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By

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In 1923 Harrison and Hood (1) reached the conclusion that the blackening of canned lobsters was of two different types, first chemical and second bacteriological. They believed that the former would account for 85 to 90% of spoiled processed lobster and about 75% of the affected cans in the case of boiled lobster.

About a year later Reed and McLeod (2) summarized their findings in the following words "The principal cause of discoloration of canned lobster is decomposition of the lobster meat previous to sterilization of the cans". This conclusion was based on the fact that the volatile sulphur from lobster muscle infected with a certain type of bacteria isolated from lobster intestine rose rapidly to a value which they found to be necessary before blackening could result. Accordingly they recommended to the lobster packers that a period of not longer than four hours should elapse between shelling and sterilization. The lobster packers, however, claim to have followed this recommendation carefully but nevertheless they found that blackening still occurred occasionally. This led to the suggestion that possibly some change affecting the discoloration of the muscle takes place during the gradual loss of vitality of the lobster but before it is actually dead.

Cook (3) began an investigation of this question during the summer of 1930 at the Fisheries Experimental Station. He was unable to find any difference as regards the quantity of sulphur which could be removed by steam distillation between live vigorous lobsters and those, which having been kept on ice for some time, were on the point of death.

This report contains the findings obtained from a study of the question originally investigated by Cook.

METHODS

Determination of volatile sulphur and accuracy of the method

The hydrogen sulphide present in muscle was determined by aeration of 4 g. of minced muscle with 1 cc. of concentrated hydrochloric acid and 5 cc. of water for 120 minutes and collection of the gas evolved in a zinc acetate solution. The hydrogen sulphide trapped in the zinc acetate was then determined by adding 10 cc. of 0.01N. iodine, 1 cc. of concentrated hydrochloric acid and titration of the excess iodine with standard thiosulphate. The zinc acetate solution was prepared by dissolving 50 g. of zinc acetate, 10 g. of sodium acetate and 0.05 g. of sodium chloride in water and making the whole up to a litre.

To test the accuracy of the method some impure zinc sulphide was prepared by bubbling hydrogen sulphide through zinc acetate, filtering the resulting precipitate and drying. This was then ground finely, approximately 4 mmg. were weighed, and the amount of hydrogen sulphide liberated from this on treatment with acid and aeration was determined in the manner outlined above. Table 1 shows the agreement obtained.

TABLE 1

Accuracy of method for determining hydrogen sulphide

Experiment number	Iodine equivalent of 1 mmg. of zinc sulphide prep.	Experiment number	Iodine equivalent of 1 mmg. of zinc sulphide prep.
1	1.30 C.C.	5	1.22 C.C.
2	1.27 C.C.	6	1.32 C.C.
3	1.38 C.C.	7	1.29 C.C.
4	1.32 C.C.	8	1.31 C.C.

By direct titration 1 mmg. of the zinc sulphide preparation was found to contain 0.223 mmg. of sulphur or to have an iodine equivalent of 1.29 cc. Hence it is apparent from the above table that approximately 0.90 mmg. of sulphur can be determined with an accuracy of about 3%.

To determine whether lobster muscle had any effect on the quantity of hydrogen sulphide which could be liberated by aeration, approximately 4 mmg. of the zinc sulphide preparation, 20 cc. of water and a few drops of capryl alcohol were added to 4 g. of minced muscle. This was then acidified with 1 cc. of concentrated hydrochloric acid and aerated into zinc acetate. After subtracting a blank for the hydrogen sulphide present in lobster muscle, the following table shows the recovery of sulphur from the zinc sulphide to be complete.

TABLE 2

Recovery of hydrogen sulphide in the presence of lobster muscle

Experiment number	Iodine equivalent of 1 mmg. of zinc sulphide prep.	Experiment number	Iodine equivalent of 1 mmg. of zinc sulphide prep.
1	1.36 C.C.	4	1.28 C.C.
2	1.31 C.C.	5	1.35 C.C.
3	1.35 C.C.	6	1.39 C.C.

By direct titration the iodine equivalent of 1 mmg. of zinc sulphide was found to be 1.35 cc. Accordingly it is found that the presence of lobster muscle does not interfere with the complete removal of hydrogen sulphide by aeration.

Determination of pH

Since the pH of raw lobster muscle decreases continually for the first few hours after killing, some method had to be devised to avoid this change. Hence before taking a pH reading the muscle was dropped into boiling water for 10 minutes, then removed, minced and 4 g. of the muscle treated with 25 cc. of distilled water. This was allowed to remain for 10 minutes when it was filtered and the pH of the filtrate was determined colorimetrically using Gillespie's method.

Determination of the volatile bases

The volatile bases were determined by aeration of the minced muscle in the presence of a considerable excess of magnesium oxide. They were collected in approximately 0.01N. acid and estimated by back titration with standard alkali. The following table and accompanying graph indicates that 60 to 90 minutes are required for complete aeration of all volatile bases.

TABLE 3

Time required for complete removal of bases by aeration from 2 g. of lobster muscle.

Period of aeration	Mmg. of nitrogen collected	Period of aeration	Mmg. of nitrogen collected
15 mins.	0.55	81 mins.	1.24
30 "	0.96	96 "	1.24
45 "	1.14	120 "	1.24
66 "	1.21		

From the above table it is seen that the removal of the volatile bases is complete in about 80 minutes. In all experimental work, however, aerations were run for a minimum period of 90 minutes.

Experimental

Influence of lobster's condition on the amount of volatile sulphur

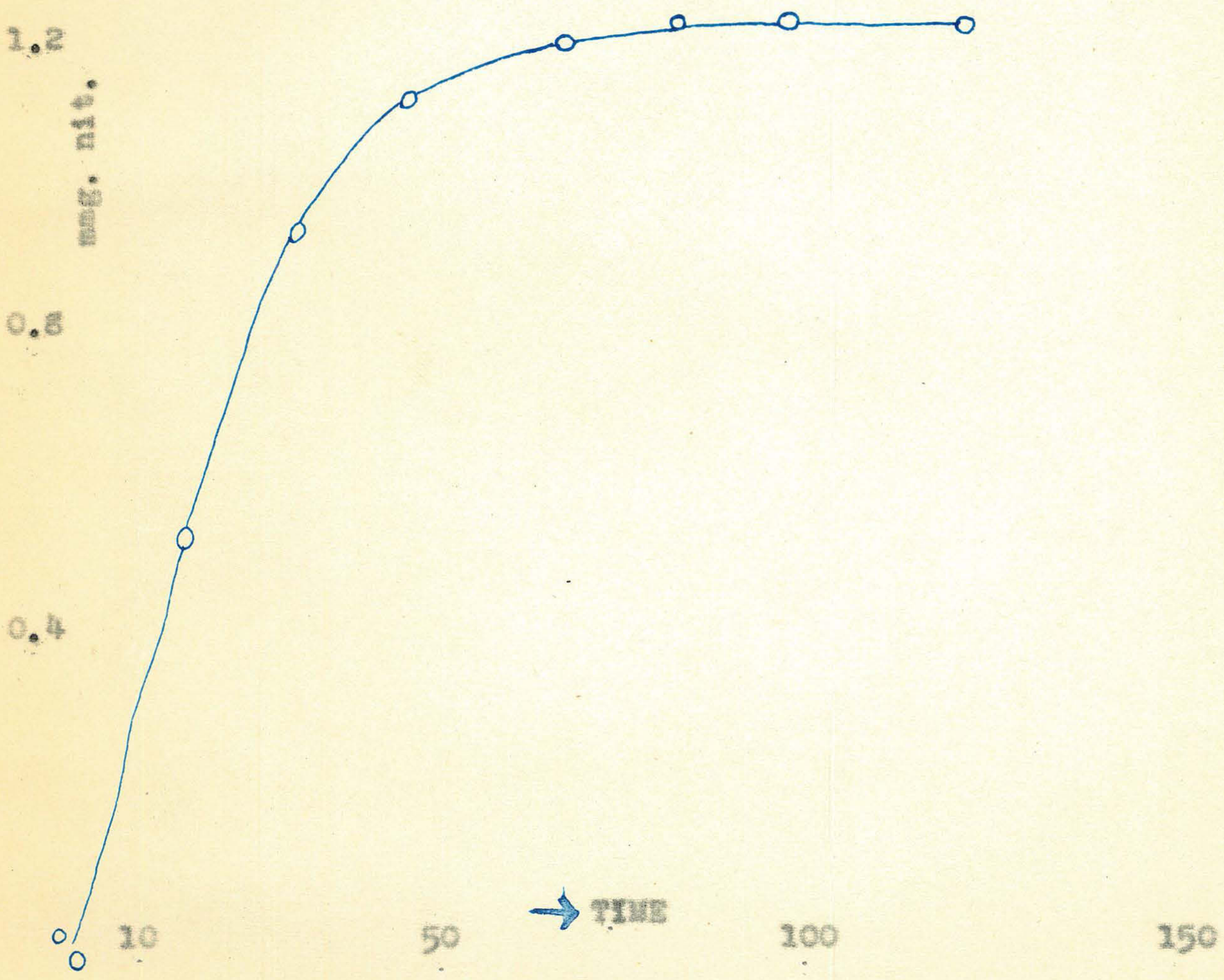
In order to study the influence of the lobster's condition on the amount of volatile sulphur present in the muscle a number of lobsters were purchased and held on ice for varying periods. After a definite period the tail from each was removed, minced by passing through a meat chopper, and 4 g. acidified with 1 cc. of concentrated hydrochloric acid. The muscle was then aerated into zinc acetate for 2 hours and the hydrogen sulphide determined by titration with iodine and sodium thiosulphate. Table 4 shows the results obtained in this manner.

TABLE 4

Volatile sulphur per 100 g. of uncooked lobster tail meat

Lobster number	Period on ice	Volatile sulphur per 100 g. flesh	Lobster number	Period on ice	Volatile sulphur per 100 g. flesh
1	0 hrs.	1.6 mmg.	6	24 hrs.	1.3 mmg.
2	0 "	2.1 "	7	24 "	0.4 "
3	0 "	0.8 "	8	48 "	0.3 "
4	0 "	0.8 "	9	48 "	0.8 "
5	24 "	0.15 "			

AERATION OF VOLATILE BASES



From the above table it is observed that no significant difference in the amount of volatile sulphur is found between live vigorous lobsters and those which having been kept on ice for varying periods were on the point of death. It is conceivable, however, that although no hydrogen sulphide was actually liberated from the muscle during the period when it was held on ice that the sulphur containing substance present in the muscle might have been so altered that increased amounts of sulphur would be liberated on cooking and autoclaving. To test this possibility 4 g. from the same lobsters, which were reported on in Table 4, were weighed into tubes and 25 cc. of distilled water added to each. These tubes were then connected to zinc acetate tubes and the former placed in boiling water for 1 hour. They were then cooled and 1 cc. of concentrated hydrochloric acid added, aerated for 2 hours and the hydrogen sulphide determined in the manner previously outlined. The results thus obtained are recorded in Table 5.

TABLE 5

Hydrogen sulphide liberated from cooked lobster muscle.

Lobster number	Period on ice	Volatile sulphur per 100 g. flesh	Lobster number	Period on ice	Volatile sulphur per 100 g. flesh
1	0 hrs.	1.7 mmg.	6	24 hrs.	1.70 mmg.
2	0 "	2.4 "	7	24 "	0.6 "
3	0 "	0.6 "	8	48 "	0.25 "
4	0 "	1.1 "	9	48 "	1.1 "
5	24 "	0.19 "			

It will be observed from an examination of the above table that in cooked lobster the amount of volatile sulphur is not greater in the case of those which have been kept on ice than from quite vigorous lobsters. By comparison of Table 4 with Table 5, it will also be seen that the volatile sulphur from cooked muscle is not greatly in excess of that liberated by the uncooked meat. Unfortunately, the pH of this muscle was not taken but as it was minced in the uncooked state, and since at least one half hour would elapse between removal of the tail from the lobster and boiling, the pH would undoubtedly be less than 7 and probably about 6.2. If, however, the pH of the muscle had been above 7 when it was boiled, as is often the case with lobsters as taken from the traps, or with those which are held on ice for some time, it is possible that much greater amounts of hydrogen sulphide would have been liberated since cystin which is probably the main source of sulphur present in muscle is quite stable in acid, but is readily decomposed in alkali with the liberation of free hydrogen sulphide. Unfortunately time did not permit of a careful investigation of this question.

Hydrogen sulphide content of normal and discolored cans.

The hydrogen sulphide content of two badly discolored and one normal can was determined. The can in good condition was packed by M. Gallant in Prince Edward Island, June 14, 1930 and was opened and examined August 10, 1931. The discolored cans were packed by Cook and Hess at the Fisheries Experimental Station in August, 1930. Unfortunately the normal can was enamelled so that a comparison between this and the latter is not entirely justified. However, no other can in good condition and of comparable age and packed without this protection was available. The hydrogen sulphide content and the pH of the pickle from each can is recorded in Table 6.

TABLE 6.

The hydrogen sulphide content of canned lobster

Condition of can on opening	Volatile sulphur per 100 g. muscle	pH of pickle
normal	1.9 mmg.	7.2
badly discolored	1.4 "	7.9
" "	2.3 "	8.1

Table 6 indicated that apparently there is no more hydrogen sulphide in cans that are badly discolored than in perfectly normal cans with a lower pH.

Since lobsters which were quite active, liberated no less sulphur either in the raw or cooked condition than those which were held for some time and would probably discolor on canning, and since the volatile sulphur from the limited number of cans which were examined was no higher in the case of discolored than normal lobsters, the conclusion has been drawn that any canned lobster would discolor slowly if given a pH above a certain definite minimum. Reed has fixed this figure at approximately 6.2 and the higher the pH the more quickly would the lobster become discolored. This contention is supported by Table 6 and also by Table 7 which shows the appearance pH of the pickle and history of the meat previous to packing of some cans put up by Cook and Hess during the summer of 1930 and opened in September, 1931.

TABLE 7

pH and condition of canned lobster when opened

Number of cans	Appearance of meat on opening	History of lobster prior to canning	pH of pickle
2	Meat discolored XXX	Canned at once	6.95
3	Meat discolored XXX	Same as 2	6.85
6	Meat discolored XXX	Lobster kept on ice 3 hours	7.0
13	Meat fair XX	Boiled at once meat kept 12 hours.	6.70
14	Meat very bad XXXX	Lobster kept on ice 12 hours	7.25
17	Meat discolored XXX	Boiled at once meat kept 24 hours	7.1
18	Meat normal X	Lobster dead before boiling	6.3
24	Meat very bad XXXX	Lobster kept on ice 36 hours	7.7

The number of X's, appearing in the column headed appearance of meat furnishes a rough index of the degree of blackening. Attention is directed to two facts, first the least discolored cans had the lowest pH and also that the lobster which was dead before canning and which is generally supposed to discolor very readily was normal in appearance. This can be attributed to the fact that the lobster must have been canned soon after dying so that the lactic acid which is rapidly produced after death had not yet been neutralized by the basic products of autolysis which do not accumulate so quickly. Thus the pH of the muscle was depressed to a value where blackening takes place only very slowly. It should also be observed that the cans from lobster which have been kept living on ice for varying periods had pH values which were higher the longer the lobsters were held. This point will be considered in greater detail later.

Effect of increasing the pH of canned lobster by the addition of ammonia

If as is suggested by our results there is sufficient volatile sulphur in all lobsters to produce blackening provided other conditions are suitable, why do lobsters which have been held on ice for varying periods discolor more readily than fresh vigorous lobsters ?

Provided that sufficient iron is always present in canned lobster, two other factors exerting great influence on the discoloration are the pH of the medium and the abundance of hydrogen sulphide. Since the amount of hydrogen sulphide is approximately equal in normal and inactive lobsters as well as in normal and discolored cans (Table 6 and also table on page 12, Manuscript Reports No.28) the conclusion is drawn that the deciding factor is a matter of pH. By adding ammonia (1.02N) to a known weight of lobster muscle the amount required to raise the pH of a quarter pound can to 7.8 was calculated as 1.8 cc. Hence in order to test the validity of the above hypothesis, 12 quarter pound cans of lobster were packed, 4 of which were held as controls (1-4) and to 4 of which were added 1.8 cc. of 1.02N ammonia (9-12). These were processed at a temperature of 242 for 35 minutes. The remaining 4 cans (5-8) were packed as controls but were processed for a period of 180 minutes. The appearance of the meat and the pH of the pickle on opening these cans which were packed September 2, 1931 and examined November 5, 1931 is recorded in the following table.

TABLE 8

Influence of increased pH and prolonged sterilization

Number of cans	Appearance of meat.	Appearance container.	pH of pickle
1	X	X	6.85
2	X	X	6.80
3	X	X	6.90
4	X	X	6.80
5	XXX	XXX	7.09
6	XXX	XXX	7.07
7	XXX	XX	7.08
8	XXX	XXX	7.09
9	XX	X	7.15
10	X	XX	7.17
11	XX	XX	7.13
12	XX	X	7.11

The number of X's. appearing after each number is a rough indication of the degree of discoloration, either on the muscle itself or on the container. One X denotes a normal can without any noticeable blackening. As may be observed those cans with the higher pH values were somewhat inferior to the controls, although the difference in hydrogen ion concentration between controls and cans 9-12 were not nearly as great as had been expected. Despite this fact, however and the fact that the period of storage was only 2 months, perceptible differences in the appearance of the muscle were observed. It will be necessary, however, to repeat this experiment at a higher pH before any absolute conclusion can be drawn.

Effect of prolonged sterilization

In 1923 Harrison and Hood reported that hydrogen sulphide was liberated at a more or less uniform rate for a period of two and one half hours in the case of lobster boiling in water. In 1930 Cook reached the same conclusion by steam distillation of muscle over prolonged periods. Accordingly the longer a lobster is sterilised the greater the amount of hydrogen sulphide one would expect to have formed and since the chemical cause of discoloration is the formation of iron sulphide, it is reasonable to suppose that the longer the lobster is processed the greater will be the resulting discoloration provided, of course, that an excess of iron is present and that the reaction of the medium is sufficiently alkaline to permit the formation of the iron sulphide. Hence to test this theory cans 5-8 Table 8 were processed at a temperature of about 242°F. for 180 minutes. The appearance of the meat and the pH of the pickle on opening are recorded in Table 8. The flavour of these cans was particularly poor and the flesh was not nearly as firm as the controls. It will be observed that the pH of these cans has risen to a value almost as high as those to which 1.8 cc. of 1.02N ammonia had been added, and this, coupled with the fact that much larger amounts of hydrogen sulphide would be liberated by the long sterilization, may account for the increased discoloration of these cans being quite obviously in a worse condition than numbers 9-12 in spite of a somewhat higher pH on the part of the latter.

Change in pH of lobster muscle

Table 8 although not conclusive would tend to confirm the suspicion that an alkaline medium will result in discoloration but does not of itself account for the fact that an increased proportion of cans are discolored if dying or inactive lobsters are used. Harrison and Hood have stated that the pH of lobster muscle may vary between the limits of 6.6 and 7.4 depending on whether the lobster was taken before or after molting. Similarly it was believed that some obscure physiological change might occur during the gradual loss of vitality of the lobster which would result in an increased alkalinity of the muscle and accordingly in an increased susceptibility to blackening.

This question was investigated from a statistical point of view. A number of lobsters generally 6 were either trapped or purchased, 2 of which were boiled immediately, 2 of which were held for about 24 hours on ice and the remainder for a somewhat longer period. The pH of the muscle was determined by boiling the lobster for 5 minutes, mincing and allowing the muscle to stand in distilled water for 15 minutes, then filtering and determining the pH of the filtrate colorimetrically. Table 9 records the pH values of lobsters immediately after procuring and after varying periods on ice. As will be observed several lots of lobsters were purchased from wholesalers and the history of these prior to receiving them in the laboratory is not known. It is possible, however, that the pH of these particular lobsters was somewhat lower just after trapping than they were when examined.

TABLE 9

pH of lobster muscle after varying periods on ice

Lot No.	pH when received	pH after 18 hours on ice	pH after 24 hours on ice	pH after 48 hours on ice
1 Purchased	7.20 7.00		7.7 7.3 (dead)	7.9
2	7.25	7.75 7.60		7.30 (dead)
3	7.20	7.30		
4	(1) 6.95 (2) 6.80	(3) 7.10	(4) 7.15	
5 Purchased	(1) 7.40 (2) 7.15		(3) 7.45	(4) 7.70
6 Purchased	(1) 7.30 (2) 7.35		(3) 7.20(dead) (4) 7.30(dead)	(5) 7.20 (dead)

Since the above table is the record of statistical experiments where the number of lobsters examined was rather limited, one might not be justified in drawing the conclusion that the pH of muscle rises during the loss of vitality yet the figures reported would lend support to this suggestion. This hypothesis is also supported by Table 7 where the pH of the pickle from lobsters which were canned by Cook and Hess after holding for various periods on ice is recorded. Accordingly it appears that the greater number of discolored cans which result from processing non-active but living lobsters might be accounted for on the grounds of an increased alkalinity.

Volatile bases from vigorous and inactive lobsters

If the pH of inactive lobsters increases as has been indicated by Table 9, two possible mechanisms among others might account for this result. There may be an increase in the volatile bases owing to some form of autolysis during the gradual loss of vitality of the lobster or there may have been a decrease in the amount of lactic acid in the muscle. The latter of these assumptions appears to be the more probable in view of the fact that during exercise considerable quantities of lactic acid make their appearance in the muscle and again disappear during rest. Accordingly, since a lobster living under natural conditions moves about considerably its muscles might conceivably have a much higher lactic acid content than one which had been placed on ice and remains perfectly still. Unfortunately time did not permit of an experimental verification of this assumption; evidence is available, however, in Table 10 to show that the former of the mechanisms suggested will not account for any increase in the pH of muscle from an inactive lobster. The following table shows the volatile bases liberated by lots 4, 5 and 6, the pH of which are recorded in Table 9.

TABLE 10

Volatile bases from vigorous and inactive lobsters

Lot No.	Mmg. vol. N. per g. muscle lobster No.1	Mmg. vol. N per g. muscle lobster No.2	Mmg. vol. N. per g. lobster No.3	Mmg. vol. N. per g. lobster No.4	Mmg. vol. N. per g. lobster No.5
4	0.213	0.159	0.191	0.182	
5	0.110	0.098	0.073	0.091	
6	0.115	0.140	0.155	0.140	0.157

The lot number and lobster number refer to the same lobsters whose pH values are recorded in Table 9. It is evident from a comparison of Table 9 with Table 10 that no correlation can be established between the pH of the muscle and the amount of volatile base liberated by that muscle. Accordingly any increase in pH of muscle which results from prolonged tenure on ice must be attributed to some cause other than the development of volatile base and possibly to the disappearance of lactic acid from the inactive muscle.

Volatile bases from normal and discolored canned lobster

A number of cans of normal and discolored lobster were examined with the object in view of attempting to correlate the appearance of the canned muscle with the amount of volatile base present. The first series examined were packed at Point Du Chene in enamelled cans on August 28, 1930, and opened September 10, 1931. The meat in all cans of this series was in good condition and the pH of the pickle below 7.0. The second series examined were packed in plain cans by Cook and Hess during the summer of 1930 and opened in September, 1931. The pH of the pickle, appearance of the meat and history of these lobsters are shown in Table 7.

TABLE 11.

Volatile bases from normal and discolored canned lobster

Series No. 1			Series No. 2		
Can	pH of pickle	Mmg. of vol. N. per g. of muscle	Can	pH of pickle	Mmg. of vol. N. per g. of muscle
1	6.50	0.312	2	pH as indicated in Table 7	0.905
2	6.50	0.248	3		0.74
3	6.35	0.332	6		0.60
			10		0.56
			14		0.84
			18		0.72
			24		0.76
			13		0.57
			17		0.63

From the above table it is evident that Series 2 which was for the most part discolored, liberated considerable more volatile base than Series 1 which was quite normal. However, as Series 1 was packed in enamelled cans and as the pH of these cans was much lower than Series 2 and since both Series were probably not processed at the same temperature for the same period, it is doubtful if this comparison is of any real significance. Comparing the cans of Series 2 with one another it is apparent that no correlation can be drawn between the appearance of the flesh as recorded in Table 7 and the amount of base liberated.

SUMMARY

1. Active lobsters and lobsters which have become inactive through varying periods on ice were examined both before and after cooking for volatile sulphur. No correlation could be demonstrated between loss of vitality and the quantity of volatile sulphur present in the muscle. This confirms the findings of Cook.

2. A limited number of normal and discolored cans were examined for volatile sulphur. No correlation could be established between the condition of the meat and the quantity of volatile sulphur found.

3. The pH of a number of normal and discolored cans was determined. It was found as a general rule that the more highly discolored cans had the higher pH.

4. A number of normal and discolored cans were quantitatively examined for volatile bases. As a rule it was found that the discolored cans had about twice as much volatile base as the normal cans. However, for reasons given in the context it is doubtful if this comparison is of much significance.

5. The effect of artificially increasing the pH of muscle prior to sterilization was determined. This experiment was not decisive but indicated an injurious effect in so far as the appearance of the meat was concerned.

6. The effect of prolonged sterilization was found to be definitely injurious both to the flavour and to the appearance of the meat. The pH of these cans was considerably higher than the controls, owing to hydrolysis produced by the long sterilization.

7. The results obtained from a statistical experiment carried out with a limited number of lobsters suggest that the pH of the flesh increases when the lobster is inactive.

8. No increase in the volatile bases was observed when the lobsters were held on ice and accordingly it is suggested without any experimental verification, however, that the increase in pH may arise from a disappearance of lactic acid from the muscle of the resting lobster.

In conclusion it must be stressed that before summaries 5 and 7 can be definitely accepted these experiments must be repeated, since the experiment on which No. 4 was based was not satisfactory owing to the fact that the final pH difference between the experimental and control was not sufficiently great. On the other hand summary No. 7 is based on a statistical experiment and as such is insecure unless confirmed with a much larger number of lobsters and preferably lobsters which have just been taken from the ocean so that their history from the time of trapping is known. It is further suggested that the data appearing in Table 11, be rechecked under more carefully controlled conditions, that is, lobsters sufficient to pack say 2 dozen cans should be trapped, one half of which would be canned at once, and the second half after a period of say 36 hours on ice, being careful to process both series at the same temperature for exactly the same periods. Then after a period of from 6-9 months storage both sets should be examined with reference to the appearance, pH of the pickle, and volatile bases, thus attempting to establish or disprove any correlation between appearance of the muscle and the extent of hydrolysis.

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