATELY ANAEROBIC BACTERIA. Leaflet III, Ed. 3, pp. 1-15. Geneva, N. Y.
- (14) WANG, SHU-HSIEN. 1941. A DIRECT SMEAR METHOD FOR COUNTING MICROSCOPIC PARTICLES IN FLUID SUSPENSION. *Jour. Bact.* 42: 297-319.
- (15) WIRTZ, R. 1908. EINE EINFACHE ART DER SPORENFÄRBUNG. *Zentbl. f. Bakt. [etc.] Originale* (I) 46: 727-728.