A STUDY OF THE BACTERIOLOGY OF THE DESALTING PROCESS IN PICKLE MANUFACTURE

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A STUDY OF THE BACTERIOLOGY OF THE DESALTING PROCESS IN PICKLE MANUFACTURE

By

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INTRODUCTION

One of three main steps in making pickles is the desalting of the salt stock. This is done by soaking the salt stock pickles in fresh water, a process in which the salt is removed from the pickle by osmosis. The removal of the excess salt, in connection with handling of salt stock pickles, is termed processing, desalting, or freshening.

Most pickle manufacturers have a definite method which is followed during processing. These methods differ somewhat in various factories depending on the facilities available and the tempo of the plant. The processing method most generally used is to place the pickles in a vat, add an equal quantity of water, and change the freshening water several times at varying intervals depending on how soon the desalted stock is needed. Alternate methods of processing include the use of heat, agitation of the water by means of compressed air or by running water continuously throughout the salt stock.

To those familiar with the salting of pickles it is a well known fact that if spoilage is to be averted the salt content of the brine must never be lowered, but always raised until the correct salt strength is obtained. However, during manufacturing the reverse process takes place and the protective action of the salt is lost as it is withdrawn from the pickles. With this in mind the present study was undertaken to determine the bacteriological

changes which take place during the desalting process in pickle manufacturing.

In conjunction with the studies on the microscopic flora, chemical analyses were made to determine any relation between changes in chemical environment and changes in the population of the micro-organisms.

In the manufacture of pickles, alum may be used as a firming and crisping agent of the pickles. There are two places where the alum may be added. It may be added a few hours before the final freshening water is withdrawn from the pickles or it may be added to the final liquor or brine used to make the finished product. Therefore, in order to obtain a complete bacteriological picture of the freshening process the effects of different alums on the micro-flora such as yeast, lactobacilli and sporogenic bacteria were investigated. In addition, the effect of alums upon a common spoilage organism in the finished product was studied. The alums commonly used by most manufacturers are "aluminum,"* ammonium, and sodium alum.

REVIEW OF LITERATURE

No study has been made previously of the bacteriology of the desalting or freshening of pickles. However, it is important to know the types of micro-organisms found in the fermenting of the cucumbers during curing and also the kind of micro-organisms associated with spoilage, so as to know what micro-flora most likely would be found on the pickles when they entered the processing tanks.

* Aluminum sulphate is not an alum according to

Rahn (12) in 1913 found several thousand bacteria per ml. in fresh brine and from 50 to 200,000,000 per ml. in barrels of fermenting brine. Le Fevre (9, 10, 11), during 1920, 1921, and 1922, reported that as the cucumbers come in from the field they carry numerous bacteria, the principal groups being Lactobacilli, aerobic spore-formers, gas-formers, yeasts and molds. He claimed that the Lactobacilli were the most tolerant of salt and were considered the most significant in acid production. Fabian and Bryan (4) found that the majority of bacteria present at the beginning were peptonizers but after the first twenty-four hours there was a decrease in the total number of bacteria due to the supression of the peptonizing bacteria while there was a gradual increase in the acid-producing bacteria. They concluded there were three factors responsible for this reduction, viz., the concentration of the salt, the presence of available food, and the acidity produced by the bacteria, themselves. Salt appears to be the most important factor responsible for the reduction of peptonizers. It is stated by Fabian and Wickerham (6) that there was a definite sequence of bacterial population in genuine dill pickle fermentation. Gram positive-cocci predominated at the beginning of fermentation and were replaced later by gram-positive short rods. Toward the end of the fermentation, long rods were in the majority. Throughout the fermentation, weak acid-producing bacteria predominated and the strong acid-producing bacteria reached a maximum in about eight to ten days. Jones, Veldhuis, Etchells and

Veerhoff (8) found the predominating micro-organisms in dill pickle fermentation to be acid-forming bacteria and yeasts. The acid-forming bacteria were present in the greatest numbers (millions/ml.) and remained in high count for approximately two weeks after the beginning of fermentation. Peptonizing bacteria were also recognized in the flora, but appeared in relatively small numbers. Etchells (1) showed how the population of yeasts changed in brines receiving different treatments with respect to salt concentration. He also reported that the yeast population decreased greatly after thirty-five days. Etchells and Jones (2) showed that there were active yeast fermentations in all brine treatments and that active fermentation by the acid-forming bacteria were restricted to the 20 and 40 degree brines. Etchells and Ohmer (3) during their bacteriological study of the manufacture of fresh cucumber pickles found that numerous populations of acid-forming bacteria and yeasts were developed during the overnight brining period of the slices. Jones (7) in his Master's thesis concerning the salting of onions, found that with increase of salt concentration there was a gradual decrease in total number of organisms present in the brine. Salt was most toxic to the peptonizers, less toxic for yeasts and the least toxic to the acid-producing bacteria.

A few workers have done experimental work on the physical and chemical aspects of the freshening process. Switzer, Richardson, and Fabian (13), in 1939, studied the rate of diffusion of salt from pickles during

the desalting process and recommended eight-hour freshening periods since 95 per cent of the salt that was capable of being removed in twelve hours was diffused out in the first eight hours. They also found that for quickest results salt stock pickles should be freshened at 70° F and above. They showed that salt stock pickles may be completely freshened in eight to ten hours if circulating fresh water is used. Fabian and Jacobs (5) found that the advantages of agitating the freshening water during desalting were slight.

PART I - METHODS FOR DESALTING PICKLES

Commercial Procedures

There is considerable variation in the freshening process of salt stock pickles among different manufacturers. The general procedure for desalting or freshening pickles commercially is to remove the pickles from the salt brine, place them in processing tanks and then cover them with cold water. The water may or may not be heated. The water may be changed every eight hours or may be left on for longer periods. The freshening water may also be kept in constant circulation by means of compressed air, a steam siphon or a pump to aid in quicker and more uniform extraction of the salt.

Addition of Alum

The texture of pickles may be somewhat crisped and firmed by the addition of an alum. Alum may be added at either one of two places in the pickle manufacture. It may be added to the final freshening water two or three hours before the water is withdrawn from the pickles or it may be added to the final liquor used to finish the pickles. If the processing involves constant circulation of the water, the alum is added at the start of the freshening. The alums generally used are aluminum sulfate, ammonium alum and sodium alum.

Experimental Procedure

Regular salt stock was obtained from four different salting stations or pickle plants. More than one size salt stock was procured from each source and the sizes designated as those adopted by the National Pickle Packers Association, i.e., the number of pickles contained in a forty-five gallon barrel. Each salt stock used was numbered and the size determined. They were listed as follows: salt stock No. 1, 2,400 size; salt stock No. 2, 1,000 size; salt stock No. 3, 2,400 size; salt stock No. 4, 15,000 size; salt stock No. 6, 1,000 size; salt stock No. 7, 4,500 size. These were kept in ten-gallon crocks at room temperature and were freshened at once to prevent any changes in the normal bacterial flora which might occur due to the formation of scum.

Four methods were used in freshening the pickles. The first method (A) was to desalt by changing the freshening water every twenty to twenty-four hours until the required amount of salt was removed. In the second method (B) the salt stock pickles were processed by heating to a temperature of 54° C (129.2° F) and left in this water for a total of eight hours at which time a second and final change of water was made. In the third method (C), the quick method, the water was changed three times at eight-hour intervals, while in the fourth method (D) the water was changed every twenty-four hours and enabled one to compare the longer with the shorter freshening period, as used in method (C). The same salt stock was used for both

method (C) and (D) and the freshening was started at the same time.

Bacterial and Chemical Analysis

Four media were used to determine the microflora present in the freshening water at various intervals throughout the desalting procedure. The media were as follows:

Double strength nutrient agar plus one ml. of sterile skim milk per plate, which was designated as skim milk agar in the tables, was used as a differential medium. A total count of all organisms present was made first. The peptonizing colonies surrounded by a clear zone were then counted, after which an estimate of the acid-producing colonies was made. The count of acid colonies was later made more accurate by adding brom creosol purple indicator (0.0032%) to the above medium. The acid-forming colonies appeared yellow against the purple background of the medium.

Nutrient agar was used to obtain a total of all bacteria present in the freshening water.

Bacto tomato juice agar was used at first to detect the number of acid-producers, especially Lactobacilli, but was later found more suitable for total counts since it did not limit itself to the acid-producers.

The number of yeasts and molds were determined by the use of acidified potato-dextrose agar. Bacterial growth on this medium was inhibited by acidifying it to

pH 3.5 by adding 1.7 ml. of ten per cent sterile tartaric acid to 100 ml. of the potato-dextrose agar just before pouring the plates.

All plates were incubated for 72 hours at room temperature and counted with the aid of a Quebec counting chamber.

Total acid as lactic acid was determined by titrating with 0.1662N NaOH, giving per cent of acetic acid directly if a one ml. sample was used and this result was multiplied by 1.5, giving the per cent of lactic acid. The per cent sodium chloride was found by titrating with a known normality of silver nitrate and using dichlorflourescein as the indicator. The amount of salt was also tested by a salometer, a commercial test based on a scale in which a saturated salt solution is considered 100 degrees salometer. The pH of the freshening water was determined with a Beckman pH meter, using a glass electrode.

Freshening Pickles by Method (A)

Portions of salt stocks No. 1, No. 2, and No. 3 were processed by adding cold tap water equal in weight to the drained pickles in five-gallon earthenware crocks which were then covered with paper and allowed to remain overnight. This was designated as the first freshening period. Two more freshening periods (second and third) were needed to remove the required amount of salt.

At the end of each freshening period the brine was tested chemically for degrees salometer, per cent salt,

per cent acid as lactic, and pH. From two to four per cent sodium chloride was allowed to remain in the pickles at the end of the freshening process and this salt content was determined by squeezing the juice from several pickles and titrating it.

At the same time bacterial results were obtained by removing a representative sample of the brine. As shown by the tables 1 to 6, inclusive, and figures 1, 2, and 3, the greatest increase in total number of micro-organisms occurred after the first freshening period. There was a direct correlation between total counts on nutrient agar, tomato juice agar, and skim milk agar. While there was a general increase in the total number of bacteria, the number of acid-producers did not increase as rapidly as the peptonizers and in some cases they actually decreased. In contrast to this the number of peptonizers increased greatly. Mold occurred in only one instance. In general there was a slight increase in the number of yeasts present.

Sodium alum at the rate of two pounds per six bushels of pickles (55 lbs. of pickles per bushel) was added to one half of the stock during the third freshening period in order to determine the effect on the total count. The other half of the stock was used as a control. This was repeated once with each salt stock used. There was a conspicuous decrease in the total number of micro-organisms when sodium alum was added as compared to the total count of the control. These results were not included

in the tables or figures because later it was found to be due to the flocculation of the alum which settled out the greater number of organisms present.

The results of bacterial and chemical analyses for salt stock No. 1 are given in tables one, two and three. Figure 1 shows graphically the plate count on nutrient agar and the degrees salometer of the three trials. The same type of results for salt stock No. 2 are shown in table 4 and figure 2 and the results of salt stock No. 3 in tables 5 and 6 and figure 3.

Freshening Pickles by Method (B)

Salt stocks No. 2, No. 4, No. 6, and No. 7 were desalted by adding equal weights of water and pickles and placing them into one-gallon jars. The jars were placed in a water bath and heated to 54° C (129.2° F). For salt stocks No. 6 and No. 7 (tables 7 and 8) the temperature of 54° C was retained for about one hour and for stock No. 2, No. 4, and No. 6 (tables 9, 10, and 11) this temperature was held for two hours. The heat was then turned off and the jars were left in the water bath from seven to eight hours while the temperature returned to that of the room. At this time the freshening water on the pickles was changed and allowed to remain overnight. Only one change of water was needed after heating to bring the salt content down to the desired amount. When the water was changed the pickles were divided equally and sodium alum added (two pounds per six bushels of pickles) to one half while

the other half was used as a control. Data for the chemical and bacterial results were taken more frequently than previously in order to obtain a more complete picture.

The skim milk agar containing brom cresol purple as an indicator usually showed a higher total count than the nutrient agar and seemed to be better suited to the organisms concerned. The total count was reduced considerably during the hot water freshening period, then it increased perceptibly during the last soaking period. Again there was an increase of peptonizers and a decrease of acid producers when this method of freshening was used.

The addition of alum again showed a marked decrease in the microbial count due to the flocculation of the alum which emeshed the bacteria and carried them to the bottom of the crocks when the aluminum hydroxide settled.

Freshening Pickles by Method (C)

Since ninety-five per cent of the salt is removed from the pickles during the first eight hours of a freshening period, method (C) consisted of desalting stock No. 4 by changing the freshening water every eight hours. Three desalting periods were needed to remove the desired amount of salt.

Sodium alum at the rate of two pounds per six bushels of pickles was added to one-half of the stock during the third freshening period and the other half used as a control.

Chemical and bacterial data were compiled as previously and the results are listed in table 12. Peptonizers occurred only once during the first freshening period. There was a decrease in acid producers. The total count on both nutrient agar and skim milk agar was reduced greatly during the first two eight-hour freshening periods and only increased appreciably during the third eight-hour desalting period. The portion of the stock to which the alum was added showed a great decrease in the number of organisms as compared with the other half of the salt stock that did not contain the alum.

Freshening Pickles by Method (D)

To compare the short eight-hour freshening periods as in Method (C) with longer freshening periods, a portion of salt stock No. 4 was desalted at the same time by using three twenty-four hour freshening periods. Alum was added to one-half of the stock during the third freshening period, while the other half of the pickles was used as a control.

The chemical and bacterial data for desalting by Method (D) are shown in table 13. The peptonizers greatly increased during the second and third freshening periods. A noticeable decrease in acid-producers occurred while there was a decided increase in the total number of bacteria on both the nutrient and skim milk agar with the greatest increase occurring after the first freshening period. The addition of alum produced a marked decrease

in the number of micro-organisms in the third desalting period when compared with the portion of stock that contained no alum.

Table 1. Chemical and bacterial analysis of salt stock No. 1, 2,400 size, at different stages of the freshening process when desalted according to method (A).

Date 1947	Freshening period	Chemical analysis					Bacterial analysis - thousands per ml.						
		Total acid as per cent lactic acid	pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Tomato juice agar	Potato dextrose agar		Skim milk agar		
							Total	Total	Ysts	Mold	Total	Acid	Peptonizers
2-28	salt stock	0.15	4.2	19.9	20.0	80	330	20	0	0	210	10	0
3-1	1st	0.15	5.1	11.0	10.8	41.5	720	310	0	0	560	11	60
3-2	2nd	0.09	6.45	6.2	3.6	16	10,000	8,000	0	0	11,000	21	800
3-3	3rd	0.09	6.5	3.3	2.1	10	15,100	10,000	2	0	13,000	3	1,200

Table 2. Chemical and bacterial analysis of salt stock No. 1, 2,400 size, at different stages of the freshening process when desalted according to method (A).

Date 1947	Freshening period	Chemical analysis					Bacterial analysis - thousands per ml.						
		Total acid as per cent	pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salo-	Nutrient agar	Tomato juice agar	Potato dextrose agar		Skim milk agar		
							Total	Total	Ysts	Mold	Total	Acid	Peptonizers
4-18	salt stock	0.15	5.3	22.6	22.65	85	550	3	0	0	25	4	0
4-19	1st	0.10	6.6	12.4	7.2	20	860	270	0	0	440	30	21
4-20	2nd	0.09	6.6	6.7	5.5	17	35,000	29,000	.5	.1	12,000	15	200
4-21	3rd	0.09	6.6	3.3	1.9	10	33,000	21,000	.4	0	20,000	18	520

Table 3. Chemical and bacterial analysis of salt stock No. 1, 2,400 size, at different stages of the freshening process when desalted according to method (A).

Date 1947	Freshening period	Chemical analysis					Bacterial analysis - thousands per ml.						
		Total acid as per cent lactic acid	pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Tomato juice agar	Potato dextrose agar		Skim milk agar		
							Total	Total	Ysts	Mold	Total	Acid	Peptonizers
5-2	salt stock	0.15	5.0	20.0	20.2	81	25	18	0	0	8	3	1
5-3	1st	0.12	5.8	11.2	9.8	38	180	5	0	0	20	2	1
5-4	2nd	0.10	6.2	6.5	3.4	16	120	*	0	0	80	10	10
5-5	3rd	0.09	6.5	3.2	2.2	8	2,500	*	0	0	2,200	2	20

* spreaders

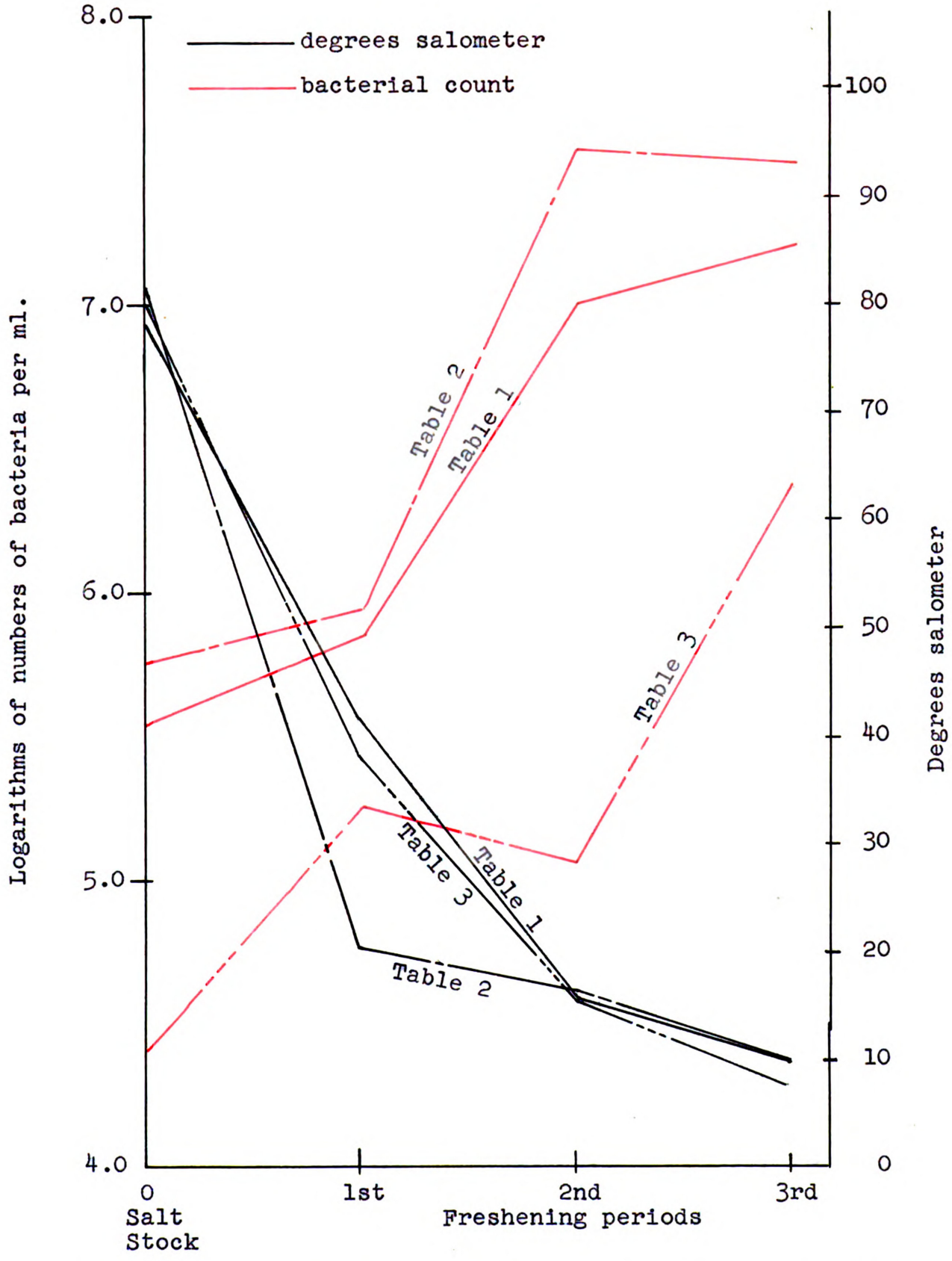

Figure 1. Showing plate count on nutrient agar and degrees salometer of salt stock No. 1 at different stages of the freshening process when desalted according to method (A).

Table 4. Chemical and bacterial analysis of salt stock No. 2, 1,000 size, at different stages of the freshening process when desalted according to method (A).

Date 1947	Freshening period	Chemical analysis					Bacterial analysis - thousands per ml.						
		Total acid as per cent lactic acid	pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Tomato juice agar	Potato dextrose agar		Skim milk agar		
							Total	Total	Ysts	Mold	Total	Acid	Peptonizers
7-28	salt stock	0.15	3.85	16.4	10.6	40	70	590			11	6	3
7-29	1st	0.18	3.9	7.4	7.4	25	1,500	2,000			2,300	8	87
7-30	2nd	0.15	5.4	3.3	3.0	10	4,000	3,200			5,800	3	150
7-31	3rd	0.15	5.4	2.1	2.0	8	4,800	4,000			5,900	2	200

Table 5. Chemical and bacterial analysis of salt stock No. 3, 2,400 size, at different stages of the freshening process when desalted according to method (A).

Date 1947	Freshening period	Chemical analysis					Bacterial analysis - thousands per ml.						
		Total acid as per cent lactic acid	pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Tomato juice agar	Potato dextrose agar		Skim milk agar		
							Total	Total	Ysts	Mold	Total	Acid	Peptonizers
7-29	salt stock	0.62	3.3	17.0	16.4	61	17	3	0	0	18	8	6
7-30	1st	0.30	3.75	6.0	8.5	31	25	120	0	0	28	5	18
7-31	2nd	0.15	4.1	3.7	3.8	15	230		5	0	34	5	20
8-1	3rd	0.15	4.0	3.4	2.6	10	510	400	410	0	540	0	120

Table 6. Chemical and bacterial analysis of salt stock No. 3, 2,400 size, at different stages of the freshening process when desalted according to method (A).

Date 1947	Freshening period	Chemical analysis					Bacterial analysis - thousands per ml.						
		Total acid as per cent lactic acid	pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Tomato juice agar	Potato dextrose agar		Skim milk agar		
							Total	Total	Ysts	Mold	Total	Acid	Peptonizers
7-29	salt stock	0.62	3.3	17.0	16.4	61	11		0	0	14	4	5
7-30	1st	0.31	3.75	10.2	8.7	31	12	13	0	2	15	0	5
7-31	2nd	0.15	4.0	4.1	4.7	16	232	43	15	0	226	1	3
8-1	3rd	0.15	3.95	3.4	2.9	11	481	410	400	0	660	0	2

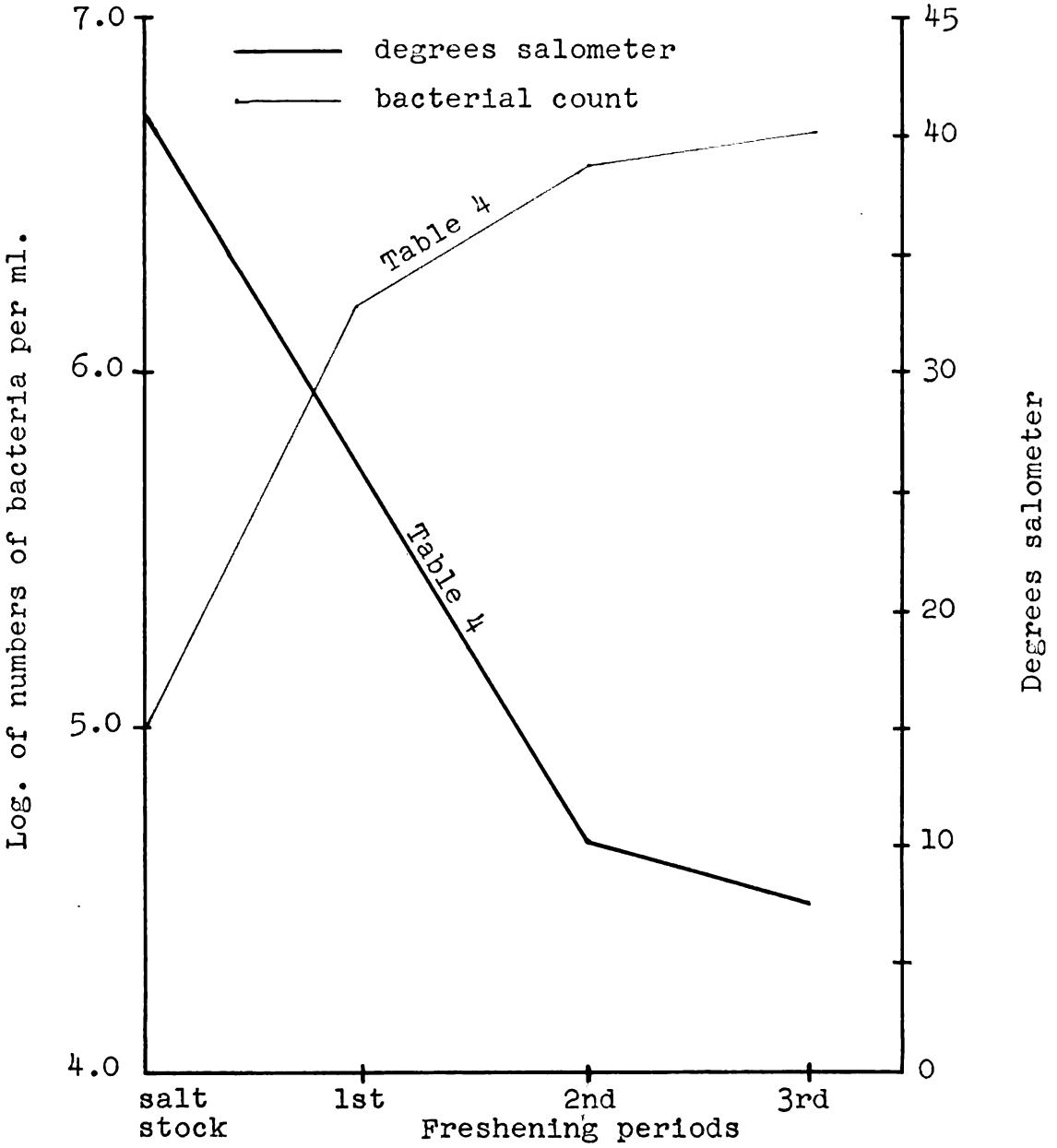

Figure 2. Showing plate count on nutrient agar and degrees salometer of salt stock No. 2 at different stages of the freshening process when desalted according to method (A).

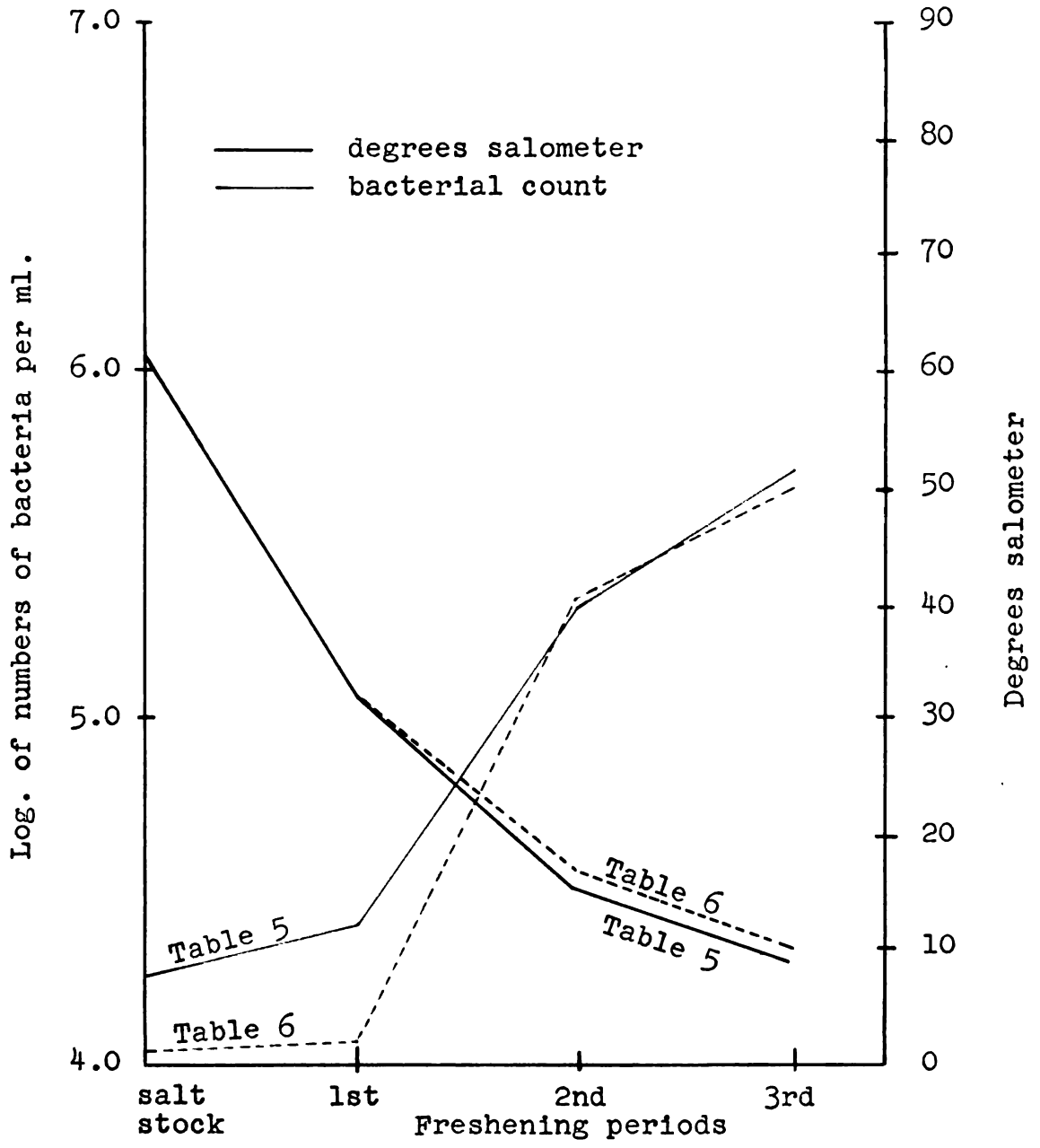

Figure 3. Showing plate count on nutrient agar and degrees salometer of salt stock No. 3 at different stages of the freshening process when desalted according to method (A).

Table 7. Chemical and bacterial analysis of salt stock No. 6, 1,000 size, at different stages of the freshening process when desalted according to method (B).

Date 1947	Time	Chemical Analysis						Bacterial Analysis - thousands per ml.					
		pH	Temperature in C°		Per cent NaCl in pickles	Per cent NaCl in brine	De-grees salo-meter	Nut-rient agar Total	Potato dextrose		Skim milk agar		
			Top	Bot.					Ysts	Mold	Total	Acid	Pepto-nizers
* 8-5	9:00 am	3.40	28.5	28.5	16.2	16.0	60	36	3	0	47	46	0
8-5	10:30 am	3.65	50	54	11.3	5.6	20	20	0	0	18	16	0
8-5	12:00 am	3.70	46	50	10.0	6.6	25	5	0	0	8	.1	0
8-5	1:00 pm	3.85	42	46	9.5	7.4	27	-	-	-	-	-	-
8-5	1:30 pm	3.85	42	42	9.5	7.9	31	.5	0	0	.7	0	.6
8-5	3:30 pm	3.85	38	39	9.2	8.0	31	.2	0	0	.4	0	.2
** 8-5	4:00 pm	3.85	37	38	9.1	8.1	32	-	-	-	-	-	-
8-6	8:00 am	5.05	28	28	7.3	2.1	8	10.2	0	0	7	0	5
*** 8-6	8:00 am	3.61	28	28	6.6	2.2	8	-	-	-	-	-	-
8-6	3:30 pm	41	27	27	4.6	4.0	15	11.6	5.5	0	9.8	0	9.8
*** 8-6	3:30 pm	3.3	27	27	3.7	4.0	15	.4	0	0	.2	0	.2

* Changed freshening water.

** Samples divided and alum added.

*** After alum added.

Table 8. Chemical and bacterial analysis of salt stock No. 7, 4,500 size, at different stages of the freshening process when desalted according to method (B).

Date 1947	Time	Chemical analysis						Bacterial analysis - thousands per ml.					
		pH	Temperature in degrees C		Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Potato dextrose		Skim milk agar		
			Top	Bot.				Total	Ysts	Mold	Total	Acid	Peptonizers
* 8-5	9:00 am	3.65	26	26	13.5	12.2	46	500	4	0	4,800	4,800	0
8-5	10:30 am	3.75	50	54	12.5	1.8	7	29	0	0	72	72	0
8-5	12:00 am	3.8	46	50	12.1	3.2	12	8.2	0	0	31	15	0
8-5	1:00 pm	3.8	43	47	11.3	4.0	16	-	-	-	-	-	-
8-5	1:30 pm	3.75	42	45	11.0	4.4	17	1.8	0	0	15	14.9	.1
8-5	3:30 pm	3.65	38	40	10.2	5.3	22	1.3	0	0	19	5	.2
** 8-5	4:00 pm	3.7	38	40	10.0	5.6	22	-	-	-	-	-	-
8-6	8:00 am	5.15	28	28	8.2	1.6	6	4.1	1	0	5.2	0	.8
*** 8-6	8:00 am	3.85	28	28	8.1	1.7	6	-	-	-	-	-	-
8-6	3:30 pm	4.5	27	27	6.6	3.2	14	7.6	3.3	0	8.1	0	1
*** 8-6	3:30 pm	3.65	27	27	6.1	3.5	14	.1	0	0	0	0	0

* Changed freshening water.

** Sample divided and alum added.

*** After alum added.

Table 9. Chemical and bacterial analysis of salt stock No. 2, 1,000 size, at different stages of the freshening process when desalted according to method (B).

Date 1947	Time	Hrs.	Chemical analysis						Bacterial analysis - thousands per ml.					
			Temperature in degrees C		pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Potato dextrose		Skim milk agar		
			Top	Bot.					Total	Ysts	Mold	Total	Acid	Peptonizers
8-12	8:30am	0	25	25	3.95	14.5	13.4	52	2.6	0	5	2.8	2.7	0
8-12	9:30am	1	54	54	Held at this temperature for two hours									
8-12	11:30am	3	54	54										
8-12	12:00am	3½	50	52	5.5	13.2	2.5	10	1.3	0	0	.5	0	.1
8-12	4:00pm	7½	39	40	4.7	7.3	4.2	16	1	0	0	.2	.1	.09
8-12	4:30pm	8	Sample divided; freshening water changed; alum added											
8-13	4:00pm	32	30	30	5.7	3.8	3.4	13	21	0	0	77	2	4
*8-13	4:00pm	32	30	30	3.65	6.1	2.8	11	0.09	0	0	.02	.01	.01

* After alum added.

Table 10. Chemical and bacterial analysis of salt stock No. 4, 15,000 size, at different stages of the freshening process when desalted according to method (B).

Date	Time	Hrs.	Chemical analysis						Bacterial analysis - thousands per ml.					
			Temperature in degrees C		pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Potato dextrose		Skim milk agar		
			Top	Bot.					Total	Ysts	Mold	Total	Acid	Peptonizers
8-12	8:30am	0	25	25	4.95	15.6	15.6	60	5,800	43	0	6,500	0	0
8-12	9:30am	1	54	54	Held at this temperature for two hours									
8-12	11:30am	3	54	54										
8-12	12:00am	3½	50	52	6.6	10.2	6.8	26	.5	0	0	.2	0	.2
8-12	4:00pm	7½	39	40	6.2	8.4	7.4	28	0.13	0	0	.12	0	.05
8-12	4:30pm	8	Sample divided; freshening water changed; alum added											
8-13	4:00pm	32	30	30	6.1	3.2	4.2	16	2,700	0	0	2,800	2,800	0
*8-13	4:00pm	32	30	30	3.75	3.2	3.8	14	.02	0	0	.05	.02	.03

* After alum added.

Table 11. Chemical and bacterial analysis of salt stock No. 6, 1,000 size, at different stages of the freshening process when desalted according to method (B).

Date	Time	Hrs.	Chemical analysis						Bacterial analysis - thousands per ml.					
			Temperature in degrees C		pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Potato dextrose		Skim milk agar		
			Top	Bot.					Total	Ysts	Mold	Total	Acid	Peptonizers
8-12	8:30am	0	25	25	3.6	16.8	17.4	66	210	0	0	250	0	0
8-12	9:30am	1	54	54	Held at this temperature for two hours									
8-12	11:30am	3	54	54										
8-12	12:00am	3½	50	52	3.9	10.0	6.4	25	.2	0	0	.3	0	.3
8-12	4:00pm	7½	39	40	3.9	7.7	7.6	29	.2	0	0	.3	.1	.2
8-12	4:30pm	8	Sample divided; freshening water changed; alum added											
8-13	4:00pm	32	30	30	3.9	4.2	4.2	16	14	0	0	14	13	0
*8-13	4:00pm	32	30	30	3.35	3.1	3.9	14	.01	0	0	.01	0	0

* After alum added.

Table 12. Chemical and bacterial analysis of salt stock No. 4, 15,000 size, at different stages of the freshening process when desalted according to method (C).

Date 1947	Freshening period	Hrs.	Chemical analysis				Bacterial analysis - thousands per ml.					
			pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Potato dextrose agar		Skim milk agar		
							Total	Ysts	Mold	Total	Acid	Peptonizers
8-14	salt stock	0	5.5	15.5	15.6	60	1,800	26	0	1,700	1,400	0
8-14	1st	8	6.25	7.8	7.1	27	29	0.5	0	30	25	2
8-14	2nd	16	6.5	4.9	3.3	13	36	3	0	24	10	0
8-15	3rd	24	6.6	1.5	1.9	8	120	0.3	0	320	3	0
*8-15	3rd	24	3.95	2.2	1.6	8	0.13	0	0.02	0.4	0.03	0

* After alum added.

Table 13. Chemical and bacterial analysis of salt stock No. 4, 15,000 size, at different stages of the freshening process when desalted according to method (D).

Date 1947	Freshening period	Hrs.	Chemical analysis				Bacterial analysis - thousands per ml.					
			pH	Per cent NaCl in pickles	Per cent NaCl in brine	Degrees salometer	Nutrient agar	Potato dextrose agar		Skim milk agar		
							Total	Ysts	Mold	Total	Acid	Peptonizers
8-14	salt stock	0	5.5	15.6	15.6	60	1,800	26	0	1,700	1,400	0
8-15	1st	24	6.15	6.5	6.7	25	150	0.4	0	1,900	800	0
8-16	2nd	48	6.1	2.0	3.3	13	18,000	0	0	20,000	100	1,500
8-17	3rd	72	6.3	1.8	2.1	9	71,000	0	0	51,000	0	2,000
*8-17	3rd	72	3.85	2.0	1.8	8	0.05	0	0	**	**	**

* After alum added.

** Spreaders.

PART II - BACTERIOLOGICAL EFFECT OF ALUMS DURING DESALTING

Experimental Procedure

In order to study the actual effect of certain alums upon micro-organisms during the desalting of pickles it was necessary to control as many factors as possible. Therefore, the organisms commonly found in pickle brines were isolated to obtain ones more suited to the natural conditions. Cultures of an acid resistant yeast, a frequently occurring aerobic, sporogenic bacterium and a Lactobacillus were used as the organisms typically found in pickle brines. No attempt was made to identify the species of these cultures.

Aluminum sulfate, $Al_2(SO_4)_3 18H_2O$; ammonium alum, $Al_2(SO4)_3(NH_4)_2SO_4 24H_2O$; and sodium alum, $Al_2(SO_4)_3Na_2SO_4 24H_2O$; designated as "aluminum" (Al), ammonium (NH_4), and sodium (Na) alums, were the alums used. As stated previously, alum was added to the freshening water at the rate of two pounds per six bushels of pickles or one pound per forty-five gallon barrel of finished stock which figures out to 2.7 grams of alum per liter. To sterilize, the alums were autoclaved for 15 minutes at 248° F under 15 pounds pressure. No breakdown of the alums was noticed.

The third freshening water from the desalting process was used for these experiments in order to have the same type of medium that the organisms and alum would be in under normal conditions. This was sterilized by passing through a Seitz filter. The freshening water

contained 2.5 per cent sodium chloride, 0.03 per cent acid as lactic acid, at a pH of 6.9. A representative part of this freshening water was plated on nutrient agar with no visible growth apparent.

Effect of Alums Upon Yeast

To determine the effect of the various alums on yeasts, 10 ml. of sterile freshening water was transferred to each test tube aseptically. Then one milliliter of sterile alum solution was added to each tube. The alum solution was made by dissolving 3.2 grams of alum in distilled water and making up to 100 ml. with distilled water. This concentration of alum was used, since one ml. of alum solution plus one ml. of yeast suspension plus 10 ml. of freshening water would give the desired final concentration of alum of 2.7 grams per liter. The yeast used was isolated from pickle brine and grown on acidified potato dextrose. Transfers were made every four days and a one ml. suspension of this growth in sterile saline was used to inoculate the freshening water containing the alum. This original yeast suspension was plated on acidified potato dextrose to determine the number of yeast cells per ml. that was used for the inoculation.

The mixture of alum plus freshening water plus yeast was plated out on acidified potato dextrose agar at intervals of 1, 7, 24, 48, 72, and 96 hours. The plates were incubated at room temperature for five days and the number of yeasts per ml. recorded. It was necessary to

multiply these results by twelve to allow for the dilution of yeasts when inoculated in freshening water. These results are shown in table 14.

Effect of Alums upon Sporogenic Bacteria

The same general procedure was followed to show the effect of alum upon aerobic sporogenic bacteria with the exceptions that nutrient agar was used as the medium and the plates were incubated at room temperature from 24 to 48 hours. The results are shown in table 15.

Effect of Alums upon Lactobacillus

Glucose tryptone yeast extract agar (G.T.Y.E.) was used as the medium for Lactobacillus; however, upon plating out at the same time intervals as before, no growth was found. Time intervals of less than one hour were tried, but without obtaining any positive results, so it was necessary to change the procedure. Since growth or no growth were of primary interest, inoculation of broth tubes at shorter time intervals was used. The effect of concentrations of the various alums upon Lactobacillus was studied at the same time and under the same procedure.

Twenty-four hour cultures of Lactobacillus grown in G.T.Y.E. broth were centrifuged and the supernatant broth removed. Sterile saline was added and the Lactobacillus resuspended.

Sterile alum solutions were pipetted into tubes of freshening water so that the final concentration of alum

in each was 2.7, 4, 6, 8, 10 and 12 grams per liter. Five-tenths ml. of the saline suspension of Lactobacillus was pipetted into each tube. Then two standard loopfuls from each tube were inoculated into other tubes of G.T.Y.E. broth at time intervals of 10, 20, 30, 40, 50, 60, 120, and 240 minutes. Each concentration of alum was run in duplicate. The control consisted of freshening water plus inoculum of Lactobacillus. The tubes were incubated at 37° C and the results recorded at the end of 24 and 48 hours. Findings are recorded in tables 16, 17 and 18.

Effect of Concentrations of Alums upon Yeast and Sporogenic Bacteria

The same general procedure was used as described above to study the effect of the concentration of the different alums upon sporogenic, aerobic bacteria and yeasts except the bacteria were grown in nutrient broth and the time intervals were lengthened to one, two four, eight, sixteen, twenty-four and forty-eight hours. The tubes containing the sporogenic bacteria were incubated at room temperature for 24 hours and the yeast cultures kept at room temperature for four days before recording the results. Neither the alum nor the concentrations used had any noticeable effect upon the sporogenic bacteria or yeast cultures used.

Table 14. Showing effect of respective alums in freshening water upon a yeast culture at different time intervals. (Count - thousands per ml.)

Time	Alums			Without
Hrs.	Al	NH_4	Na	alum
0	1,725	1,725	1,725	1,725
1	1,850	1,965	1,995	2,065
24	1,370	1,400	945	2,075
48	995	1,000	272	1,810
72	600	284	134	1,945
96	304	134	28.5	1,710

Table 15. Showing effect of respective alums in freshening water upon a common sporeformer at different time intervals. (Count - thousands per ml.)

Time	Alums			Without
Hrs.	Al	NH_4	Na	alum
0	8,300	8,300	8,300	8,300
24	6,200	5,500	6,800	11,000
48	6,600	6,850	6,700	6,900
72	4,950	6,400	6,700	8,650
96	5,150	5,100	6,250	7,300
120	6,100	6,200	8,350	6,750

Table 16. Showing effect of various concentrations of aluminum alum upon Lactobacillus in freshening water.

Conc. of Al "alum" in grams/liter	Time in minutes							
	10	20	30	40	50	60	120	240
2.7	+	+	+	+	-	-	-	-
4.0	+	+	+	+	-	-	-	-
6.0	+	+	+	+	-	-	-	-
8.0	+	+	+	+	-	-	-	-
10.0	+	+	+	+	+	-	-	-
12.0	+	+	+	+	+	+	-	-
control	+	+	+	+	+	+	+	+

Table 17. Showing effect of various concentrations of Ammonium alum upon Lactobacillus in freshening water.

Conc. of NH_4 alum in grams/liter	Time in minutes							
	10	20	30	40	50	60	120	240
2.7	+	+	+	+	-	-	-	-
4.0	+	+	+	+	-	-	-	-
6.0	+	+	+	+	-	-	-	-
8.0	+	+	+	+	-	-	-	-
10.0	+	+	+	+	+	-	-	-
12.0	+	+	+	+	+	-	-	-
control	+	+	+	+	+	+	+	+

Table 18. Showing effect of various concentrations of Sodium alum upon Lactobacillus in freshening water.

Conc. of Na alum in grams/liter	Time in minutes							
	10	20	30	40	50	60	120	240
2.7	+	+	+	+	-	-	-	-
4.0	+	+	+	+	-	-	-	-
6.0	+	+	+	+	-	-	-	-
8.0	+	+	+	+	-	-	-	-
10.0	+	+	+	+	+	-	-	-
12.0	+	+	+	+	+	-	-	-
control	+	+	+	+	+	+	+	+

Effects of Alums upon Yeast in Finishing Liquors

For these experiments, a true yeast was isolated from a jar of spoiled processed dill pickles and grown on acidified potato dextrose agar. The composition of liquors used were made up according to table 19, and approximate in composition those found on finished pickles. Control liquors were made containing only sodium chloride, acetic acid or sucrose. Sterile alums of a final concentration of 2.7 grams per liter were inoculated into tubes of the different liquors. Then a known amount of yeast was inoculated into the tubes and proper dilutions were made at the end of the time intervals shown in table 20 to 26. At the end of these time intervals they were plated out on acidified potato dextrose agar. The plates were kept at room temperature for 96 hours and then the number of yeasts per ml. recorded. There was usually a slight decrease after the first twenty-four hours, but no appreciable effect was noticed.

Table 19. Showing general liquor compositions of finished pickles and composition of control liquors.

Test liquor	Type of finished pickle	Per cent NaCl	Per cent acetic acid	Per cent sucrose	Degrees Baume
1.	Fresh pasteurized dills	2.5	1.0	-	-
2.	Bread and butter	2.5	1.2	16.0	10
3.	Low Baume and low acid	2.5	1.2	21.7	12
4.	Sweets	2.5	2.0	32.6	18
5.		2.5	-	-	-
6.		-	1.0	-	-
7.		-	-	32.6	18

Table 20. Showing effect of respective alums in liquor No. 1 (table 19), upon a resistant yeast culture at different time intervals (Count - thousands per ml.)

Time Hrs.	Alums Al	Alums NH_4	Alums Na	Without alum
0	2,320	2,320	2,320	2,320
1	2,240	2,120	1,870	2,190
4	2,110	1,880	1,600	2,150
7	1,790	1,690	1,450	1,920
24	2,040	2,050	1,650	1,590
48	1,310	1,380	1,390	800
96	219	222	210	304
168	2.2	2.6	1.9	24.9

Table 21. Showing effect of respective alums in liquor No. 2 (table 19), upon a resistant yeast culture at different time intervals. (Count - thousands per ml.)

Time hrs.	Alums Al	Alums NH_4	Alums Na	Without alum
0	2,320	2,320	2,320	2,320
1	1,230	1,470	1,215	1,180
4	1,290	1,180	1,370	940
7	1,020	1,180	920	840
24	750	595	710	1,680
48	670	300	460	435
96	34.4	22	29.2	176
168	12.4	10.5	15.6	55

Table 22. Showing effect of respective alums in liquor No. 3 (table 19), upon a resistant yeast culture at different time intervals. (Count - thousands per ml.)

Time hrs.	Alums Al	Alums NH_4	Alums Na	Without alum
0	2,732	2,732	2,732	2,732
1	2,250	2,295	2,390	2,310
4	1,750	1,590	1,865	1,810
7	1,515	1,365	1,400	1,480
24	620	680	439	556
48	344	335	357	413
96	205	199	206	126
144	102	98	76	53

Table 23. Showing effect of respective alums in liquor No. 4 (table 19), upon a resistant yeast culture at different time intervals. (Count - thousands per ml.)

Time hrs.	Alums Al	Alums NH_4	Alums Na	Without alum
0	2,732	2,732	2,732	2,732
1	2,050	2,225	2,025	1,640
4	1,975	1,705	1,515	450
7	1,210	820	1,150	1,675
24	265	323	206	146
48	13	10	13	3.5
96	2	4.5	3.3	1
144	0	0	0	0

Table 24. Showing effect of respective alums in liquor No. 5 (table 19), upon a resistant yeast culture at different time intervals. (Count - thousands per ml.)

Time Hrs.	Alums Al	Alums NH_4	Alums Na	Without Alum
0	4,180	4,180	4,180	4,180
1	2,355	2,330	2,480	2,990
4	2,940	3,345	3,270	4,260
7	2,875	3,105	2,650	2,770
24	2,650	3,402	3,390	2,306
48	2,075	3,705	3,580	1,845
96	1,830	2,800	2,220	1,340
120	465	1,000	867	1,185

Table 25. Showing effect of respective alums in liquor No. 6 (table 19), upon a resistant yeast culture at different time intervals. (Count - thousands per ml.)

Time Hrs.	Alums Al	Alums NH_4	Alums Na	Without alum
0	4,180	4,180	4,180	4,180
1	2,820	2,230	2,325	2,980
4	2,685	2,610	2,915	2,145
7	2,315	2,080	2,140	2,410
24	1,635	1,280	1,040	1,190
48	1,125	680	371	640
96	520	340	201	53
120	76	86	26	0.1

Table 26. Showing effect of respective alums in liquor No. 7 (table 19), upon a resistant yeast culture at different time intervals. (Count - thousands per ml.)

Time Hrs.	Alums Al	Alums NH_4	Alums Na	Without alum
0	3,450	3,450	3,450	3,450
1	2,200	2,310	1,340	665
4	2,445	2,210	2,115	2,560
7	2,125	1,345	1,570	1,710
24	1,585	1,310	1,000	14.6
48	1,000	1,085	830	0.7
120	515	496	22	0.01

DISCUSSION

There are many alternate methods of freshening cucumber pickles. One procedure is to change the freshening water every twelve to twenty-four hours until the necessary amount of salt has diffused out. The first group of experiments under Method (A) consisted mainly of this procedure. From tables 1 through 6, and figures 1, 2 and 3, it will be seen under the bacteriological analysis that the number of micro-organisms even in the same salt stock fluctuated so that it was necessary to determine the number of bacteria in the salt stock at the beginning of each desalting experiment. The data in these same tables also show that there was a very rapid increase in total count as the amount of salt in the brine was decreased. This increase occurred from a few thousand to many millions of bacteria per ml. The greatest increase in organisms usually occurred in the second freshening period and is undoubtedly due to the lower amount of salt present. The amount of salt present after the first desalting period was ten per cent or less and the common types of micro-organisms found in pickle brines were able to tolerate and reproduce at these concentrations.

With this increase in total number of bacteria during processing, it was observed that while there was a slight increase or in some cases a decrease in the number of acid-producers there was an enormous increase in the number of peptonizers.

There seemed to be very few yeasts or molds present in the salt stock and only a very slight increase in either occurred during freshening.

There was a slight increase in pH, and where this increase approached the optimum for growth there was the greater increase in the total number of micro-organisms.

Since ninety-five per cent of the salt capable of being removed in twelve hours was removed in the first eight hours, a comparison was made of the increase in numbers of organisms during 24 hour (Method D) and eight hour (Method C) freshening periods. The same procedure that was used in Method (A) for desalting was used in Method (D) except the time interval was increased to 24 hours. The same salt stock was used for both the quick and slow methods and the freshening was started at the same time. In the slow or twenty-four hour procedure, the total count increased as before, with a decrease in acid-producers and a decided increase in peptonizers (table 13). During the quick, or eight-hour freshening method, peptonizers occurred only once and there was a substantial decrease in the number of acid-producers. The total number of organisms was reduced considerably during the quick desalting and only a slight increase occurred during the third period of freshening (table 12).

Method (B) was carried out to see the effect of heat upon the number of organisms present. The temperature was increased only during the first freshening period (tables 7 through 11). Heat was not only helpful in speeding

up desalting but also for keeping the total bacterial count down. During the second freshening period, where no heat was applied, there was a noticeable gain in total count of bacteria. The same decrease in acid-producers occurred and again there was an increase in peptonizers with the biggest increase in the last period. From the results in this method it is evident that by the use of heat during desalting the number of micro-organisms may be greatly inhibited.

When alum was added to the final freshening period there was a remarkable decrease in the number of organisms present. In order to find the actual effect upon the viability of the micro-organisms during processing, a group of experiments was carried out as shown in Part II. Tables 14 and 15 revealed that the common alums at the concentration of two and seven-tenths used by most manufacturers of pickles had very little or no effect on resistant true yeasts or sporogenic bacteria found in pickle brines. There was no effect on the same yeast or bacteria at concentrations through twelve grams of alum per liter of freshening water. However, alums did have a decided effect upon Lactobacillus cultures isolated from pickle brine. These results are found in tables 16, 17 and 18. Between fifty and sixty minutes was required to inhibit growth of Lactobacillus when it was subjected to various concentrations of aluminum, ammonium and sodium alums in freshening water. There seemed to be very little difference in the inhibiting powers of the different alums or

in the concentrations of alums from 2 to 12 grams per liter.

As mentioned previously, there was an appreciable decrease in the number of organisms when alum was added to the final freshening water. This was undoubtedly due to the aluminum hydroxide that was formed which enmeshed the bacteria and carried them to the bottom of the container as it settled. The same thing occurs in the mechanical purification of water when alum is added for that purpose. Here it might be mentioned that the pickle manufacture may lose the crisping effect of the alum since it settles to the bottom of the tanks as aluminum hydroxide.

In addition, a yeast causing spoilage in finished pickles was isolated and submitted to the same alums, at the same concentration, and in the same liquors as used by pickle makers (table 19). No appreciable effect was noticed (tables 20 through 26).

The work done on the effect of alums on bacteria in this investigation is a small part of that which should be done in order to understand better what is actually happening when alums do inhibit the growth of bacteria. However, during this study there was greater concern with obtaining a more complete bacterial picture of the desalting process in the pickle industry.

SUMMARY

The results of a bacteriological study of the desalting process in pickle manufacture have been presented and may be summarized in the following paragraphs.

During the ordinary desalting process (Method A) when the freshening water was changed every twenty-four hours, there was a marked increase in the number of micro-organisms as the amount of salt in the brine and in the pickles was decreased. The largest increase in organisms occurred after the greatest percentage of salt was removed in the first freshening period. Possibly the most important fact was the noticeable increase in peptonizers in comparison with a decrease or very slight increase of acid-producing bacteria. Shortening the time of desalting to eight hour freshening periods (Method C) resulted in a sharp reduction of organisms.

Where heat was used in combination with the eight hour freshening period (Method B), to hasten the freshening process of the pickles, the number of bacteria did not increase appreciably and most of the results showed an actual decrease in the total number when compared with the initial count of the salt stock. Heat also inhibited the increase of peptonizers.

When sodium alum was added to the last freshening water the total number of organisms was greatly reduced due to the sedimentation of the aluminum hydroxide. Alums in the concentration used by pickle manufacturers had no inhibiting effect upon yeasts or aerobic sporogenic bacteria

isolated from pickle brines as used in these experiments. Furthermore, concentrations of alum as high as twelve grams of alum per liter of solution did not have a noticeable effect. However, there was a decided inhibition in one hour of the Lactobacillus group found in pickle brines when alums were added at the concentration ordinarily used by pickle men and at concentrations as high as twelve grams per liter.

Alums added to liquors having the same composition as that used on finished pickle products had no inhibiting effect on a true yeast culture isolated from spoiled processed dill pickles.

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