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CARL L. ALSBERG, Chief

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A STUDY OF SOME OF THE CHEMICAL CHANGES WHICH OCCUR IN OYSTERS DURING THEIR PREPARATION FOR THE MARKET.

By EDWARD E. SMITH, *Junior Chemist*.¹

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SCOPE OF INVESTIGATION.

This bulletin gives the results of an investigation to determine the amounts of ammoniacal nitrogen, amino-acid nitrogen, moisture, total solids, ash, and sodium chlorid present in oysters under the various conditions through which they pass in ordinary commercial practice in the oysterhouse, and to ascertain the effect of washing and soaking on both the chemical composition and physical condition of the oysters. The investigation was conducted during the fall and winter of 1914-15, in certain oysterhouses in Connecticut, selected as being representative of the oyster industry throughout the North Atlantic States. The oysters used were grown in various beds, ranging from New Haven, Conn., on the east, to Raritan Bay, N. J.; on the west. As work was done in several houses, each using a different method of treatment of the stock, the methods given represent commercial practice at that time and in that locality, but may not be representative of later practice or other localities. The experiments which were made to show the results of various special treatments do not represent commercial practice.

¹ The writer wishes to express his indebtedness to Dr. E. D. Clark and Mr. L. H. Almy, who instructed him in the methods of determination of ammoniacal nitrogen and amino-acid nitrogen; to Mr. Carleton Bates and Dr. Lester Round, who assisted materially in planning the work and under whose direction it was carried out; and especially to Mr. R. W. Lamson, without whose help Series V would have been impossible.

COMMERCIAL TREATMENT OF OYSTERS.

As a slight knowledge of the methods of handling oysters is necessary for a clear understanding of the various subjects touched upon in this bulletin, a brief outline of the essential features involved in the preparation of oysters for the market, beginning with the dredging of the mature oysters, that is, those which are three or four years of age, will be given first.

Oysters are grown in water of a salinity ranging from 1.5 to 3.5 per cent, calculated as sodium chlorid, the average being about 2.5 per cent, and are covered to a depth of from 5 to 50 feet at mean low tide. In many cases the beds are so far from the oysterhouse that upon arrival the oysters may have been out of water for several days. For the beds near the house the period is shortened to a few hours. "Shucking" oysters, the process of opening the shells and removing the meats therefrom, is always done by hand, and those engaged in the work are called "shuckers." The shucker puts the oyster meats into a perforated dipper holding 1 gallon. From the shuckers' dippers the oysters are emptied upon the "riffles," inclined, corrugated metal boards, down which a thin stream of water runs. The slight bumping caused by the corrugations tends to remove the bits of shell which may cling to the body of the oyster, and the water both hastens its progress and washes off most of the dirt which it has acquired during the process of opening. From the riffles the oysters slide through metal chutes to the sorting table, where they are graded and those not fit for food are culled out. In Connecticut the following grades of oysters are recognized: (1) "Straights," the name given to the entire output of the plant, not graded, with only those unfit for food removed; (2) "counts," very large oysters, not mutilated in opening, perfect in shape and color; (3) "selects," smaller than "counts," but larger than the ordinary run of stock, un mutilated and perfect in shape and color; (4) "standards," the ordinary run of stock from which the "counts," "selects," and "culls" have been removed.

From the sorting table the oysters slide down metal chutes to the washing and chilling tanks, where they are thoroughly washed in running water, and chilled by means of ice, if the weather is warm enough to require it. The method of washing and chilling oysters had changed materially in the years immediately preceding the time of this investigation, and, instead of only one method, several methods were in use. The old method of preparing shucked oysters for the market consisted in washing the stock as received from the shucker upon a perforated "skimmer board," by means of running water from a hose, the oysters being moved about with a paddle during the process. The oysters were then placed in tanks of ice water with chunks of ice, and chilled for a period of time not exceeding 20 or 30 minutes. Mechanical devices, however, had been invented

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for the washing and chilling of the shucked oysters, and in 1914-15 the air-agitation system of washing was in general use in the larger plants. In this system, from 20 to 30 gallons of oysters are placed in a tank of water, ice is added if necessary, and the whole is agitated by means of a blast of air blown in through the perforated bottoms of the tanks. A constant flow of fresh water is maintained. In this way a very thorough washing is secured. With the change in the method of handling, however, the oysterman did not modify the length of time for washing and chilling, which resulted in a great gain in the bulk of the oysters, due to osmotic action. For in the old method of washing, the oysters remained stationary in the bottom of the tank, their mass was slowly pervious to water, and osmosis could not take place to any extent, whereas in the latter method each individual oyster was constantly exposed to the action of fresh water for a period of from 20 to 30 minutes. As a result, the oysters lost a marked proportion of their soluble constituents, and due to the process of osmosis became greatly distended.

From the washing and chilling tanks, the oysters were run out upon "skimmers," which are perforated metal tables, to drain. They were stirred about upon the skimmers with metal paddles until they were thoroughly drained, then were run off into cans, which were immediately sealed and stored in a refrigeration room at a temperature of about 33° F. Stock was not usually kept in the refrigerator more than one or two days; in fact, it was usually shipped the morning after packing.

EXPERIMENTAL WORK.

METHODS OF ANALYSIS.

1. *Preparation of sample*.—The sample of oysters is drained in a colander for two minutes, with gentle shaking or stirring to facilitate drainage, the liquor being discarded. After draining, one pint of the drained meats is ground in a meat chopper, No. 2, using the next-to-finest blade, and thoroughly mixed. The required amount of sample is weighed out immediately, as evaporation causes serious errors in a short time.

2. *Amino-acid nitrogen*.—Weigh out 100 grams of the finely-ground oyster meat upon the rough balance, wash into a 500 cc volumetric flask, make up to the mark with distilled water, and shake thoroughly every 10 minutes for 1 hour. Allow to settle, and pour off the supernatant liquid. Upon this liquid the determination for amino-acid nitrogen is run according to Sørensen's method.¹

3. *Ammoniacal nitrogen*.—This determination was made by Folin's method,² aerating for exactly 2 hours, and using 20 grams of the

¹ Allen's Commercial Organic Analysis, 4th ed., 7:262.

² Am. J. Physiol., 1903, 8:343.

finely-ground oyster meats, weighed to within 0.1 gram. Tenth normal solutions of acid and alkali were used.

4. *Total solids*.—Place a thin layer of dried shredded asbestos on the bottom of a thin lead dish, add a short glass rod, and weigh the whole. Add about 5 grams of the ground sample, and weigh as quickly as possible. Evaporate to dryness on the steam bath, and dry for exactly 4 hours in a water-jacketed oven at the temperature of boiling water. Weigh, and calculate the percentage of total solids. During evaporation, the meat is stirred occasionally with the glass rod. This stirring with the asbestos prevents the formation of a crust which would hinder evaporation.

5. *Moisture*.—Determined by difference from the total solids results.

6. *Ash*.—Weigh about 5 grams of the finely-ground sample as quickly as possible into a previously ignited and weighed platinum dish or crucible, drive off moisture at a moderate heat, and ignite at the dullest red heat until the ash is gray in color. Extraction with water, followed by a second evaporation and ignition, may be necessary to secure a clean gray ash. Weigh, and report as percentage of original sample.

7. *Salinity*.¹—Extract the ash obtained in the preceding determination with hot water, cool, and titrate against silver nitrate solution containing 29.0575 grams of silver nitrate per liter of solution at room temperature. As indicator, use one or two drops of a 10 per cent solution of potassium chromate. One cc of this silver nitrate solution is equivalent to 10 mg of sodium chlorid. Calculate all chlorin as sodium chlorid, and report as percentage present in original sample.

SERIES I.

In this series, determinations for amino-acid nitrogen and ammoniacal nitrogen only were run. A summary of results is given in Table 1.

The column headed "Shucker" represents oysters as taken from the shuckers' dippers; "Riffle," oysters taken from the chute below the riffle-boards; "Commercial package," oysters after washing and chilling, packed into cans ready to go into the refrigerator; "Refrigerator," oysters from the same day's run as those upon the same line in the preceding columns, but sampled from the cans remaining in the refrigerator one or two days later.

¹ These results are not exactly accurate, due to the fact that all the chlorin is not in the form of sodium chlorid. As no analysis for oyster ash could be found in the literature, and there was no time to make such analyses, the plan of calculating all the chlorin as sodium chlorid and reporting it as salinity was adopted. Since all the data have been prepared on this basis, the results are comparable, and the error probably is negligible, as the amount present is small in any case, and it is certain that a large proportion of the chlorin is present in the form of sodium chlorid.

TABLE 1.—Percentages of nitrogen obtained at various stages of handling.

Date.	Amino-acid nitrogen.				Ammoniacal nitrogen.			
	Shucker.	Riffle.	Commer- cial package.	Refriger- ator.	Shucker.	Riffle.	Commer- cial package.	Refriger- ator.
1914.	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Oct. 5	0.183	0.166	0.128	0.120	0.00200	0.0018	0.0012	0.0025
6	.173	.159	.1267	.085	.00189	.0015	.0015	.0004
8	.171	.1495	.115	.146	.00042	.0004	.0000	.0010
10	.148	.149	.106	.116	.00074	.0011	.0000	.0022
12	.155	.152	.106	.089	.00154	.0011	.0004	.0000
13	.1658	.1534	.1183	.1237	.0007	.0006	.0007	.00077
14	.1439	.1433	.1142		.0007	.0007	.00042	
15	.1551	.1512	.1232	.1378	.00077	.0003	.00024	.00087
16	.1580	.1542	.1198		.00038	.0005	.0000	
Nov. 9	.1522	.1582	.1249	.1272	.0000	.0000	.0000	
10	.1484	.1400	.1215	² .0946	.00032	.0002	.00115	² .00024
11				.1210				.00017
12	.1703	.1670	.1064	.1232	.00021	.00007	.0000	.00073
16	.1813	.1692	.1305		.00098	.00021	.00073	
17			.1311				.00073	
18				.1540				.00035
21				.1540				.00035
.....	.1932	.1727	.1318	² .1084	.00084	.0002	.00098	² .00031
.....			.1429				.00056	
.....	.1635	.1585	.1204		.00049	.00011	.0000	
.....	.1652	.1630	.1142		.00014	.0000	.00024	
.....	¹ .1658	¹ .1429			¹ .00053	¹ .00025		
.....		² .1170				² .00025		
Dec. 10	.1739	.1558	.1188	.1177	.00014	.0000	.00032	.0000
11	.1456	.1479	.1367	.1177	.00035	.00007	.00007	.00011
12	.1702	.1685	.1317	.1250	.00014	.0000	.00008	.00011

¹ Selects.

² Counts.

From this table it is apparent that a considerable amount of amino-acid nitrogen is present in the oyster as it comes from the shell. It is probable that a certain percentage of amino-acid nitrogen exists in the tissues of the oyster body, being either absorbed or formed in the process of metabolism. Some of this may be due to bacterial activity within the oyster itself.

The comparatively slight washing of the oyster upon the riffle board removes approximately 10 per cent of the amino acids present, which shows that a certain proportion of them are present in the mucus surrounding the oyster. An additional amount of approximately 25 per cent is removed in the washing and chilling process proper, which may be explained partly by solution in the wash water, partly by the fact that most of the remaining mucus is washed from the outside of the oyster, and partly by the increase in volume of the oyster due to osmosis.

From the figures in the "Refrigerator" column it is seen that the relative figures vary widely. In some cases the percentage of amino-acid nitrogen apparently increased, in others remained constant, and in still others decreased. This variation is due largely to difference in stock used, as it was found impossible, in a large plant, to be sure that the oysters stored were from exactly the same stock as those analyzed in the preceding columns. However, if the amino-acid content be taken as indicative of decomposition, it is evident that

no appreciable amount takes place while the oysters are stored in the refrigerating room of the oysterhouse for a short time. It is interesting to note that the figure for the larger oysters, selects and counts, is uniformly smaller than that for the standards of the same stock.

From the results obtained in this series, there appears to be no relation between the amino-acid nitrogen and the ammoniacal nitrogen present. In general, the figures for ammoniacal nitrogen change in the same way as do those for amino-acid nitrogen; but the former are so inconsistent, even in duplicates run on the same sample, that the whole table is practically meaningless in this connection. Similar determinations were run in all the following series of experiments, but as they are in all cases as conflicting as those in Tables 1 and 2, they have been omitted from all subsequent tables.

SERIES II.

In order to prove that the amount of amino-acid nitrogen and ammonia present would not indicate decomposition in oysters if the water over them was changed occasionally, the following series of experiments was run.

A 5-gallon can of oysters was divided into two lots, and the oysters in each were covered with water. One lot was stored at room temperature, the other in a small ice box at a temperature of about 45° F., and the water over each lot was changed every day. Determinations were run upon each lot every day for one week. A summary of results obtained is given in Table 2.

TABLE 2.—*Amino-acid and ammoniacal nitrogen as index of decomposition.*

Date of analysis.	Amino-acid nitrogen in portion stored at—		Ammoniacal nitrogen in portion stored at—	
	Room temp.	45° F.	Room temp.	45° F.
1914.	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Oct. 5.....	0.1290	0.1290	0.00123	0.00123
Oct. 6.....	.1280	.1280	.00116	.00116
Oct. 8.....	.1244	.1272	.00329	.00259
Oct. 9.....	.1075	.1120	.00165	
Oct. 10.....	.1250	.0687	.00392	.000105
	.1250	.0676	.00185	.000098
	.1215	.0638	.00473	.000840

At the end of this time the oysters soaking at room temperature were badly decomposed. In spite of this condition, the amino-acid nitrogen content decreased, and the figure for ammoniacal nitrogen, though larger than in the same oysters at the beginning of the experiment, was smaller than that found in several samples of fresh oysters. The oysters stored in the refrigerator kept much better than those stored at room temperature, though they also were slightly decom-

posed at the end of the week. In this lot, the values for both amino-acid and ammoniacal nitrogen were smaller at the end than they were at the beginning of the experiment.

Of the two determinations intended to show the amount of decomposition which has occurred in oysters, it appears that determination of ammoniacal nitrogen by the Folin method is worthless in commercial oyster work for the following reasons:

- (a) The free ammonia and ammonium salts are washed out of the oysters nearly as fast as they form.
- (b) The very small amount of standard acid neutralized by the ammonia makes the probable error relatively large.
- (c) With alizarin red as an indicator, the color change obtained is not sharp, under such conditions.

For the last two reasons, duplicate determinations often varied widely, and no great reliance is to be placed upon the accuracy of the determinations; hence, very little weight should be attached to the meaning of the results obtained. Often higher ammoniacal nitrogen figures were obtained from fresh oysters than from others which had undergone decomposition.

It is believed that the amino-acid nitrogen determination is much more consistent and reliable. It is more consistent in that a larger amount of solution is taken for titration, which lessens the probable error, and in that the end point is easier to locate. The principal objection to this determination is that the amino-acid nitrogen content is not a true indication of the amount of decomposition which has taken place in the oysters, for the following reasons:

- (a) Amino acids, being soluble, are washed out of the oysters by each change of water in the same way as are the ammonia and its compounds.
- (b) Amino acids present a very suitable medium for bacteriological growth, and are, therefore, being continuously broken down into some of the simpler compounds already mentioned. Therefore, the amount of amino acids present is never a measure of the total amount of decomposition, and in many cases is a measure of only a very small percentage of it. However, the results obtained on oysters undergoing washing treatments are comparable, and are of great value in indicating the amount of soluble matter removed.

In the study of washing oysters, the greatest reliance is to be placed upon the determinations for amino-acid nitrogen, total solids, and ash. The figures for moisture are very useful in showing the amount of water added to the oysters in the washing processes. The determinations for salinity were of little assistance, as the salt was removed quite completely in a comparatively brief period of washing.

SERIES III.

Since the amino acids present in oysters had been regarded as decomposition products, it was assumed that their amounts would be at a minimum in oysters fresh from the beds, and would increase with the length of time the oysters were out of the water. As some of the experiments indicated that this was not the case, the following series of experiments was carried out.

By means of a portable apparatus carried aboard an oyster boat, amino-acid determinations were run upon liquors and meats of oysters as they came from the water at Princess Bay, New York. Two bushels of the same stock were transported to the laboratory at South Norwalk the same night. Determinations were run upon representative samples of this lot every day for six days, the oysters being shucked in the laboratory immediately before analysis. On the last day, analyses were also run upon a lot of shell oysters from the same locality, which had been stored in the laboratory in baskets for four weeks. The results of this series are given in Table 3.

TABLE 3.—*Percentage of amino-acid nitrogen present in meats and liquors of shell oysters, and its variation with age.*

Date.	Meats.	Liquors.	Remarks.
1914.	<i>Per cent.</i>	<i>Per cent.</i>	
Nov. 30.....	0.1938	0.0588	Perfectly fresh.
	.1970	.0625	Oysters immediately after dredging.
	.1976	.0598	
Dec. 1.....	.1640	.0336	
	.1626	.0353	
2.....	.1709	.0280	
	.1709	.0263	
3.....	.1737	.0224	
	.1647	.0258	
4.....	.1645	.0380	
	.1594	.0342	
5.....	.1651	.0302	
	.1651	.0300	
5.....	.1720	.0370	Oysters from same locality stored in laboratory 4 weeks.
	.1728	.0377	

This series leaves no doubt that the percentage of amino acids in perfectly fresh oysters is much greater than has been supposed. This value decreases to a minimum in the first day or two that the oysters are out of water, and remains practically constant as long as the oysters remain in the shell but do not lose shell liquor. Shortly after losing the shell liquor from any cause death ensues with attendant decomposition.

As no further work was done on this very interesting point, no conclusions can be drawn at this time. This series seems to indicate, however, that some reason other than decomposition must be sought to explain the presence of amino acids in fresh oysters.

SERIES IV.

In this and the following series, determinations for total solids, ash, moisture, and salinity were made, in addition to those for ammoniacal and amino-acid nitrogen, as those were found to be the most satisfactory determinations for showing the effects of commercial washing processes. These determinations were run upon oysters in all the various stages of preparation in several oysterhouses. The results are shown in Table 4.

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TABLE 4.—*Effect of washing.*

WASHING BY TANK METHOD, WITH AIR AGITATION.

Date.	Experi- ment No.	Amino-acid nitrogen.			Total solids.			Moisture.			Ash.			Salinity.		
		Shell.	Shucker.	Com- mercial package.	Shell.	Shucker.	Com- mercial package.	Shell.	Shucker.	Com- mercial package.	Shell.	Shucker.	Com- mercial package.	Shell.	Shucker.	Com- mercial package.
1915. Jan. 15	1	Per ct. 0.1650	Per ct. 0.1660	Per ct. 0.1232	Per ct. 22.3	Per ct. 24.7	Per ct. 16.4	Per ct. 77.7	Per ct. 75.3	Per ct. 83.6	Per ct. 1.67	Per ct. 1.88	Per ct. 1.17	Per ct. 0.36	Per ct. 0.37	Per ct. 0.02
	3	.1600	.1615	.1025	21.7	23.0	15.8	78.3	77.0	84.2	1.79	1.82	.71	.52	.54	None.
	26	.1630	.1740	.1280	21.0	22.0	14.0	79.0	76.4	86.0	1.80	1.74	.74	.65	.61	None.
	6	.1336	.1606	.1290	21.4	21.4	15.3	78.6	78.6	84.7	1.75	1.75	.87	.56	.63	Trace.
Feb. 5	12	.1370	.1520	.1185	20.8	19.2	15.3	79.2	80.8	84.7	1.55		1.15	.55	.95	0.17
	16	.1476	.1684	.0873	16.7	18.9	15.2	83.3	81.1	84.8	1.95	1.85	.62	.58	.50	None.
	23	.1723	.1748	.1242	17.1	20.7	16.4	82.9	79.3	83.6	1.89	1.95	.94	.62	.60	0.03
	26	.1535	.1571	.1087	19.2	20.9	16.2	80.8	78.5	86.3	1.89	2.10	1.81	.63	.63	.05
Mar. 2	13	.1542	.1675	.0826	20.2	21.4	14.8	79.8	78.6	85.2	2.00	2.03	.71	.74	.63	None.
	5	.1723	.1748	.1242	19.0	21.3	12.6	81.0	78.7	87.4	1.64	1.63	.77	.52	.52	None.
	9	.1605	.1747	.1100	19.5	20.3	15.3	80.5	79.1	84.6	1.84	1.88	.82	.65	.52	None.
	12	.1597	.1635	.1176	18.7	22.6	15.5	80.8	77.4	84.5	1.64	1.98	.96	.41	.64	0.04
.....		.1566	.1662	.1130	19.2	19.8	15.1	80.6	80.2	84.9						
					19.79	21.25	15.06	80.21	78.75	84.94						

It had been assumed previously that the composition of oysters in the shell would be identical with that of oysters taken from the shuckers' dippers. Table 4 shows that this is not the case, that there is a slight but well-defined difference in composition. The percentage of total solids and other constituents except water is greater in the samples from the shuckers than in the sample of the same stock shucked in the laboratory immediately before analysis, while the percentage of water is correspondingly less. This difference probably is due to the fact that the oysters drain for half an hour or more while in the shuckers' dippers, and tend to lose a certain amount of their body fluids, shell liquor, and adhering mucus. The shell stock, being opened in the laboratory, had had less time in which to lose water, and, therefore, was always poorer in solids. This marked difference in composition between oysters which it would seem at first glance should be of identical composition, shows very plainly that the stock for each determination must be handled in exactly the same way every day if the results are to be comparable.

Table 4 is of great interest in showing the composition of oysters during the various stages of progress through the oysterhouse, and it shows also in a very striking manner the difference between the old and the new methods of washing oysters, to the discredit of the new methods, if amount of total nutrients is a proper criterion. With the purpose of plainly showing this difference, the table has been arranged in two parts, the analyses of oysters washed by the modern method of air agitation in tanks and the analyses of stock washed by the old method upon a perforated skimmer.

Of the substances determined, amino-acid nitrogen and total solids are taken for comparative purposes, as they are the most consistent determinations made and most clearly represent the condition of the oysters examined. The amino-acid nitrogen represents a truly soluble constituent which would be dissolved out of the oysters by the wash water. The total solids, though including most or all of the amino-acid nitrogen, are in the main insoluble substances whose apparent diminution in quantity is due largely to the distention of the bodies of the oysters because of osmosis and loss of particles of solid meat in handling or washing. A glance at the average values of amino-acid nitrogen and total solids for the two classes of washing shows that the content of both is much higher in oysters washed by the old method than in those washed by the modern one. A brief analysis of the results will show exactly how the two sets of figures compare. Taking the amount of each of these substances present in the shell stock as a basis upon which to calculate the losses, this figure may be called 100 per cent. Thus, the amounts of each of the constituents are expressed in percentages of the amount present in the same stock while in the shell. For the shucked stock and the commercial package, then, the figures in Table 5 are obtained.

TABLE 5.—Average values of amino-acid nitrogen and total solids of oysters washed by both methods.

(Shell stock, 100 per cent.)

Determination.	Old method.	New method.
	<i>Per cent.</i>	<i>Per cent.</i>
Shucker:		
Amino-acid nitrogen.....	104.8	106.2
Total solids.....	109.4	107.3
Commercial package:		
Amino-acid nitrogen.....	95.0	72.2
Total solids.....	94.0	76.0

Considering the comparatively small number of determinations made, and the multiplication of error involved in expressing the various fractions as percentages, the figures in Table 5 are very satisfactory checks. They show that in the old method of washing the finished product contains, in a given volume, about 94 per cent of the food value possessed by the same oysters in the shell, whereas in the modern method of washing this figure falls to a percentage of about 74 per cent.

This series, however, does not indicate in any way the cause of the loss, as the amino acids disappear at the same rate as do the total solids. This loss may be due almost entirely to osmosis, or it may be due in part to solution in the wash water of soluble parts of the oyster, or to both. That there is a loss of soluble matter in the wash water there is no doubt. This is proved by the complete disappearance of sodium chlorid after a comparatively short period of washing. Sodium chlorid and other crystalloids would wash out much faster than the soluble protein compounds, which are colloids, but if the sodium chlorid disappears it is certain that other soluble constituents tend to disappear also.

SERIES V.

In order to determine, if possible, just what proportion of the apparent loss of the various constituents is due to osmotic distention and what proportion is due to solution in the wash water, and also the general chemical effect of all kinds of oyster washing, a series of experiments was run in which measured volumes of oysters were subjected to various washing processes and were both measured and analyzed at various stages of washing. The results of this series are given in Table 6, and a mathematical analysis of the results obtained is given in Table 7.

Experiment 1.—Five gallons of standards were taken from the chute immediately before they entered the agitation tank, carefully measured, put into an equal volume of water, stirred thoroughly, and allowed to stand. Measurements were made of both water and oysters every 6 hours for the first day, and every 12 hours thereafter for 3 days. The water was renewed after each measurement. Chemical determinations were made once a day upon samples taken in the

morning. In all these tables allowance has been made for all samples removed. Due to the fact that the oysters settled to the bottom of the tank and formed a compact mass which was almost impervious to water, very little opportunity was offered for osmotic action to take place, and the volume of the oysters remained practically constant throughout the experiment. In the periods of disturbance, however, which occurred while the oysters were being measured and the water was being removed fresh water came into contact with the oysters, and some of it remained with them in the bottom. Thus, a little osmotic action other than that at the top of the mass took place, and solution progressed continuously, though somewhat slowly. Since the volume remained practically constant throughout this experiment, it is evident that all the loss observed in this case must be due to solution or mechanical loss. In this case mechanical loss was small. In commercial practice oysters never are subjected to such long continued action of water, but this experiment proves that a large percentage of the substance of the oyster is soluble in fresh water, and that osmotic distention is negligible in oysters washed in a tank without agitation. As is to be expected, the purely soluble constituents show a much more marked decrease than do the total solids, which include both soluble and insoluble substances.

Experiment 2.—This experiment was carried out in the same way as was Experiment 1, with the same purpose in view. On the third day, however, the samples were taken from the bottom of the tank instead of from a representative sample of the whole, as was usually done, in order to determine whether or not the bottom oysters were being affected by the water. The results show very clearly that they were not being so affected, as the percentages of total solids, etc., did not change during 24 hours of soaking. The percentages show the same relative decrease as in Experiment 1.

Experiment 3.—Having established the fact that a marked proportion of the solid substance of the oyster is soluble in fresh water, it becomes desirable to discover what proportion of the apparent loss of the various substances is due to actual loss in the wash water, and what is due to the increase in volume of the oyster. In this experiment, 20 gallons of oysters were taken from the chute immediately before entering the agitation tank, carefully measured into one of the tanks, and washed in the regular way, except that the washing was continued for one hour. Samples were taken before the process was started and at 15-minute intervals during washing. Detailed discussion of this and following experiments will be taken up later in connection with Table 7.

Experiment 4.—This experiment was conducted in an oysterhouse in which the old system of washing was used. Five gallons of shucked oysters were measured out and placed upon the skimmer.

They were then washed for about 5 minutes by means of water from a 1½-inch hose, the oysters being constantly stirred about with a wooden paddle. They were then drained and packed for shipment. This represents regular commercial practice in this particular oyster-house.

Experiment 5.—This experiment was conducted in a plant which uses the old method of washing, but in which the oysters are subjected to the action of water in a tank, with occasional agitation by means of a paddle, for 20 or 30 minutes, then drained upon a skimmer. Seven gallons of oysters were placed in the tank and treated as already described.

Experiment 7.—This experiment was run in a tank, but, instead of air agitation, the oysters were stirred constantly by means of a large metal paddle. They were drained, measured, and replaced in the tank at such intervals that the period of washing between measurements was 10 minutes. Samples were taken before washing was begun, and each time the oysters were measured. Washing was continued until the oysters began to lose markedly in volume, which was observed after 50 minutes of washing.

Experiment 8.—This experiment was conducted in the same manner as Experiment 7, except that it was done in a different oyster-house and that the regular air-agitation method was used.

Experiment 9.—The object of this experiment was to determine whether or not oysters lose appreciably in volume on account of repeated handling and measurement as done in the preceding experiments. Six gallons of drained oysters were placed in one tank and washed for 30 minutes by the regular method, with air agitation. Seven gallons of the same stock were washed in the adjoining tank with air agitation, but were drained and measured at 10-minute intervals, as in the foregoing experiment.

Experiment 10.—Having shown that handling oysters during washing has a marked effect in decreasing the osmotic distention and increasing the actual loss of substance, it was decided to run an experiment to compare the volumes and chemical composition of oysters washed and handled under conditions identical except for the time of washing. This experiment was also designed to find out what the maximum of osmotic distention would be. Five gallons of drained oysters were placed in each of three adjoining tanks and washed with the regular water current and air agitation, Tank A for 30 minutes, Tank B for 60 minutes, and Tank C for 90 minutes. A representative sample of the whole lot was taken before the washing was begun, and from each tank at the expiration of its period of washing.

Experiment 11.—This experiment was made under regular shipping conditions, and was simply a measurement and analysis of

oysters before and after washing with the regular water current and air agitation for 30 minutes.

Experiment 12.—This experiment is of the same nature as the preceding one, simply measurement and analysis of a commercial shipment before and after washing with regular water current and air agitation.

Experiment 13.—This experiment was carried out in the same manner as was Experiment 12, except that enough dairy salt was added to the wash water to make the salinity of the solution 2.53 per cent. The water over the beds where the oysters were grown varied from 2.48 to 2.70 per cent salinity, so no osmosis should take place. The object of this experiment was to test the correctness of the belief commonly held that all the salt must be washed out of oysters to have them "keep." It has been observed since the beginning of the industry that oysters tended to spoil if the salt was not washed out to below the point where it could be tasted in the water; hence the spoiling was connected with the salinity. This phenomenon appears to be merely a coincidence, due to the fact that the putrefactive bacteria are washed out at about the same rate as is the salt.

Experiment 14.—The oysters in this experiment were washed upon a skimmer with a hose for 10 minutes, and were stirred constantly with a paddle.

Experiment 15.—In this experiment the oysters were washed in a regular agitation tank by the regular method, except that instead of being constantly agitated by a current of air they were stirred every 5 minutes with a large metal paddle. Thus, the oysters were affected by a constant stream of cold water from the bottom of the tank, and were thoroughly agitated six times during the experiment.

TABLE 6.—*Washing experiments (Series 5).*

Date.	Method of washing.	Experi- ment No.	Interval of examination.	Volume.	Increase in volume.	Amino- acid nitrogen.	Total solids.	Moisture.	Ash.	Salinity.
1915. Jan. 15	Stationary, in tank with equal volumes of water and oysters. Laboratory conditions. Fresh water added (waters renewed) after each measurement. No agitation except that caused by measurement.	1	At start.....	Liters. 19.15	Per cent.	Per cent. 0.1232	Per cent. 16.41 15.80	Per cent. 83.50 84.20	Per cent. 1.17	Per cent. 0.02
			After 6 hours.....	18.30						
			After 12 hours.....	19.50						
			After 24 hours.....	19.10		.0948	14.49	85.51	.87	None.
			After 36 hours.....	19.08		.0967	14.23	85.77		
19	Same method as No. 1, except that on third day samples were taken from bottom of tank, showing impermeability of mass of oysters.	2	After 48 hours.....	19.20		.0519	10.93	89.07	.50	None.
			At start.....	19.10		.1475	11.45	88.55	1.29	0.02
			After 12 hours.....	20.00	4.72	.1018	15.20	84.80	.83	Traces.
			After 24 hours.....	20.16	5.55	.1009	15.40	84.60		
			After 36 hours.....	20.44	7.01					
26	Mechanical washer, with air agitation and constant flow of water. Commercial conditions.	3	After 48 hours.....	20.36	6.60	.1054	15.60	84.40	.90	Traces.
			At start.....	20.40	6.80	.1040	15.50	84.50	.93	
			After 60 hours.....	20.40		.1647	18.50	81.50	1.07	0.14
			After 15 minutes.....			.1270	18.10	81.90	.66	.03
			After 30 minutes.....			.1238	15.20	84.80		
29	Washed on skimmer with hose and paddle. Commercial conditions.	4	After 45 minutes.....			.1015	15.50	84.50	.67	None.
			After 60 minutes.....			.1015	14.90	85.10	.65	None.
			After 60 minutes.....	116.06,	27.73	.0040	15.10	84.90	.65	None.
			At start.....	Gallons. 5			13.40	86.60	.56	None.
			From commercial package...	5	None.	.1605	13.20	86.80		
Feb. 9	Hand agitation in tank. Commercial condition.....	5	After 30 minutes.....	8.75	25.00	.0977	21.30	78.70	1.77	0.47
			At start.....			.1298	21.10	78.90		
			After 30 minutes.....			.1605	20.60	79.40	1.28	.17

TABLE 6.—*Washing experiments (Series 5)*—Continued.

Date.	Method of washing.	Experi- ment No.	Interval of examination.	Volume.	Increase in volume.	Amino- acid nitrogen.	Total solids.	Moisture.	Ash.	Salinity.
1915. 12	Washed with flowing water; agitated with paddle.....	7	At start.....	Liters. 10.53	Per cent.	Per cent. 0.1520	Per cent. 20.90	Per cent. 79.10	Per cent. 1.55	Per cent. 0.95
			After 10 minutes.....	11.55	9.68	.1355	20.70	79.30	1.47	.36
			After 20 minutes.....	12.03	14.24	.1185	17.10	82.90	1.15	.17
			After 30 minutes.....	12.86	22.12	.0973	16.20	83.80		
			After 40 minutes.....	12.73	20.89	.0836	16.40	83.60	.91	.00
			After 50 minutes.....	12.36	17.40	.0765	14.60	85.40	.66	Trace.
16	Regular commercial practice, with air agitation, except in measuring every 10 minutes.	8	At start.....	6.00		.1677	13.30	86.70	.66	None
			After 10 minutes.....	7.10	16.66	.1344	13.50	86.50	1.85	0.50
			After 20 minutes.....	7.41	23.33	.1062	21.50	78.50	.97	.06
			After 30 minutes.....	7.37	22.83	.0992	16.40	83.60	.79	None.
			After 40 minutes.....	7.26	21.07	.0874	15.20	84.80	.67	None.
							14.90	85.10	.62	None.
23	Mechanical washer, with air agitation: Tank A, 30 minutes continuous..... Tank B, drained, and measured at intervals.....	9	At start.....	6.00		.1534	13.60	86.40		
			After 30 minutes.....	7.37	22.80	.1068	13.70	86.30	1.95	0.60
			At start.....	7.00		.1534	20.40	79.60	.94	.026
			After 10 minutes.....	7.67	9.57	.1326	15.60	84.40	1.95	.60
			After 20 minutes.....	7.92	13.14	.1077	20.40	79.60	1.28	.19
			After 30 minutes.....	8.12	16.00	.0968	20.50	79.50	.92	.037
			After 40 minutes.....	8.23	17.57	.0794	16.90	83.10	.78	None.
			After 60 minutes.....	8.24	17.71	.0623	17.40	82.60	.67	None.
							15.80	84.20	.60	
							15.40	84.60	.60	

24	Washed in regular tanks, with air agitation: Tank A, 30 minutes.....	10	At start..... After 30 minutes..... At start..... After 60 minutes..... At start..... After 90 minutes..... At start..... After 30 minutes.....	5.00 6.32 5.00 7.08 5.00 7.37 5.00 6.08	 26.40 41.60 49.40 21.60	.1765 .1142 .1765 .0930 .1765 .0906 .1571 .1087	21.20 20.90 15.30 15.40 21.20 20.90 14.00 13.90 21.20 20.90 13.20 13.40 21.40 21.00 14.80 15.10 21.80 22.60 15.30 15.50 21.80 22.60 21.20 21.10 19.70 19.80 15.70 15.90 19.70 19.80 15.00 15.10	78.80 79.10 84.70 84.60 78.80 79.10 86.00 86.10 78.80 79.10 86.80 86.60 78.60 79.00 85.20 84.90 78.20 77.40 84.70 84.50 78.20 77.40 78.80 78.90 80.30 80.20 84.30 84.10 80.30 80.20 85.00 84.90	1.93 .92 1.93 .70 1.93 .69 2.10 1.81 1.88 .82 1.88 1.72 1.98 .93 1.98 .96	0.46 .03 .46 None. 0.46 None. 0.63 .05 .52 None. 0.52 .65 .64 None. 0.64 .04
26	Regular commercial shipment. Washed with water and air, 30 minutes.	11	At start..... After 30 minutes.....	5.00 6.08	 21.60	.1571 .1087	21.40 21.00 14.80 15.10 21.80 22.60 15.30 15.50 21.80 22.60 21.20 21.10 19.70 19.80 15.70 15.90 19.70 19.80 15.00 15.10	78.60 79.00 85.20 84.90 78.20 77.40 84.70 84.50 78.20 77.40 78.80 78.90 80.30 80.20 84.30 84.10 80.30 80.20 85.00 84.90	2.10 1.81 1.88 .82 1.88 1.72 1.98 .93 1.98 .96	0.63 .05 .52 None. 0.52 .65 .64 None. 0.64 .04
Mar. 9	Regular commercial shipment. Washed with water and air, 30 minutes.	12	At start..... After 30 minutes.....	7.00 9.50	 35.70	.1747 .1100	21.80 22.60 15.30 15.50 21.80 22.60 21.20 21.10 19.70 19.80 15.70 15.90 19.70 19.80 15.00 15.10	78.20 77.40 84.70 84.50 78.20 77.40 78.80 78.90 80.30 80.20 84.30 84.10 80.30 80.20 85.00 84.90	1.88 1.72 1.98 .93 1.98 .96	0.52 .65 .64 None. 0.52 .65 .64 None. 0.64 .04
9	Carried out in parallel with No. 12, except that wash water was 2.53 per cent salinity.	13	At start..... After 30 minutes.....	5.00 5.00	 None.	.1747 .1610	21.80 22.60 21.20 21.10 19.70 19.80 15.70 15.90 19.70 19.80 15.00 15.10	78.20 77.40 78.80 78.90 80.30 80.20 84.30 84.10 80.30 80.20 85.00 84.90	1.88 1.72 1.98 .93 1.98 .96	0.52 .65 .64 None. 0.52 .65 .64 None. 0.64 .04
12	Washed on skimmer with hose and paddle, for 10 minutes.	14	At start..... After 10 minutes.....	5.00 5.43	 8.60	.1635 .1222	19.70 19.80 15.70 15.90 19.70 19.80 15.00 15.10	80.30 80.20 84.30 84.10 80.30 80.20 85.00 84.90	1.98 .93 1.98 .96	.64 None. 0.64 .04
12	Washed in tank, with regular water current, but no air. Agitated by paddle, every 5 minutes.	15	At start..... After 30 minutes.....	6.00 7.25	 20.30	.1635 .1176	19.70 19.80 15.00 15.10	80.30 80.20 85.00 84.90	1.98 .96	0.64 .04

From Table 6 it is apparent that oysters lose a marked proportion of their total solids, and therefore their nutritive value, during the process of washing. In speaking of these losses the following terms are used:

- (a) "Apparent loss" denotes the entire loss shown by the analysis. This loss is due to both osmotic distention and solution or suspension in the wash water.
- (b) "Osmotic loss" denotes the loss due to osmotic distention of the tissues of the oysters. This is not an actual loss of substance, but a replacement by an equal amount of water.
- (c) "Actual loss" denotes the loss caused by solution or suspension of part of the oyster substance in the wash water. This loss is actual, as the substance thus dissolved or suspended goes to waste.

These losses have been calculated for the more important experiments, using as a basis the amount of each substance present in the shucked stock. The figures are tabulated in Table 7.

TABLE 7.—Losses expressed in percentages of original amounts present.

Experi- ment No.	Figure of osmosis. ¹	Amino-acid nitrogen.			Total solids.			Ash.		
		Appar- ent. ²	Os- motic. ³	Actual. ⁴	Appar- ent. ²	Os- motic. ³	Actual. ⁴	Appar- ent. ²	Os- motic. ³	Actual. ⁴
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
3.....	78.3	27.0	21.7	5.3	40.9	21.7	19.2	47.7	21.7	26.0
4.....	100.0	None.	None.	None.	5.6	None.	5.6	27.6	None.	27.6
5.....	80.0	24.6	20.0	4.6						
7.....	81.8	36.0	18.2	17.8	30.4	18.2	12.2	41.4	18.2	23.2
8.....	81.4	40.8	18.6	22.2	33.0	18.6	14.4	63.8	18.6	45.2
9-A.....	81.5	30.5	18.5	12.0	23.4	18.5	4.9	51.8	18.5	33.3
9-B.....	85.0	59.4	15.0	44.4	34.7	15.0	19.7	69.2	15.0	54.2
10-A.....	79.0	35.3	21.0	14.3	23.5	21.0	2.5	52.3	21.0	31.3
10-B.....	70.6	47.3	29.4	17.9	30.5	29.4	1.1	63.8	29.4	35.4
10-C.....	67.8	48.7	32.2	16.5	33.6	32.2	1.4	64.2	32.2	32.0
11.....	82.2	30.8	17.8	13.0	29.5	17.8	11.7			
12.....	73.7	37.0	26.3	10.7	30.6	26.3	4.3	56.4	26.3	30.1
13.....	100.0	7.8	None.	7.8	4.7	None.	4.7	8.5	None.	8.5
14.....	92.0	25.2	8.0	17.2	20.0	8.0	12.0	53.0	8.0	45.0
15.....	82.7	28.1	17.3	10.8	23.8	17.3	6.5	51.5	17.3	34.2
Averages..			17.60	14.30		17.46	8.59		17.46	32.77

¹ Percentage of original amount of each substance which would be present if the apparent loss were due to osmosis alone.
² The entire loss shown by analysis. This loss is due to both osmotic distention and solution or suspension in the wash water.
³ The loss due to osmotic distention of the tissues of the oysters. This is not an actual loss of substance, but a replacement by an equal amount of water.
⁴ The loss caused by solution or suspension of part of the oyster substance in the wash water. This loss is actual, as the substance thus dissolved or suspended is washed off and goes to waste.

Experiment 3.—Taking the amount of each substance present in the shucked stock as 100 per cent for a basis of calculation, it is seen that if the decrease in percentage of the various substances were due to osmotic distention alone each of these substances would have a value of 78.3 per cent at the end of the process, since the volume of the oysters increased from 90.86 liters to 116.06 liters. The actual figures obtained, however, are as follows: Total solids, 73

per cent; amino nitrogen, 59.1 per cent; ash, 52.3 per cent. Thus in this experiment the actual loss of total solids is only 5.3 per cent of the original amount present, probably soluble amino compounds and inorganic salts, while the actual loss of amino acids is 19.2 per cent and that of ash-forming substances is even greater, amounting to 26 per cent. As the osmotic loss is in each case 21.7 per cent, it is clear that the actual loss of total solids is relatively slight compared to it, while the actual loss of amino acids and other soluble constituents is as great as, or greater than, the osmotic loss. These figures do not represent commercial practice, however, for, in general, oysters are washed for about 30 minutes.

Experiment 4.—In this experiment the increase in volume and, therefore, the osmotic loss are zero. The actual loss of amino acids is also zero, and that of total solids is only 5.6 per cent. The actual loss of ash is rather high but is far lower than when the washing is done by the other method.

Experiment 5.—The results of this experiment show a gain in volume of 25 per cent. This makes the percentage figure for each substance 80 per cent and the osmotic loss 20 per cent. As the derivation of these figures already has been given in detail (Table 7), it will not be enumerated for this and the following experiments.

Experiment 7.—The maximum osmosis was observed at the end of 30 minutes of washing in this method. The maximum osmotic increase was 22.1 per cent, about 5 per cent less than that observed in the case of air agitation. As will be shown later, this lower value is due solely to the excessive handling inherent in this method of repeated measurement and not to any difference in the methods of agitation. The percentage of losses will be seen to correspond rather closely to those observed in the air-agitation experiments. It is interesting to note, however, that at the end of 40 minutes the loss of total solids stopped, probably because of shrinkage in volume caused by the excessive handling already mentioned. The percentage of amino acids, however, continued to fall. The ash was apparently reduced to a minimum, the remainder being practically all insoluble. The percentages in Table 7 are calculated from the results at the end of 30 minutes, so that they closely represent commercial practice.

Experiment 8.—In the calculated results of this experiment the 30-minute results are used, as these are representative of commercial practice. It will be observed that these results agree very closely with those obtained in Experiment 7, except in the case of ash. This difference is probably explained by the fact that the stock came from widely scattered beds, and the inorganic constituents were probably dissimilar.

Experiment 9.—These results show clearly that handling has a great effect in reducing the amount of increase. The contents of Tank A increased 22.8 per cent in volume in 30 minutes of continuous washing, while the contents of Tank B increased only 17.71 per cent in 60 minutes of equally vigorous but intermittent washing. The difference is caused by the intervals of draining and stirring about upon the skimmer, in which the oysters lose water.

Experiment 10.—From the results of this experiment it is apparent that oysters are capable of enormous distention and that the limit is not reached even with 90 minutes of washing. This experiment also seems to indicate that the great actual losses observed in Experiments 3, 7, 8, and 9-B were due to the excessive handling inherent in the method of measurement during washing. It is seen from this experiment that, even after 90 minutes' washing, the actual loss of total solids is comparatively slight and is probably accounted for almost entirely by the great actual losses of amino acids, ash, and salt. The osmotic losses, however, are very great, as 32.3 per cent of the total solids originally present have been replaced by water, and the apparent loss is 33.6 per cent. As in the hard northern oysters very little of this added water is given off in shipment, it is clear that the consumer may be paying oyster prices for a great deal of added water. In one case a 5-gallon commercial package of oysters which had been increased in volume 35.7 per cent was shipped to a market four days away, and arrived and was sold in apparently the best of condition. The meats were sound and plump, and the liquor above the oysters was only the usual thick, dense mucus.

Experiment 11.—These results are in accord with other results of similar experiments, showing a marked loss of solids. The ash figures are omitted, as the ash analysis showed a doubtful value which is not in accord with the other figures. It was too high, probably caused by the presence of a small fragment of shell.

Experiment 13.—The results of this experiment are very interesting, in that they show the increase in volume of oysters during washing to be due purely to osmosis, and the soluble parts of the oysters to be less soluble in salt water than in fresh.

Experiment 14.—This represents commercial practice in some oysterhouses, and the results of this experiment are comparable with those of Experiment 4, except in that the washing was continued for 10 instead of 5 minutes. A comparison of the two sets of results shows that the losses are much greater in the second case, as is to be expected. As has been pointed out, in this type of washing some of the solid matter of the oysters is forced through the perforations of the skimmer during washing and is thus lost.

Experiment 15.—By comparison of the results of this experiment with those of Experiment 9-A, which was run on the same kind of

stock but with air agitation, it is seen that they are in very close agreement throughout. This shows that the air has little or no effect as such, its effect being only to keep the oysters in motion, so that osmosis will attain a maximum value. The results of Experiments 9-A, 10-A, 11, 12; and 15 all agree fairly well, though differences in stock and in methods of handling cause rather large discrepancies in some cases.

SUMMARY.

1. The determination of ammoniacal nitrogen by the Folin method is of very little value in estimating the amount of decomposition which has occurred in oysters during preparation for the market, because of the repeated washings to which they are subjected.

2. For the same reason the determination of amino-acid nitrogen also is useless in estimating decomposition in oysters undergoing commercial treatment, although it is a reliable index of the amount of washing or soaking which the oysters have received.

3. A marked loss of oyster solids and of ash constituents occurs on washing oysters with fresh water.

4. Oysters covered with water, but not agitated, are not appreciably affected by osmosis, except in relatively long periods of time. Solution proceeds slowly, but amounts to a large percentage of the solids originally present in the course of two or three days.

5. If oysters are agitated in fresh water, either by mechanical means or by means of a blast of air, a large increase in volume results in a short space of time, amounting to as much as 35 per cent in 30 minutes, and to as much as 50 per cent in 90 minutes in these experiments. This increase is believed to be due to osmotic action. As many shucked oysters prepared for the market are washed by this method and sold by measure, it follows that the consumer may be buying added water.

6. When oysters were washed in unpolluted water of approximately the same salinity as that in which they were grown, no increase in volume was found to occur, the actual loss of nutrients was slight, and the oysters were cleaned as effectually as they were by being washed in fresh water.

7. The old method of washing oysters on a skimmer with a hose and paddle gives much less osmotic loss, and, therefore, a much higher content of total solids than does the method of washing by a water current and air agitation.

8. The amount of osmotic distention of which oysters are capable has not been determined. It is at least 50 per cent of the original volume in the oysters under experimentation.

9. These experiments seem to indicate that there is no connection between soaking and "bleeding" in the commercial package (that is,

giving off the water, which forms a layer in the upper part of the container).

10. Interrupted washing (draining and measuring during washing) results in a smaller osmotic increase than does continuous washing for the same length of time.

11. The relation of osmotic loss to actual loss varies with the solubility of the substance in question. Thus, in the average of all the experiments, in the case of total solids made up of insoluble and soluble constituents, the ratio of osmotic loss to actual loss is approximately 2 : 1; for amino-acid nitrogen, all of which is soluble, but which goes into solution slowly, the ratio is approximately 1 : 1; and for ash-forming substance, which is composed largely of soluble inorganic compounds, the ratio is approximately 1 : 2.

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