A BACTERIOLOGICAL AND BIOCHEMICAL STUDY OF DIFFERENT METHODS OF SALTING ONIONS

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INTRODUCTION

Under normal conditions salting is one of the four principal methods for preserving food. During the war and since, there has been a shortage of critical materials needed for canning, dehydrating and freezing foods, so that various methods of brining have been used extensively for preserving large amounts of vegetables. Some of the methods used have not been satisfactory resulting in considerable loss due to spoilage. One of the vegetables that has always been salted extensively and which gives trouble to the salter is onions. This study was undertaken to compare different methods of salting onions and to determine, if possible, which method produced the best onions with the least waste.

Bacteriological studies were used to obtain a picture of the relative numbers of different types of organisms as they developed during the process of brining.

Chemical analyses were run in conjunction with the studies on the microscopic flora to determine any relation between changes in chemical environment and changes in the population of the microorganisms.

With these data a much better idea may be obtained of the biological changes which take place during the preserving process in each of the different methods used, and also the types of microorganisms developing during the process.

REVIEW OF LITERATURE

The literature dealing with the preservation of vegetables by the use of salt is quite extensive. Fabian, Bryan and Etchells (6) reported extensively on the influence of salting upon cucumber fermentation and upon the incidence of certain types of micro-organisms related to the fermentation. Fabian and Erickson (7) stated that 30° salometer brine to which has been added 2.5 per-cent dextrose by weight is the best method for the salting of green tomatoes. Fabian and Blum (5) found that it takes at least a 70° salometer brine to preserve peas; corn, green string beans and okra being preserved in low brine concentrations (40° salometer), whereas, a brine strength of 60° salometer appeared to be on the borderline between preservation and spoilage for green lima beans. They found that vegetables with a high protein content were not salted as successfully as those with a low protein content. Etchells and Jones (3) in a study of commercial brining methods stated that green beans, lima beans and peas were satisfactorily preserved by coverning with a 60° salometer brine. Leafy vegetables, such as; kale, mustard greens, spinach and turnip greens were kept in good condition treated with a 20° salometer acidified brine. Fabian and Wadsworth (9) reported that blanched whole kernel corn, green snap beans, carrots, spinach and beets brined in 18 per-cent salt gave the best results with the products comparing favorably with similar products canned at the same time. Wadsworth and Fabian (13) studied methods of salting peas. They recommended at least 18 and not over 20 per-cent salt. Fabian and Hontz (8) described a method for

preserving red mango pepper hulls which gave excellent results. They found that covering them with 70° salometer brine and then adding 15 pounds of dry salt per 100 pounds of pepper hulls produced a good quality product and allowed practically no fermentation.

Literature pertaining to the salting of onions is limited and conflicting. Campbell (2) described a method for salting onions which consisted of soaking them in water for several days, draining and adding 40° salometer brine. This was allowed to stand for 4 days, then to be replaced with 60° salometer brine and finally by 80° salometer brine. This method allowed an active fermentation. However, Fabian (4) stated that lactic acid fermentation will injure the texture of onions. He showed that a 20° salometer brine allowing fermentation, loosened the outer layers of the onion. For this reason the salt concentration should be sufficiently high to prevent fermentation. Until 15 per-cent salt concentration is reached, fermentation will take place, and spoilage may occur until more than 20 per-cent salt is present. The amount of salt to add to suppress spoilage bacteria and permit large numbers of lactic-acid producing bacteria to grow is from 2½ to 5 per-cent.

Certain bacteria, fortunately not directly responsible for spoilage of vegetables, have been reported to grow in very high concentrations of salt. Falk (10) stated that micrococci and sarcinae, because of their form of growth, could withstand up to 15 per-cent concentrations of salt. Wyant and Normington (14) reported that Clostridium botulinum is not affected by 10 per-cent salt concentration. Fischer (11) reported that the "hog bacillus" grew well on an infusion containing 9 per-cent salt.

Halophiles have been described by Browne (1) which grew luxuriantly in a saturated solution of salt, and which would not grow on media containing less than 16 per-cent salt by weight. LeFevre and Round (12), while studying the microorganisms concerned in the fermentation of cucumber pickles, isolated a group of bacteria from pickle scum containing 10 per-cent salt, and were grown readily on a medium containing 25 per-cent salt.

METHODS

General Salting Procedure

All the onions were salted according to a general salting procedure. The onions were allowed to become dry by standing at room temperature for several days. The stem and root ends were trimmed, adhering dirt removed, and the rough outer skins removed. They were then weighed into three crocks and treated with three different brining procedures which will be outlined in detail later.

In salting onions, as in other vegetables, it is necessary to compensate for the water present in them, some of which will be removed when the brine is added. To determine the amount of salt that is necessary to compensate for the water in the onion, the following formula was used: (5)

$$X = \frac{A \times \frac{s}{100}}{1 - \frac{s}{100}}$$

in which X = weight of salt in pounds per 100 pounds of onions,

A = per-cent of moisture in onions,

and s = per-cent of salt desired, or in terms of salometer degrees desired it is: $\frac{\text{degrees salometer} \times 26.5}{100}$

To use this formula, it is necessary to know the weight of the onions and the amount of moisture they contain. For commercial purposes the amount of moisture listed in tables of chemical composition is satisfactory, but in this work the moisture was determined by drying an accurately weighed sample in an electric drying oven at 100° C. until a constant weight was reached. The loss of weight was taken as the amount of moisture present and the average for five samples was found to be 87.6 per-cent.

Bacterial and Chemical Analysis

Four media were used to determine the microscopic flora present in the brine at various intervals throughout the salting procedure.

Bacto nutritive caseinate agar was used as a differential medium. On these plates a total count of all organisms present was made first. Next, the strong acid forming colonies were counted, which were distinguished by a white zone of precipitated casein surrounding the colonies. The plate was then flooded with brom cresol purple indicator solution for five minutes and a total count made of the acid forming colonies which were yellow against the purple background of the medium. To determine the number of weak acid formers, the number of strong acid formers was subtracted from the total number of acid forming colonies. The plate was then flooded with dilute acetic acid, and the peptonizing colonies were counted, which were surrounded by a clear zone.

Bacto tryptone glucose extract agar was used to obtain a total count of all bacteria present in the brine. Bacto tomato juice agar was used to detect the number of acid-producers, especially Lactobacilli. To determine the number of yeasts and molds present in the brine, potato dextrose agar was used. Bacterial growth on this medium was inhibited by acidifying it to pH 3.5 ± with a sterile solution of tartaric acid.

All plates were incubated 48 hours at 30° C. and counted with the aid of a Quebec counting chamber.

Total acid as lactic was determined by titrating with N/10 NaOH. The pH of the brine was determined with a Beckman glass electrode pH

meter. Salt was determined in per-cent by titrating with N/10 $AgNO_3$ using dichlorofluorescein as an indicator. Salt strength was also tested by using a salometer which is a commercial test based on a scale in which a saturated salt solution is considered 100° salometer. The degrees salometer are measured by a graduated hydrometer calibrated on a 0 to 100° scale, reading direct in terms of degrees salometer.

Method A

To the first lot of onions (50 pounds) sufficient 40° salometer brine was added to cover them and then an additional 5.19 pounds of salt added to compensate for the water present in the onions. This amount was calculated from the formula described previously. Sufficient salt was then added to raise the salometer reading at various intervals (refer to Table 1) until a final concentration of 60° salometer was reached.

The bacterial and chemical results obtained from the brine of this lot of onions treated by this method are found in Table 1. The number of yeasts reached a maximum on the second day, after which they gradually decreased in numbers until the twentieth day where a slight use was evidenced. The total plate count on tryptone-glucose extract agar was not large, the highest count, occurring on the second day, was two hundred thousand per ml. This count decreased to nine thousand on the eleventh day and later built itself up to eighteen thousand at the end of twenty-six days. The ratio of acid producing organisms to the total count on nutritive caseinate agar was approximately 1:2 at the beginning with the final ratio being approximately 1:16.

No visible fermentation of the onions in this lot occurred. However, the acidity did increase from .01 percent and pH 6.50 in the first day to .14 percent and pH 4.85 on the twentieth day, after which a sharp decline was noted. The pH curve seemed to follow that of the percent acidity quite regularly and seemed to bear a direct relationship. Tomato juice agar generally gave higher counts than the tryptone-glucose extract agar and produced larger, more distin-

guishable colonies. The bacterial counts on this medium also followed the general curve more closely than the tryptone-glucose extract agar and was generally found to be a medium more suited to the optimum conditions of the organisms concerned.

Table 1. Showing number of microorganism on the respective media and pertinent chemical data for onions salted by Method A.

TABLE 1. - METHOD A

Days	Bacterial Analysis - thousands per ml. of brine								Chemical Analysis			
	Nutritive caseinate agar					Tryptone glucose agar	Tomato juice agar	Potato dextrose agar yeasts	Total acid as per cent lactic	pH	per cent NaCl	degrees Salometer
	Total	Strong Acid	Weak Acid	Total Acid	Pepto-nizers							
1	70	0	41	41	.1	85	94	0	.01	6.50	11.6	40
2	150	0	85	85	0	200	220	15	.04	6.30	11.4	40
3	50	3	16	19	0	165	69	5.5	.06	5.70	11.1	40
5	50	9	19	28	0	117	88	4.2	.09	5.52	11.8	42
7	24	2.9	2.1	5.0	0	41	40	.15	.09	5.46	11.5	42
9	3.4	.55	.62	1.17	0	10.3	13.5	.03	.10	5.50	11.8	42
11	8.5	.5	.8	1.3	0	9.6	9.0	0	.10	5.00	11.8	42
14	13.8	2.2	5.6	7.8	0	16.2	14.2	1.38	.10	5.00	11.9	42
17	25.1	3.3	5.3	8.6	0	24.7	21.0	.48	.12	4.86	12.2	45
20	15.3	0	.2	.2	0	9.7	12.4	15.7	.14	4.85	13.6	50
23	14.7	.56	.2	.76	0	15.8	15.8	13.0	.075	5.20	14.5	55
26	16.1	.40	.6	1.0	0	18.6	25.9	14.6	.09	4.80	16.5	60

Method B

The second lot of onions (50 pounds) was covered with an 80° salometer brine and 11.78 pounds of salt added to maintain the 80° brine strength for the duration of the preserving process.

No visible fermentation occurred in these onions. Starting with an initial acidity of 0.07 per cent and a pH 5.70, the maximum acidity reached was only .11 per cent with a pH 4.87 on the tenth day and falling off after that to .02 per cent and pH 5.35 on the twentieth day. The microbial picture showed considerably smaller numbers of organisms than in Method A. A maximum of ten thousand occurring on the first day which was gradually reduced to disappearance on the twenty-second day. Six hundred peptonizers were present on the second day after which they disappeared. Yeasts which were present at the beginning, reached a maximum at the end of the second day and disappeared by the sixth day. The ratio of the number of acid-formers to the total number of bacteria present on nutritive caseinate agar reached 1:2 on the second day, 1:4 on the sixteenth day and disappeared entirely on the nineteenth day.

The results of bacterial and chemical analyses are given in Table 2. Tomato juice agar again shows higher counts and more consistent results than the tryptone-glucose extract agar.

Table 2. Showing number of microorganisms on the respective media and pertinent chemical data for onions salted by Method B.

Days	Bacterial Analysis - thousands per ml. of brine								Chemical Analysis			
	Nutritive caseinate agar					Tryptone glucose agar	Tomato juice agar	Potato dextrose agar yeasts	Total acid as per cent lactic	pH	per cent NaCl	degrees Salometer
	Total	Strong Acid	Weak Acid	Total Acid	Pepto-nizers							
1	5.9	0	0	0	0	11.0	10.0	.23	.07	5.70	23.0	80
2	10.4	0	4.6	4.6	.6	15.4	5.4	7.6	.08	5.50	21.6	78
4	2.5	.04	.26	.30	0	15.1	8.0	.36	.08	5.35	23.0	79
6	1.7	.38	.11	.49	0	4.5	1.5	0	.09	5.15	21.6	78
8	.4	.06	.03	.09	0	2.74	.63	0	.10	4.95	23.9	80
10	.75	.10	.26	.36	0	1.18	.14	0	.11	4.87	24.5	80
13	.42	.09	.12	.21	0	.64	.34	0	.11	5.10	23.6	80
16	.98	.12	.13	.25	0	.51	.20	0	.10	5.00	24.0	80
19	.05	-	-	-	-	.04	.15	0	.09	5.00	24.5	80
22	-	-	-	-	-	-	-	-	.02	5.35	24.7	80
25	-	-	-	-	-	-	-	-	-	-	-	-

Method C

The third lot of onions (50 pounds) was treated in a somewhat different manner designed to allow fermentation to proceed. The onions were first covered with tap water and allowed to soak for three days. On the second day a maximum bacterial count on tryptone-glucose extract agar of over six billion bacteria per ml. was attained. The soaking in water removed considerable odor and strong taste from the onions and resulted in a cleaner looking brine later on. This water was drained off and the onions were covered with a 40° salometer brine which resulted in a considerable reduction in the numbers of bacteria.

After standing three days, this brine was replaced with 60° salometer brine with a resulting further decrease in numbers of organisms. Again this brine was drained off and replaced with 80° salometer brine which was maintained throughout the remainder of the process. Each time the brine was changed, salt was added to compensate for the water present in the onions. The 80° salometer brine resulted in a drastic reduction in numbers of organisms so that at the end of twenty-four days only three-thousand viable organisms per ml. remained in the brine. It is interesting to note here that although the total number of bacteria was reduced and remained small in number, the number of acid-forming organisms decreased irregularly, showing alternate diminishing and increasing numbers. This phenomenon resulted in curves with three peaks, each successively lower than the other. This has been graphically illustrated in Figure 1. A summary of the results of bacterial and chemical analyses appears in Table 3. Peptonizers predominated in the second and third days

while the onions were in water. The first day after the 40° salometer brine was added there were six thousand peptonizers present, after which they were completely inhibited.

The lactic acid bacterial count on tomato juice agar, Table 3, column 7, rose very high during the period the water was on the onions and were reduced considerably after the addition of the salt but managed to survive in considerable numbers and persisted throughout the duration of the experiment. Yeasts, as shown by the potato dextrose agar, Table 3, column 8, were abundant from the beginning. They increased considerably while the water was on the onions but were reduced upon the addition of the 40° salometer brine; decreased still further when the 60° salometer brine was added and disappeared entirely upon the addition of the 80° salometer brine.

These data confirm the bacteriological findings of previous work on the influence of salt on microorganisms by various investigators. Salt is very toxic to the proteolytic bacteria, less toxic to yeasts and only mildly toxic to the lactic acid group of bacteria. Certain members of this latter group are evidently able to develop strains that will withstand very high concentrations of salt.

Tryptone glucose extract agar gave higher counts with the water than did the tomato-juice agar, however, with the brine, the tomato-juice agar again gave higher and more consistent results. A comparison of the bacterial counts of brines plated on tomato-juice agar is given in Figure 2.

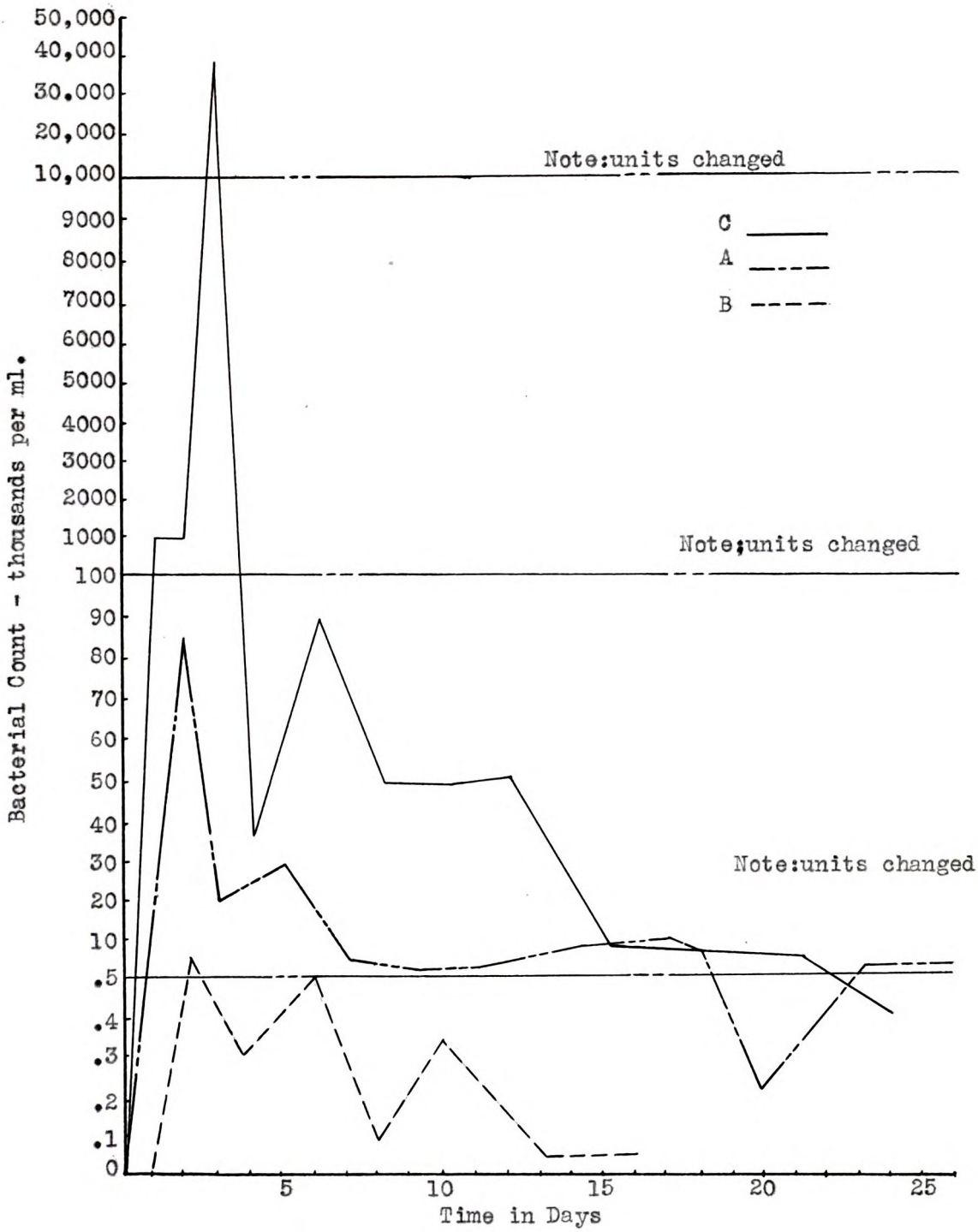

Fig.1. Plate count on nutritive caseinate agar of the brine of onions preserved at different salt concentrations.

Table 3. Showing number of microorganisms on the respective media and pertinent chemical data for onions salted by Method C.

Days	Bacterial Analysis - thousands per ml. of brine								Chemical Analysis			
	Nutritive caseinate agar					Tryptone glucose agar	Tomato juice agar	Potato dextrose agar yeasts	Total acid as per cent lactic	pH	per cent NaCl	degrees Salometer
	Total	Strong Acid	Weak Acid	Total Acid	Pepto-nizers							
1	47,000	0	1,120	1,120	0	630,000	3,900	460	-	-	-	-
2	136,000	0	1,000	1,000	10,000	6,250,000	550,000	139	-	-	-	-
3	200,000	0	40,000	40,000	20,000	176,000	168,000	1,420	.20	4.20	-	-
4	86	0	33	33	6	6,250	9,620	480	.23	4.65	10.5	40
6	470	30	60	90	0	386	820	248	.26	4.40	11.1	40
8	58	44	4	48	0	291	240	46	.24	5.02	16.5	60
10	82	40	8	48	0	93	97	37	.23	5.16	21.3	80
12	104	10	40	50	0	32	31.6	0	.11	4.92	22.8	80
15	25	0	8	8	0	28	41	0	.12	4.87	21.8	80
18	22.5	1.1	6.8	7.9	0	22.5	28.8	0	.14	4.60	23.0	80
21	2.03	.05	.58	.63	0	3.1	4.3	0	.15	4.62	22.7	80
24	1.96	.2	.2	.4	0	2.12	3.0	0	.02	5.00	23.0	80

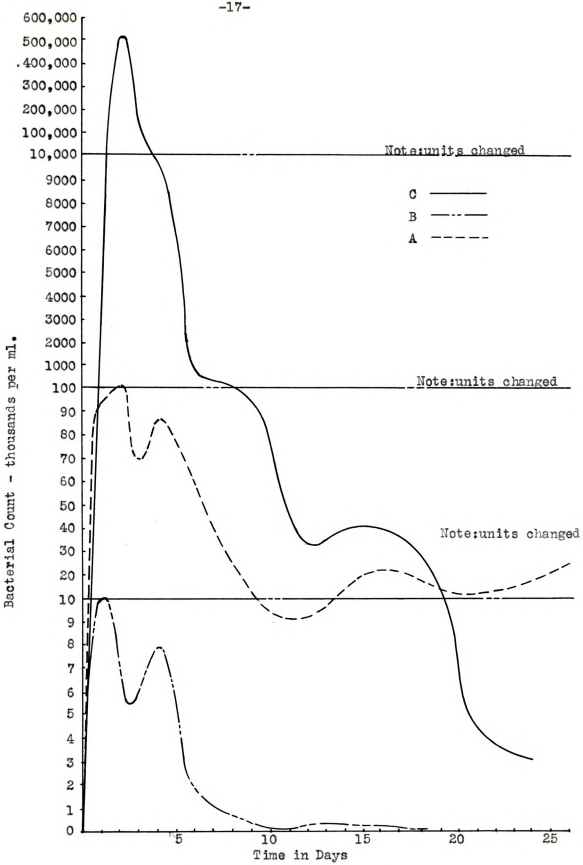

Fig.2. Total bacterial plate count on tomato juice agar of the brine from onions preserved at different salt concentrations.

PHYSICAL CONDITION OF ONIONS SALTED BY THREE METHODS

The results obtained in this series of experiments tend to show that of the three methods employed, Method A appeared to be the most satisfactory. This is easily shown by the results of examination of the physical appearance of the three lots of onions. To general visual inspection little difference was observed between the three methods. However, upon manual examination of each onion, it was found that a considerable difference existed between the different methods. It was found that Method C created a product with considerable injury. This injury specifically consisted of a loosening and softening of the outer scales of the product. This injury to the scales was generally concomitant with a soft, mushy interior of the onion. Counts were made on each lot for injured onions. Method C produced 86.85 per cent injured onions. Method B resulted in a lot containing 44.21 per cent injured onions. Method A gave the best results with only 2.59 per cent injury. The loss of these injured onions cannot be attributed to one specific cause. However, it may be caused by fermentation occurring with Method C, or a too high salt concentration at the beginning of the process in Method B.

The results indicate that a brine concentration of 80° salometer is not satisfactory as an initial covering for preserving onions. The high salt concentration apparently draws the moisture out of onions too fast to allow the brine to enter the onion and, thereby, make it firm and crisp. This fact precludes the possibility of dry-salting onions. The results also indicate that fermentation of

onions is not desirable to produce the best product.

It was also evident that the color of the brine has no bearin on its preservative action on onions. The brine of Method C was much cleaner-looking due to the numerous changes but was least effective in preserving the onions.

Discoloration of all the onions in all three lots was observed. However, this blackening occurred over the entire surface of the onion preserved according to Methods B and C. The onions salted according to Method A were merely discolored about the cut edges. This led to the recommendation that the onions be only trimmed at the root and stem ends, leaving at least one rough outer scale present to protect the onion from discoloration. Those onions near the surface of the crock showed the most discoloration indicating an oxidation possibly with the production of tannin or deposition of iron salts.

Freshening the products of the three methods by running water resulted in a whiter, firmer and crisper onion with those salted according to Method A.

DISCUSSION

The data presented indicate that of the three methods used for salting onions, Method B was the least satisfactory, whereas, both Methods A and C had merit and certain distinct advantages. Method A resulted in a product of good quality but the microscopic flora was not reduced sufficiently to warrant storage over a long time. Method C gave a less desirable product but storage for considerable time without spoilage could be depended upon because of the almost complete inhibition of bacterial growth. It would seem plausible, therefore, on the basis of the data obtained, that a combination of these two methods could be used to distinct advantage. Such a method as to embody the advantages of both methods and remove the undesirable factors present could be recommended. This method would consist of covering the onions with a 40° salometer brine, allowing this to remain for two days or longer, after which it would be replaced with a 60° brine. This would be allowed to remain on the onions for two days after which time it would be replaced with an 80° salometer brine. Based on data presented, it seems probable that this method would result in the firm, crisp, white product which was produced by Method A and would give the chemical and bacteriological results comparable to Method C in which bacterial growth was completely inhibited at the end of the preserving process.

SUMMARY

Three methods of preserving onions by salting were studied. The method that gave the best results was Method A which consisted of covering the onions with 40° salometer brine, increasing the salt concentration gradually to 50° salometer and finally to 60° salometer. It was found that the other two methods which utilized 80° salometer brine resulted in an almost complete inhibition of bacterial growth. Method B, which consisted of covering the onions with an 80° salometer brine at the beginning had a deleterious effect on the physical appearance of the onions. Method C allowed fermentation to occur and also produced an undesirable product. With Method A, the onions had a good color, were firm in texture and only 2.59 per cent showed any injury to the outer scales of the onions. Bacterial analysis showed the presence of fairly large numbers of bacteria at the termination of the experiments with the lot of onions preserved according to Method A.

For this reason a combination of methods A and C is recommended which embodies the advantages of each. This method is to initially cover the onions with a 40° salometer brine, to be replaced in two days with a 60° salometer brine, which in turn is replaced in two days with an 80° salometer brine. This method will insure the production of good quality onions with a maximum of safety from spoilage during storage.

Notable effects of salt on the development of various types of microorganisms were in evidence. With the gradual increase in salt concentration there was a gradual decrease in the total number of organisms present in the brines. The peptonizers affected most by

the salt were always the first group to disappear. The toxicity of the salt to yeasts was less than that to the peptonizers but sufficient to cause them to disappear shortly thereafter. The acid forming organisms were the most resistant of any of the organisms to the salt and maintained a relatively high ratio to the total number present throughout the duration of the process.

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