FISH STORAGE EFFECTS

Sodium Ion, Potassium Ion, and Weight Changes in Fish Held in Refrigerated Sea Water and Other Solutions

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When sockeye salmon, halibut, herring, lemon sole, and brill were stored in refrigerated sea water for a week, analyses of the flesh revealed a rise in sodium ion and a drop in potassium ion over the natural levels. Weight increases in the fish ranging from 2.4% for salmon to 17.5% for a group of lemon sole were recorded after storage. The outer layer of the flesh of sockeye salmon showed a slow rise in sodium ion and a drop in potassium ion content over the first 48 hours of immersion in chilled sea water, after which time much more rapid changes in the flesh levels of these ions occurred. When solutions were prepared containing sodium and potassium ions at concentrations approximating those calculated for the total tissue water of the fish, the sodium and potassium ions level of the flesh of fish immersed in them remained close to their natural values. It was found that the weight changes of the fish could be prevented by adding 2% of polyvinylpyrrolidone to the immersing solutions.

HOLDING SALMON AND HALIBUT in chilled sea water prior to processing is a procedure which is rapidly gaining acceptance in the fishing industry of the northwest Pacific coast. Chilling units and reefer tanks have been installed on trollers, seiners, tenders, and packers, and in canneries in Canada and the United States, including Alaska (*3*, *14*).

A French patent assigned to LeDanois in 1920 (*9*) covered the holding of fish on boats in sea water or brine, but at −4° C. (24.8° F.) a temperature which would freeze the fish. Hess reported in 1933 that fish kept twice as well at 30° F., the freezing point of fish muscle, as at 36° to 37° F. and that a temperature of 30° F. could be attained using circulating sea water containing ice (*7*). The engineering, bacteriological, chemical, and biochemical aspects of holding fish in refrigerated sea water have been the subject of considerable study at the Technological Station over the past several years.

The concentrations of sodium and potassium ions in the flesh of living fish differ little from those prevailing in animal tissues (*11*). Sodium and potassium ions in sea water are present at approximately 20 and 0.07 times, respectively, the concentration of these ions in fish flesh. Living fish maintain these concentration gradients by expending energy. Dead fish, on the other hand, if maintained in sea water, tend to equilibrate with their surroundings. The sodium ion concentration of the flesh rises, the potassium ion drops (*13*), and the fish increase in weight (*2*).

In this paper the sodium and potassium ions and the weight changes in fish stored in refrigerated sea water are recorded for various species. The effect of varying the salt concentrations and osmotic pressure of the immersing solutions on the extent of the changes observed is also reported.

Methods

Unless otherwise indicated, fish were cleaned and then sampled according to a slight modification of the official method (*1*). Samples of homogenized fish were dissolved in nitric acid and analyzed for sodium and potassium ions using a flame photometer attachment for a Beckman DU spectrophotometer. Details of the sampling and analytical procedures used have been described (*11*). Two methods were employed to maintain fish in refrigerated sea water or other salt solutions for the desired length of time. In one case a laboratory scale model of equipment designed for use on a fishing boat was employed. Fish were held in the immersing solution in a plywood box painted on the inside with Terecote, a Thiokol-type water-resistant coating (Technical Research Co., Seattle, Wash.). The immersing solution was continuously circulated at a rate of 4.5 gallons (U. S.) per hour through the box and over pipes containing refrigerant in an outside chilling unit. The temperature of the immersing solution was maintained at 30° ± 0.5° F. When the effect of a number of different immersing solutions on fish was being compared, 5-gallon plastic pails equipped with stirrers were used and the experiments were conducted in a refrigerated room maintained at approximately 32° F.

The ratio of fish to immersing solution was maintained as closely as possible to one part by weight of fish to four of solution. Chlortetracycline was added as an antibacterial agent to all immersing solutions at a level of 10 p.p.m. Artificial rather than natural sea water was used throughout this study because the salt concentrations were more readily reproducible than with the natural sea water available to this laboratory. The artificial sea water had the following composition (*10*) in grams per kg.: NaCl, 23.476; $NaSO_4$, 3.917; $NaHCO_3$, 0.192; KCl, 0.664; KBr, 0.096; $MgCl_2$, 4.981; $CaCl_2$, 1.102; $SrCl_2$, 0.024; H_3BO_3, 0.026. Half-strength sea water was prepared by diluting the artificial

sea water with an equal volume of fresh water.

Results

The effect on the sodium and potassium ion content of the flesh of storing eviscerated coho salmon (*Oncorhynchus kisutch*) in refrigerated sea water for periods up to 4 weeks has been reported (*13*).

In the present study, the earlier observations were extended in the following manner. Sockeye salmon (*Oncorhynchus nerka*) were used in place of coho. Artificial rather than natural sea water was employed as the immersing solution to provide more readily reproducible salt concentrations. As sea water along the coastline is frequently diluted with fresh water, particularly near points where rivers discharge into the sea, the artificial sea water was tested at both full and half strength. As fish are frequently eviscerated immediately after capture, a comparison was made of the extent of the changes occurring in whole and eviscerated fish. Weight changes in the fish were also determined. The results are recorded in Table I. For this experiment, sockeye salmon freshly caught in a gill net at the mouth of the Fraser River were quickly transported to the laboratory and immediately immersed in previously prepared solutions. Less than 4 to 6 hours elapsed between the time of capture of the fish and the beginning of the experiment.

The results show (Table I) that half-strength sea water raised the sodium ion level of the flesh of the fish approximately half as high as full-strength sea water, but produced little difference in the potassium ion concentration. Although the eviscerated fish presented a greater surface exposed to the salt solution than did the whole fish, surprisingly little difference was observed in the sodium and potassium ion concentrations of the flesh from the two groups. Fish held in full-strength sea water tended to gain more weight than those in the half-strength solution, while the increase observed in eviscerated fish exceeded that in whole ones.

In the experiment just described, the first analyses were made after immersion of the fish for 1 week in the sea-water solutions. In previous experiments with coho salmon in which fish were immersed for short intervals in sea water, the natural levels of sodium and potassium ions were maintained in the fish for a period of approximately 24 hours, after which time a rather abrupt rise in sodium ion and fall in potassium ion occurred (*13*).

To determine whether a similar phenomenon could be observed in another species, an experiment was conducted using sockeye salmon. In an effort to cast further light on the nature of the phenomenon, the flesh in a 1-inch slice taken through the center section of the fish was divided into inner and outer layers. The inner layer represented the flesh from the backbone to a point midway between the backbone and the outer surface of the fish, while the outer layer was made up of the flesh from the mid-point to the outer surface. The layers were rendered skin- and bone-free and then homogenized. The flesh from a second 1-inch slice through the fish taken at a point adjacent to the first was pooled in the usual manner for analysis. For this experiment sockeye salmon were caught in gill nets at the mouth of the Fraser River and transported immediately to the laboratory. Approximately 4 hours elapsed from the time of capture of the fish until the whole fish were immersed in the experimental sea water tank. At each 6-hour interval three fish were removed from the tank for sampling and analysis. The results obtained are recorded in Figure 1. The outer layer of flesh showed a slow rise in sodium ion concentration and fall in potassium ion which was evident from the earliest analyses. After approximately 72 hours these changes began to take place much more rapidly. No change occurred in the ion levels of the inner layer of flesh during the first 48-hour period. As can be seen, the increase in the rate of change of the ion concentrations in the outer layer occurred after changes in sodium and potassium ion levels had begun to take place in the inner layer of flesh. As might be expected, values obtained from

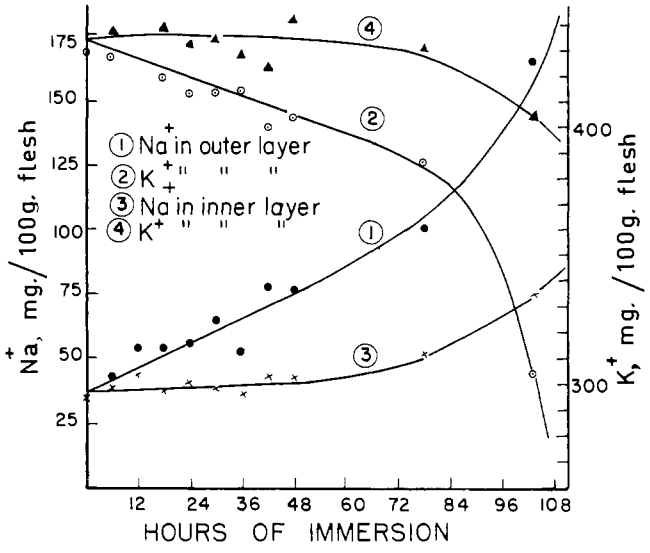

Figure 1. Na^+ and K^+ changes in inner and outer layers of muscle tissue of sockeye salmon at various intervals after immersion of the fish in full strength refrigerated sea water

Each recorded point represents average value for three fish

Table I. Sodium and Potassium Ions and Weight Changes in Sockeye Salmon Held Whole and Eviscerated in Refrigerated Sea Water

Sea Water Concentration	Immersion Time, Weeks	Mg./100 G.[b] Na^+	Mg./100 G.[b] K^+	Wt. Increase,[a] %
Control (not immersed)	...	33.5 ± 1.1	411 ± 15	...
		Whole fish		
Full strength	1	277 ± 13	276 ± 5	2.98 ± 0.79
	2	422 ± 18	198 ± 6	4.33 ± 1.25
Half strength	1	149 ± 12	265 ± 9	2.38 ± 0.58
	2	221 ± 20	201 ± 17	3.45 ± 0.65
		Eviscerated Fish		
Full strength	1	297 ± 38	272 ± 19	4.63 ± 1.39
	2	487 ± 26	193 ± 19	8.16 ± 1.69
Half strength	1	178 ± 29	257 ± 34	3.89 ± 0.99
	2	250 ± 21	178 ± 25	5.60 ± 1.00

[a] Of entire fish.
[b] Based on wet weight of muscle tissue. Each value represents average and average deviation of four fish.

a flesh sample representing a composite of the inner and outer layers, were approximately the average of the two sets of values shown.

The initial slow rise in sodium ion and concomitant fall in potassium ion in the outer layer of the flesh (Figure 1) may well have been due to equilibration of the immersing solution with the extracellular fluid of the tissue, while the more rapid changes occurring subsequently could represent equilibration with intracellular fluid. Studies with much smaller sections of tissue would be required to test this hypothesis further.

Information on ion and weight changes in fish immersed in refrigerated sea water has so far been restricted to studies on various species of salmon. As a step toward extending these observations to include other commercially important species of fish, an experiment was conducted with halibut (*Hippoglossus stenolepsis*). For this purpose, halibut ranging in weight from 10 to 20 pounds were caught at sea and packed in ice for transport to the laboratory. The experiment was begun within 24 hours of the time of capture of the fish. In this study a slice 1 inch wide was taken from the center section of the fish and divided into inner and outer layers. As in the previous salmon experiment, the inner layer represented the flesh from the backbone to a point midway between the backbone and the outer surface, while the outer layer was composed of flesh from this mid-point to the outer surface. The layers were freed of skin and bone and then homogenized. The results (Table II) show that after 1 week in half-strength sea water, the inner layer of flesh of the halibut was almost unchanged with respect to sodium and potassium ion concentration, while the values for the outer layer had changed very appreciably. After 2 weeks the two layers had still not equilibrated. The weight increases recorded after 1 and 2 weeks are slightly higher than for sockeye salmon held under comparable conditions.

It was of interest to know whether sea water could be modified or solutions devised which would minimize or eliminate the changes observed in the preceding experiments. To investigate this possibility, smaller species of fish were selected to enable a reasonable number of different solutions to be tested at the same time with the facilities available.

In Table III are recorded the results obtained when herring were immersed for 1 week in various solutions. For experiments with herring the fish were obtained from seine boats as soon as possible after capture, which was usually within 24 to 36 hours. January-caught herring were used. The whole herring were weighed individually before being immersed in the various solutions. In the case of immersion in full-strength sea water the results show (Table III) that the uptake of sodium ion was somewhat higher for herring than had been observed for sockeye salmon under comparable conditions (Table I). The potassium ion loss for herring was a little less and the weight changes were slightly higher.

As the sodium ion uptake by and the potassium ion loss from the cells could possibly be due to exchange (*6*), it seemed not unlikely that if potassium ion loss could be prevented, sodium ion uptake might not occur. Accordingly potassium ion was added to the sea water until the concentration equaled that calculated for the potassium ion concentration of the total tissue water in the flesh of the fish. Although this concentration of potassium ion in the immersing fluid not only maintained but somewhat increased the potassium ion concentration in the flesh, it failed to prevent the uptake of sodium ion (Table III). Similar results were obtained when potassium ion was added to half-strength sea water.

From a knowledge of the moisture content of the flesh and its content of sodium and potassium ions, one can calculate the concentration of sodium and potassium ions in the total tissue water. If the immersing fluid contained sodium and potassium ions in this same concentration, it should be possible to maintain the flesh levels of these ions approximately constant. Solution 5 in Table III was prepared to test this possibility. The total tissue water of an average herring was calculated to contain 1.09 grams of sodium ion and 6.44 grams of potassium ion per liter. These levels were provided in the immersing solution using appropriate amounts of sodium chloride and potassium chloride. Although some changes did occur as a result of immersion of the fish in this solution, a reasonable approximation of the starting values was maintained (Table III).

A series of solutions was tested using lemon sole (*Parophrys vetulus*) as the experimental fish. This species was chosen because it is a small flat fish with a high ratio of surface area to body weight. In addition, as it never accumulates any

Table II. Sodium and Potassium Ion Changes in Inner and Outer Layers of Muscle Tissue of Halibut and Weight Increases in Fish Preserved in Half-Strength Refrigerated Sea Water

Time, Weeks	Portion of Tissue Analyzed	Mg./100 G. Na^+	Mg./100 G. K^+	Wt. Increase,[a] %
0	Inner layer	45.6 ± 6.1	510 ± 9	
	Outer layer	47.7 ± 5.1	493 ± 15	
	Average	46.7 ± 5.6	501 + 12	
1	Inner layer	67.4 ± 5.1	501 ± 15	3.7 ± 0.7
	Outer layer	149.3 ± 22.9	389 ± 17	
	Average	108.4 ± 14	445 ± 16	
2	Inner layer	137.4 ± 19.1	413 ± 22	5.1 ± 0.9
	Outer layer	210.8 ± 19.1	302 ± 23	
	Average	174.1 ± 19.1	358 ± 23	

[a] Of entire fish.
[b] Based on wet weight of muscle tissue. Each value represents average and average deviation of four fish.

Table III. Changes in the Sodium and Potassium Ion Content of the Muscle Tissue and in the Weight of Herring Held for 1 Week in Various Immersing Solutions

Solution No.	Composition of Solution[a]	Mg./100 G.[b] Na^+	Mg./100 G.[b] K^+	Wt. Increase, %
...	Control, not immersed	68.4 ± 1.9	446 ± 8	
1	Sea water (full strength)	365 ± 20	305 ± 23	3.4 ± 0.6
2	Solution 1 + K^+	412 ± 21	522 ± 3	3.8 ± 0.6
3	Sea water (half strength)	227 ± 30	267 ± 10	4.3 ± 1.0
4	Solution 3 + K^+	229 ± 9	554 ± 7	5.3 ± 0.6
5	Na^+ and K^- to maintain flesh level constant	79 ± 5	542 ± 19	7.7 ± 0.8

[a] Solution 2. Sea water + 11.61 grams/l. of KCl. Solution 4. Half-strength sea water + 11.97 grams/l. KCl. Solution 5. 2.76 grams/l. of NaCl + 12.33 grams/l. of KCl.
[b] Based on wet weight of muscle tissue. Each value represents average and average deviation of six fish.

appreciable levels of fat as compared to herring or salmon, it can be considered also as representative of the nonfatty fish. When lemon sole were immersed in half-strength sea water for 1 week (Table IV), the sodium ion uptake and potassium ion loss greatly exceeded those for sockeye salmon and herring held under comparable conditions (Tables III and VI) and the weight increase, 17.5%, far exceeded that for any of the other species of fish examined. A solution of sodium and potassium chlorides designed to maintain the flesh levels constant again came close to achieving the desired effect (solution 2). Such a solution can be readily prepared from sea water by mixing 1 part of sea water with 9 parts of fresh water and adding potassium chloride. When tested (solution 3) it caused some rise in sodium ion, but maintained the potassium ion level in the flesh of the lemon sole close to the value at the start of the experiment.

Little difficulty is experienced in preparing solutions which will maintain the level of sodium and potassium ions in the flesh at or near their natural values. Fish immersed in such solutions, however, usually gained more weight than when stored in sea water. As no significant net uptake of salt was involved when these solutions were used, the weight increase observed could only be due to uptake of water by the flesh. Water uptake would be expected to occur if the osmotic pressure of the immersing solution was lower than that of the tissue fluids of the fish. It was therefore desirable to find an agent which would be sufficiently soluble to provide the required osmotic pressure in the solution but be impermeable to the tissue membranes of the fish. Such an agent would be expected to have properties similar to those of the compounds employed to maintain osmotic pressure in plasma substitutes (*4*). A compound used for the latter purpose, polyvinylpyrrolidone (PVP) was tested to determine its capacity to prevent weight increases in lemon sole (Table V). Only 2% of this compound prevented water uptake from a solution of sodium and potassium chlorides and from full and half-strength sea water. Separate experiments showed that higher concentrations of polyvinylpyrrolidone actually caused the fish to lose weight.

Discussion

To compare the extent of the changes occurring in various species of fish immersed in refrigerated sea water, the results for fish held under comparable conditions for 1 week are summarized in Table VI. The smallest changes in sodium and potassium ions occurred in halibut. As the changes recorded represent the average for the flesh of the fish taken in cross section, the larger the fish the less the change that would be expected in such values, if insufficient time had elapsed for complete equilibration of the flesh with the immersing solution. As Table II showed, the inner layer of the flesh of halibut had not reached the sodium ion content of the outer layer at the end even of the second week of immersion.

For fish of the same weight one might expect the greatest changes to occur with fish having the highest ratio of surface area to body weight. Although this would appear to be borne out by a comparison of the changes in herring and the first group of lemon sole (Table VI), a second group of lemon sole showed a weight increase similar to that in the herring and much less than in the first group of fish. The first group of lemon sole were caught in March and contained no visible milt or roe, while the second group were captured in November and contained well developed gonads. Such marked differences in the physiological state of the living fish could well have an influence on the extent of changes occurring in storage. It may be of significance that the skin of herring (*12*) and salmon (*15*, *17*) has been observed to

Table IV. Variations in Sodium and Potassium Ion Content of Flesh and Weight Changes in Lemon Sole Held for 1 Week in Solutions of Various Compositions

Solution, No.	Composition of Solution[a]	Mg./100 G.[b] Na^+	Mg./100 G.[b] K^+	Wt. Increase, %
...	Control (not immersed)	89 ± 1.0	387 ± 2	...
1	Sea water (half strength)	311 ± 9	138 ± 4	17.5 ± 1.6
2	Na^+ and K^+ to maintain flesh level constant	113 ± 1.4	405 ± 3	18.2 ± 1.4
3	1 part sea water + 9 parts fresh water + K^+ to maintain flesh level constant	115 ± 2	378 ± 18	22.9 ± 4.3

[a] Solution 2. 2.35 grams of NaCl and 10.24 grams of KCl per l. Solution 3. Indicated amounts of sea water + 10.3 grams/l. of KCl.
[b] See Table III.

Table V. Capacity of Polyvinylpyrrolidone to Prevent Weight Increases in Lemon Sole Immersed in Salt Solutions

Solution, No.	Salt Composition of Immersing Solution[a]	Polyvinylpyrrolidone Addition, %	Wt. Change[b]
1	Na^+ and K^+ to maintain flesh level constant	0	+ 8.5 ± 1.3
2	Same as solution 1	2	+ 0.7 ± 0.9
3	Sea water (half strength)	0	+ 7.8 ± 1.5
4	Sea water (half strength)	2	+ 0.1 ± 1.4
5	Sea water (full strength)	0	+11.5 + 2.4
6	Sea water (full strength)	2	+ 0.8 ± 0.6

[a] Solution. 1. 2.37 grams of NaCl and 7.9 grams of KCl per l.
[b] Each value represents average and average deviation of 10 fish.

Table VI. Sodium and Potassium Ions and Weight Changes in Various Species of Fish Held 1 Week in Half-Strength Refrigerated Sea Water

Species	Weight, Lb.	Change Recorded,[a] Mg./100 G. Na^+	Change Recorded,[a] Mg./100 G. K^+	Wt. Increase, %
Sockeye salmon	5.29 ± 0.23	+115	−146	2.4 ± 0.6
Halibut	15.2 ± 1.7	+ 61	− 56	3.7 ± 0.7
Herring	0.33 ± 0.04	+159	−179	4.3 ± 1.0
Lemon sole				
1	0.65 ± 0.08	+222	−249	17.5 ± 1.6
2	1.0 ± 0.05	+273	−266	5.5 ± 1.9
Brill (*Eopsetta jordani*)	1.7 ± 0.27	+223	−213	7.8 ± 1.4

[a] Change recorded in Na^+ and K^+ levels represents difference between amounts present before and after immersion for 1 week in half-strength sea water.

constitute a greater proportion of the body weight of the fish just prior to spawning than at other stages in their cycle of maturation.

In relation to the other fish (Table VI) both the herring and salmon appear to have increased in weight less than might have been expected from the extent of the changes in their sodium and potassium levels. Whether this means that the osmotic pressure of the intracellular fluids is lower in these fish than in the others or that the higher fat content of the two species specifically impeded the uptake of water cannot be determined from the data.

The fact that storage of fish in refrigerated sea water produces an uneven distribution of sodium ion in the flesh indicates that analyses based on the official method of sampling (*1*) in this case provide insufficient information for marketing purposes. A situation could conceivably arise wherein the outer layer of flesh of a fish contained an unpalatable level of sodium ion, the inner layer remained almost unchanged, while a value based on the cross-sectional sample specified in the official method indicated a level of sodium ion within the limits of palatability.

There have been many studies in which hydration of mammalian tissues has been observed in salt solutions isoosmotic with blood and tissue fluids, and there are conflicting explanations to account for the phenomenon (*8, 16, 18*). In this study, where the ion concentrations of the immersing solution were adjusted to maintain the flesh levels constant, water uptake must have been due primarily to the hypertonicity of the intracellular fluids of the flesh. This was further substantiated by the observation that a nondiffusible substance capable of increasing the osmotic pressure of the immersing solution prevented water uptake by the fish.

Polyvinylpyrrolidone is a chemically inert, hydrophilic, water-soluble macromolecule essentially nontoxic for humans by all routes of administration. The compound is widely used in the formulation of pharmaceuticals and has been recommended for use as a beverage clarifier (*5*).

In the practical application of the results reported here it would be feasible to premix and package sodium chloride, potassium chloride, and polyvinylpyrrolidone for convenient addition to tanks of fresh water on fishing vessels or in shore plants.

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COFFEE VOLATILES

Analysis of the Volatile Constituents of Coffee

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A METHOD OF SAMPLING roast and ground coffee for analysis of coffee volatiles by gas chromatography has previously been reported (*3*). A few modifications in procedure and equipment have made it possible to obtain quantitative data on 19 volatile constituents of coffee.

Data on the green beans and beans roasted at various temperatures have been obtained for seven trade varieties of coffee. Analysis of 13 retail brands of coffee has been performed and the data presented for comparative purposes.

Brewed coffee has been analyzed using the same sampling technique. By use of comparative analysis of brewed coffee and coffee suspended in water, it has been possible to calculate the percentage of each constituent which is extracted and retained in freshly brewed coffee. Concentrations of volatiles in brewed coffee are shown to be in the range of a few parts per billion to 1 to 2 p.p.m.

Eighteen of the chromatogram peaks have been assigned tentative identifications.

Evaluation of the final roast data for the seven trade varieties indicates a possible relationship between the degree of roasting and the ratio of diacetyl to acetyl propionyl. The green bean analysis shows differences which may be helpful in typing or grading green coffee.

Experimental

Apparatus and Procedure. The apparatus used in this work is essentially that previously described (*3*). The major modifications are: Original column replaced by a 4-meter column of 15% Carbowax 1500 on 30- to 60-mesh Chromosorb. Recorder sensitivity increased from 10 mv. full scale to 2.5 mv. full scale.

The sampling procedure was changed only to the extent of a decreased carbon