ON THE CHANGES IN THE MAIN MINERAL CONSTITUENTS OF BALTIC HERRING FILLETS WHILE STANDING IN SALINE SOLUTIONS

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In fish muscle proteins harmful changes may take place during prolonged frozen storage; this is observable as loss of water binding capacity, which causes so-called thawing drip, as decrease of the solubility of the myofibrillar proteins, and as a drop in the organoleptic quality (development of toughness). Similar changes also occur when fish is kept in saline solutions (see e.g. LINKO & NIKKILÄ 1961). These changes may be prevented through the use of certain protective substances, such as phosphates and citrates (NIKKILÄ et al. 1960, MAHON 1962, NIKKILÄ et al. 1964). The basis of these protein changes and their prevention is at present still unsettled, although some hypotheses have been put forward. One assuption is (HAMM & GRAU 1958) that in meat, such compounds as phosphates and citrates improve the water binding capacity by binding with calcium, perhaps also zinc and magnesium. On the other hand, it has been suggested that the favorable effect were due to the dissociation of actomyosin into actin and myosin, connected with the influence of adenosine triphosphate and pyrophosphate (BENDALL 1954, KOTTER & PRÄNDL 1956, YASUI et al. 1964 a, YASUI et al. 1964 b). The final answer to these questions is still lacking. However, several authors have criticized the calcium binding hypothesis. Thus, in some still unpublished experiments LINKO and NIKKILÄ have found that EDTA, which binds calcium more strongly than phosphates and citrates, is unfavorable for the solubility of the fish proteins. Similarly, some recent results obtained by the present authors have shown that EDTA causes a decrease in the solubility of fish myofibrillar proteins. Also HELLENDOORN (1962) stated that although pyrophosphate and tripolyphosphate improve the water binding ability of the sausage mass, oxalate and EDTA on the contrary decrease the water binding in spite of their strong calcium binding. As the alkali and alkaline earth metals in the muscle show certain characteristic changes postmortally, and it is known that in general calcium is connected with the contraction of the muscle, and magnesium again shows relaxing or anesthetic effects, it was considered

useful to perform a series of experiments, where the changes in the most important metals in the fish muscle were investigated. The metals in question were the alkali metals Na and K, and the earth alkali metals Mg and Ca, and the fate of the metals was studied while standing in salt solutions containing either NaCl or Na pyrophosphate, or both salts. Moreover, at the same time the fate of the anions of the standing solutions, i.e. chloride and phosphate, was investigated. It is known from earlier experience that the NaCl solution causes a decrease in the solubility of the myofibrillar proteins, but if the solution in addition contains pyrophosphate, this decrease in solubility is prevented (LINKO & NIKKILÄ 1961, NIKKILÄ et al. 1967). The purpose of the present experiments was to investigate whether the changes in the ions investigated might be related to the protective effect of the pyrophosphate.

Material and methods

Spring Baltic herring (*Clupea harengus* var. *membranus*) which was obtained fresh from the market served as material. At this season the fat content of Baltic herring is low (approx. 2 %). In the experiments, unskinned fillets were used. The amount of fish was 45 g/450 ml of the storage solution. Storage took place at + 4°C. The fillets were analyzed in the beginning, and again after 1 and 5 days' storage. The standing solutions were the following:

I 3 % NaCl
II 1 % $Na_4P_2O_7$
III 3 % NaCl + 1 % $Na_4P_2O_7$

In all cases the pH of the solution was adjusted to 7.2.

First, the ash content was assayed by dry ashing. In the series of metal analyses, the ashing was performed at 520°C for 4 hrs (HECKMAN 1967); according to THOMPSON (1964) losses in K take place at 550°C. In the series of phosphorus analyses, ashing was performed at 550—600°C for 2 hrs, according to the AOCS method (official and tentative methods . . . 1967). The ash content of the samples is calculated as the average of these two values.

The assay of the metals was performed by atomic absorptiometry. This method is modern and rapid; it has one disadvantage, namely that the presence of phosphate lowers the values of Ca and makes the assay of this metal inaccurate. As in the present experiments the fillets were allowed to stand in pyrophosphate solution, the danger of phosphate influence might be worse than usual. For this reason, phosphate elimination before atomic absorptiometry was considered necessary. The method used for this purpose was a modification of the method of HAMM (1955), developed for animal tissues; the principle of the method was ion exchange. The modification was as follows. Of the fillets, which were first drained, a sample of 5 g was taken for ashing as explained above. The ash was dissolved in 0.1 n HCl and an aliquot was taken for ion exchange. Strongly acid cation exchanger Amberlite IR—120 in H-form was used as the resin; the exchange capacity is approx. 2—10 m-ekv./g. The resin was thoroughly washed with acid before use. The height of the column was 16 cm and the diameter 12 mm. The volume of the sample was 20 ml and the rate of flow 0.6 ml/min. After the sample had been put in, the column was washed with 30 ml distilled water. The metals were eluted with 2 × (10 ml 5 n HCl + 50 ml H_2O) and 1 × (10 ml 5 n HCl + 25 ml H_2O). At the preliminary trials it was

confirmed that elution was complete; moreover, in the actual runs an additional elution was performed to check that no more metals were left behind. The metals in the eluates were assayed using a Perkin Elmer 303 Atomic absorptiometer. The HCl strength of the samples and the standards was 1 n. In the Ca assays an addition of La basic solution was performed for all the solutions in proportions 5 ml/25 ml (the basic solution contained 58.65 g La_2O_3 + 250 ml conc. HCl/1000 ml); this addition prevents disturbances caused by phosphorus up to 200 ppm. In preliminary experiments it was found that elimination of phosphorus by ion exchange was 95—100 %; thus, elimination of the effect of the small amount of phosphorus still left was considered useful. In assays of K it is known that the presence of Na is disturbing; consequently, Na was added to the standard solutions to make them comparable to the sample solutions, with an accuracy of 20 %.

In the assay of chloride GRAUS method (1960), developed for meat products, was used. In this method, the extract of the sample is clarified with Carrez solutions, and afterwards an argentometric titration is performed. In checking experiments it was stated, that added chloride was found quantitatively, and that the presence of phosphate did not cause significant disturbance. It may be mentioned that BARNETT & NELSON (1968) have also used a rapid assay method, where ashing is omitted; in this case, extraction is effected by 10 minutes' boiling. This method, which was specifically developed for marine products, was found to be reliable and rapid.

Assay of phosphorus was performed after the ashing explained above according to the Nordisk metodik-kommittee method (1954). Samples ashed were of 3 g weight.

Results and their evaluation

Initial values. For the initial fillets, the following values were obtained:

Total ash	1491.2	mg/100 g
Na	101.3	—„—
K	463.5	—„—
Mg	31.8	—„—
Ca	89.4	—„—
Cl	94.4	—„—
P_2O_5	680.5	—„—
(P, calculated	297	—„—)

In the literature, comparatively few values are available concerning Baltic herring. For this reason, values for herring are also used for comparison; as Baltic herring is a smaller variant of herring, this is considered justifiable.

The ash content value of Baltic herring is comparable with that given by TURPEINEN & ROINE (1960): in the edible part 1500 mg/100 g. The value of herring is, according to SOUCI et al. (1962), 1260 mg/100 g, the range being 1160—1370 mg/100 g. LUDORFF (1960) gives for herring (Ostsee) 600 mg/100 g. The ash content of the edible part of fish is of course influenced by the amount of bones remaining in the flesh; this amount may be greater in small fish, such as Baltic herring, than in the larger form, herring.

For Na content, values for Baltic herring were not available in the literature. For herring, SOUCI et al. (1962) give the value 118 mg/100 g, the range being 106—130 mg/100 g. In the Food Industries Manual (1957) the value 130 mg/100 g is given; for Pacific

herring (*Clupea pallasi*) McBride & McLeod (1956a, 1956b) report 74 mg/100 g, range 73—74.5 mg/100 g. The value obtained for Baltic herring is somewhat lower than that of herring, which may be due to the fact that Baltic herring lives in brackish water.

Similarly, K content values of Baltic herring were not available. For herring, Souci et al. (1962) report 317 mg/100 g, range 300—400 mg/100 g. In the Food Industries Manual (1957), too, 317 mg/100 g is given. For Pacific herring, McBride & McLeod (1956a, 1956b) report 367 mg/100 g, range 355—380 mg/100 g. The value obtained here for Baltic herring is somewhat higher than the values for herring.

For Mg content, the Food Industries Manual (1957) reports 31.7 mg/100 g for herring, which corresponds well to the value obtained here for Baltic herring.

The value of Ca in Baltic herring is according to Turpeinen & Roine (1960) 16 mg/100 g. For herring, Souci et al. (1962) report 57 mg/100 g, the range being 31—100 mg/100 g; the Food Industries Manual (1957) reports 101 mg/100 g. The value obtained here for Baltic herring is somewhat high, but still remains within the limits given for herring.

For Cl content, no values are available for comparison in the literature concerning Baltic herring. For herring, the Food Industries Manual (1957) gives the value 122 mg/100 g. The value obtained here for Baltic herring is somewhat lower, perhaps due to the adaptation to brackish water.

Again, no values for comparison have been found concerning the phosphorus content of Baltic herring. For herring, Souci et al. (1962) give 240 mg P/100 g, the range being 200—270 mg/100 g. In the Food Industries Manual (1957), 272 mg/100 g is reported; Atwater (1892) gives 244 mg/100 g. The value obtained here for Baltic herring is somewhat higher (297 mg P/100 g); perhaps the remaining bones in the fillets are the reason for this high value, as a similar feature is also noted in ash and Ca values.

Influence of the standing in saline solutions on the mineral compounds. In the table 1 the analytical results of the fillets kept in the different standing solutions for the periods of one and five days are presented. All the values are given in mg per 100 g of original or drained fillets.

Table 1. *Ash components in Baltic herring fillets, kept in NaCl, Na pyrophosphate, and NaCl + Na pyrophosphate solutions for various periods.*

	Fresh fillets	NaCl		Pyrophosphate		NaCl + pyrophosphate	
Standing time, days	0	1	5	1	5	1	5
Ash average	1491.2	2532.5	2824.1	1249.5	1243.0	3172.9	3357.8
Na	101.3	847.4	1077.0	273.2	380.7	1096.6	1168.9
K	463.5	96.1	48.0	124.0	52.1	69.6	45.1
Mg	31.8	13.5	11.9	14.7	12.2	12.5	10.6
Ca	89.4	59.1	67.4	70.8	75.1	57.8	67.4
Cl	94.4	1239	1456	13.6	0.4	1309	1389
P_2O_5	680.5	330.1	153.5	595.7	572.6	593.3	563.5

To find out how well the sum of the components assayed corresponds to the total ash, calculations were carried out. If it is assumed that the ash components are either metal

chlorides or oxides, the latter amount being that part of the metals which remains after the chlorides are subtracted, together with phosphorus pentoxide, the following comparison is obtained:

	Fresh fillets	NaCl		Pyrophosphate		NaCl + pyrophosphate	
Standing time, days		1	5	1	5	1	5
Ash average	1491.2	2532.5	2824.1	1249.5	1243.0	3172.9	3357.8
Components together	1542.9	2616.7	2887.4	1181.8	1211.3	3205.2	3319.1
Difference	—51.7	—84.2	—63.3	+67.7	+31.7	—33.1	+37.9
Difference, % of ash average	3.5	3.3	2.2	5.4	2.6	1.0	1.1

The comparison shows that the sum of the components, calculated in this way, corresponds quite well to the value of total ash, the difference being in the mean 2.7 % and maximally 5.4 % of the ash average. Of course, ash contains some minor components in addition to the components assayed. The fact that the sum of the components assayed is in most cases somewhat higher than the ash average, gives the impression that the separate assays have given values that are slightly too high. It may be mentioned that in the assay of chloride, no ashing was performed, and the value thus differs from the others in that respect. It is evident, however, that agreement may be considered satisfactory.

The results for each series of analyses separately. The changes in total ash depending on the different standing conditions are illustrated in Fig. 1. It can be seen that the ash content of the fillets increases markedly when the fillets are kept in 3 % NaCl solution; the increase is greatest during the first day. The increase is still greater when the solution also contains pyrophosphate; here, too, the change is greatest during the first day. In contrast, in the fillets kept in 1 % pyrophosphate solution, small decrease in the ash content takes place; here, again, the change is greatest during the first day.

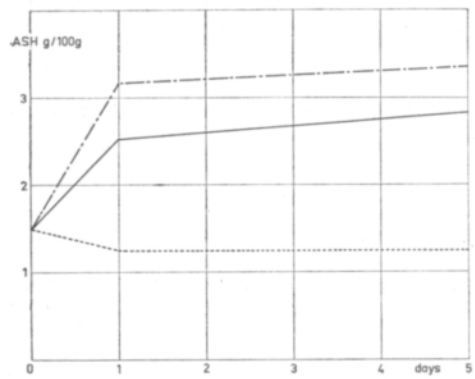

Fig. 1. Changes in the ash content of Baltic herring fillets, kept in various salt solutions.

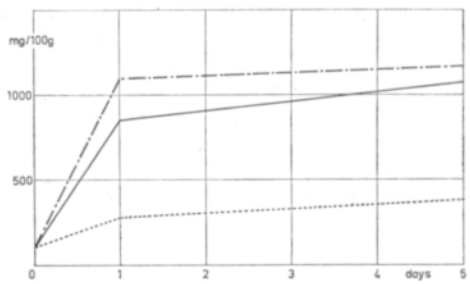

Fig. 2. Changes in the sodium content of Baltic herring fillets, kept in various salt solutions.

Explanation of the curves:
——— 3 % NaCl
- - - - - - 1 % Na pyrophosphate
— - — - 3 % NaCl + 1 % Na pyrophosphate

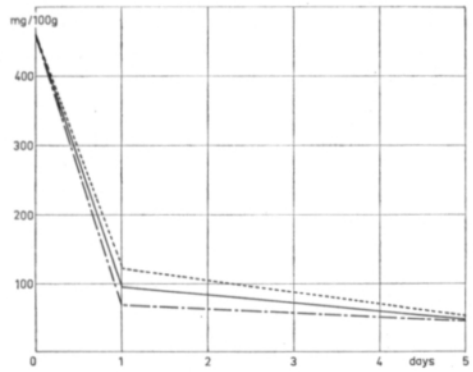

Fig. 3. Changes in the potassium content of Baltic herring fillets, kept in various salt solutions.

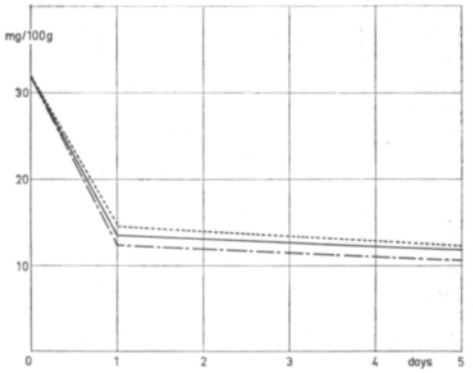

Fig. 4. Changes in the magnesium content of Baltic herring fillets, kept in various salt solutions.

In these results, it seems astonishing that a decrease in the ash value occurs when the fillets are kept in 1 % pyrophosphate solution. The ionic strength of this solution is, if it is assumed that dissociation takes place quantitatively (VUJICÍC et al. 1967), 0.376, which is higher than that of most fresh- and brackish water fish (WIKGREN 1953). Thus, if the ash content of the fillets were primarily dependent on the ionic strength of the standing solution, the value should also increase in this solution. It may here be important that the salts in the standing solutions can affect the water binding capacity of the fillets, and thus the drained weight, against which the ash values have been calculated, is also influenced. Consequently, checking experiments were performed to find out the changes of the ash values, if they are calculated against the initial weights instead of the drained weights of the fillets. These checking experiments showed that when the ash values were calculated against initial weights, the ash contents also increased in the fillets kept in the pyrophosphate solution, although the increase was only small.

The changes are then considered concerning the metals under investigation. Na is the only metal originally present in the standing solutions, as both chloride and pyrophosphate were used as Na salts. Correspondingly, in all series the Na content of the fillets has increased during standing, as is shown in Fig. 2. The increase has been smallest in the pyrophosphate solution and greatest in NaCl + pyrophosphate solution. In all cases, the increase is greatest during the first day.

In the other metals studied, the change is in the opposite direction, i.e. the amounts in the fillets become progressively less. The changes in K are shown in Fig. 3, those in Mg in Fig. 4 and those in Ca in Fig. 5. In all cases, loss of metals has taken place; the loss is greatest for K. The greatest part of the loss is observed during the first day; of the different standing solutions, NaCl + pyrophosphate shows the greatest and pyrophosphate the smallest loss. However, the final values are almost the same. The final values are approx. 9.7—11.2 % of the original value. For Mg, the type of change is similar to that of K: strong decrease during the first day and slower loss thereafter. Here, too, loss is greatest in NaCl + pyrophosphate solution and smallest in pyrophosphate solution, although the differences are small. In percentage, loss of Mg is smaller than that of K, the final values being 33.3—38.4 % of the initial value. A decrease is also observed for Ca, although only small. The order of influence of the standing solutions is similar to that of K and Mg.

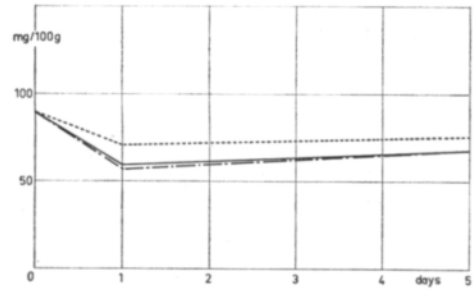

Fig. 5. Changes in the calcium content of Baltic herring fillets, kept in various salt solutions.

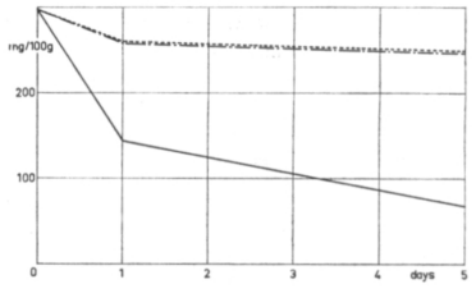

Fig. 6. Changes in the phosphorus content of Baltic herring fillets, kept in various salt solutions.

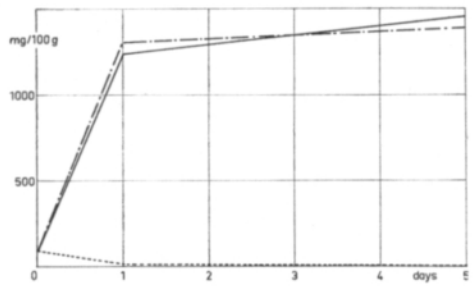

Fig. 7. Changes in the chloride content of Baltic herring fillets, kept in various salt solutions.

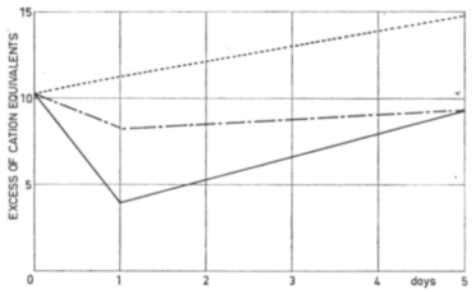

Fig. 8. Changes in the excess of cation equivalents in ash of Baltic herring fillets, kept in various salt solutions.

In contrast, the type of curve is different here: for Ca, the values are minimal after one day, and after 5 days they have again increased, although not up to the initial level. After one day, the minimal values were 64.7—79.2 % of the initial level, and after 5 days, the range was 75.4—84 % of the initial level.

The results concerning the anions investigated are shown in Figs. 6 and 7. The phosphorus content of the fillets (Fig. 6) has in all cases decreased, during the first day rapidly and thereafter more slowly. The loss has been greatest in NaCl solution, where the final value after 5 days is 22.6 % of the initial value, whereas in both solutions containing pyrophosphate the loss is much smaller and nearly the same, the final value after 5 days being 82.8—84.2 % of the initial value. Thus, the presence of pyrophosphate has here almost completely prevented the loss of phosphorus caused by NaCl. The chloride content again has in both solutions containing chloride strongly increased, almost independently of the presence of pyrophosphate; here, too, the increase is greatest during the first day (Fig. 7). Pyrophosphate solution alone has extracted the chloride from the fillets progressively and effectively, so that after 5 days only traces remain.

Change of the cation and anion equivalents of the fillets during standing. As the components assayed correspond well to the total ash values, it might be possible that comparison of the sum of the cation equivalents on the one hand and the sum of the anion equivalents on the other would perhaps give some hint of the effects of NaCl and Na pyrophosphate solutions on the fillets. For this reason, in each series the amounts of metals and anions assayed were calculated as mg equivalents,

Table 2. *The amounts of cation and anion equivalents calculated as sums, and the amount of cation excess in Baltic herring fillets kept in saline solutions.* The values are given as mg equivalents/100 g of fillets.

	Fresh fillets	NaCl		Pyrophosphate		NaCl + pyrophosphate	
Standing time, days	0	1	5	1	5	1	5
Na	4.41	36.9	46.9	11.9	16.6	47.7	50.9
K	11.85	2.46	1.23	3.17	1.33	1.78	1.15
Mg	2.62	1.11	0.98	1.21	1.01	1.02	0.87
Ca	4.46	2.95	3.37	3.54	3.75	2.88	3.36
Sum	23.34	43.42	52.48	19.82	22.69	53.38	56.28
Cl	2.66	34.94	41.07	0.38	0.01	36.91	39.18
PO_4	10.42	4.55	2.12	8.21	7.89	8.18	7.77
Sum	13.08	39.49	43.19	8.59	7.90	45.09	46.95
Cations	23.34	43.42	52.48	19.82	22.69	53.38	56.28
Anions	13.08	39.49	43.19	8.59	7.90	45.09	49.65
Cation excess	10.26	3.93	9.29	11.23	14.79	8.29	9.33

and the sums of both were then calculated and compared with each other. The comparison is given in the table 2.

The changes taking place in the cation excess in fillets kept in various standing solutions are shown in Fig. 8. It has already been seen from the table, that throughout the series the sum of cation equivalents is greater than the sum of anion equivalents; the size of the cation excess, however, differs greatly according to the different standing solutions. In the fillets kept in the pyrophosphate solution, the cation excess increases steadily during the whole period, from 10.26 until 14.79 as the final value. In contrast, in NaCl solution, the value falls steeply during the first day to the value 3.93, after which an increase is noted; however, the final value, 9.29 has not yet reached the original level. When the standing solution contains pyrophosphate in addition to the NaCl, there is similarly a decrease of the cation excess during the first day, but much less marked than in NaCl solution alone, the value being 8.29. After this the value increases again, the final value being practically the same as in NaCl solution alone (9.33). Thus, standing in NaCl solution has strongly decreased the cation excess, particularly after twentyfour hours; here, presence of pyrophosphate has clearly reduced this effect. This fact may be connected with the effect of pyrophosphate preventing the harmful influence of NaCl on the properties of fish structure proteins.

Discussion

In the literature some data are available for comparison with the present results. It would be best, of course, to compare with results obtained specifically for fish. Unfortunately, in many respects far more information exists concerning muscle in general than specifically fish muscle; the general results are mostly based on results obtained in physiological investigations or with meat.

Some comparison on this basis may also be useful, although it is evident that fish and meat differ in some respects, thus e.g. the decrease of pH is in meat much stronger than in fish; see among others PARTMANN (1964). However, with necessary precautions the background of general information may still be used.

The data in the literature concerning postmortal changes of the metals investigated here are rather scanty. TOMLINSON (1964), TOMLINSON et al. (1965) have investigated changes of metals in trout and salmon kept in refrigerated artificial sea water and state that penetration of sodium in the white muscle did not begin until the adenosine triphosphate of the muscle had been largely destroyed; loss of potassium became appreciable at the exhaustion of the muscle as judged by cessation of lactic acid formation. With magnesium, the authors did not detect any relation to the postmortal biochemical changes. In the Baltic herring, investigated here, the rigor usually occurs very rapidly, being already over after the first 3 hours (AMLACHER 1961). Therefore, with this species the experiments are of necessity nearly always performed after the rigor has already passed. Consequently, the changes in the metals noted in the present experiments do not reflect the postmortal phases, as the factors preventing the transfer phenomena are no longer at work, and thus the transfer follows the general diffusion laws. One effect of the postmortal biochemistry may, however, still have influence, namely that the loosening of the metals from the muscle is generally increased, as the pH is lowered due to the formation of lactic acid (HAMM 1958).

The transfer of salts to the fish has been the object of several investigations. Thus, the investigators at Torry Research Station (1961) have stated that when regularly-shaped pieces of fish muscle are studied, the immigration follows simple diffusion laws. However, the results were clearer with cod than with herring, where no simple relationship existed between salt uptake and time (1963). In herring and Baltic herring, fat content influences the transfer of salt (NIKKILÄ 1950, NIKKILÄ 1951): immigration is slower with fatty than with lean fish. In Baltic herring, the fat content is minimal in spring; moreover, Baltic herring always contain less fat than herring. Several investigators have noted that skin is a factor which restricts the immigration of salts (TOMLINSON et al. 1965; SCHEURER 1968); this fact was also stated in the earlier investigations of the present authors (KUUSI et al. 1965, NIKKILÄ et al. 1967). Interesting investigations on the principles of fish salting have been carried out by DEL VALLE & NICKERSON (1967a, 1967b). Here, small rectangular pieces of swordfish were investigated; this fish has a particularly firm and cohesive flesh, in which respect it definitely differs from the Baltic herring. The above-mentioned authors assumed that the salt content of the fish and solution was equilibrated after 2 days' standing; then the salt content of the tissue water was the same as that of the brine. In the present experiments the standing time was maximally 5 days; the main change always took place during the first day, but slow changes were still observable during the later period.

The transfer of the pyrophosphate from standing solution to fish fillets has already been studied by the authors, using radioactive pyrophosphate (KUUSI et al. 1965, NIKKILÄ et al. 1967). When the fillets were kept in the solution, the tracer phosphate had in one day immigrated, being distributed overall. When the fillets were only dipped in the pyrophosphate solution quickly and afterwards frozen, the tracer remained in the periphery of the fillets. It is interesting to compare these results with Scheurer's present study (SCHEURER 1968), where similarly radioactive tracers were used to follow the immigration. The usefulness of the tracer method was stated; in Scheurer's experiments, immigration of nitrite was more rapid than that of tripolyphosphate. The pieces of fish were, after dipping, kept in a moist exiccator for one day; then, the salt content of the inside

was still lower than that of the periphery. Also SUTTON & OGILVIE (1968) have studied immigration of tripolyphosphate in small pieces of fish in dipping experiments. The dipped pieces were frozen, and later the pieces were thawed and the drip loss measured, as well as the sodium and phosphorus content of the pieces. Since the ratio Na:P did not change, it was assumed that the tripolyphosphate molecule remained intact during processing. — In the present experiments, as well as in the authors' earlier studies, there was a transfer of phosphorus compounds from the fillets to the brine, as the phosphorus values decreased in all cases. However, immigration of pyrophosphate from the standing solution to the fillets also took place. As noted earlier (NIKKILÄ et al. 1967), the immigration of chloride and phosphate seem to be nearly independent; the amount of phosphate in the fillets is not influenced by the presence of chloride, and similarly the amount of chloride in the fillets is not influenced by the presence of phosphate.

There are only a few data to be found in the literature concerning the loosening of the metals investigated from the fillets, except in the investigations by TOMLINSON et al. (1965) already mentioned. However, the general feature seems to be, that the strength of the binding of metals in the muscle decreases in the order Ca > Mg > Na > K (HAMM 1958, ASSAF & BRATZLER 1966). In the present study the fate of Na in the muscle cannot be evaluated, as the standing solutions all contain Na as the only cation. By contrast, the other metals investigated show a similar type of binding strength as the data in the literature report. As mentioned above, the binding of the metals in these experiments cannot be connected with the postmortal phases due to the early occurrence of rigor in Baltic herring.

If the changes in the amounts of Ca and Mg are specifically considered in relation to the presence of pyrophosphate, it is stated that the loss of the metals is greatest in the solution containing both NaCl and pyrophosphate, although the losses vary only slightly in the different solutions. Thus the fact that pyrophosphate prevents the loss in solubility of the proteins does not seem to be connected with elimination of Ca or Mg from the muscle. This result is in agreement with that of e.g. INKLAAR (1967), according to which in meat, added phosphate did not change the binding of Ca and Mg. Earlier studies have already put forward the opinion that polyphosphate does not remove the bound Ca from the muscle (BOZLER 1955); instead, polyphosphate may detach one part of the Mg, which causes an increase of the swelling. BOZLER (1955) found that Ca may be removed by aid of EDTA, whereby the water binding ability was increased; similar results were obtained by HAMM (1958), who used ion exchange to remove Ca. By contrast, HELLENDOORN (1962) and SHERMAN (1962) found that although oxalate and EDTA are strong Ca binders, their effect on the water binding ability of meat is unfavorable. YASUI et al. (1964a, 1964b) assume that pyrophosphate and tripolyphosphate are bound to actomyosin through earth alkali metals; the affinity is enhanced by high ionic strength and the presence of earth alkali metal ions. The authors' explanation of the improvement in the solubility of myofibrillar proteins by phosphates is that the latter dissociate actomyosin into the components actin and myosin. Particularly in freezing this explanation may be applicable: as the ionic strength of the cell sap increases because of crystallization of water, and the cells in general contain plenty of potassium and in addition calcium, the conditions are favorable for the binding of phosphates by actomyosin; this again dissociates actomyosin, and this phenomenon corresponds to relaxation and improved solubility of

myofibrillar proteins. The results obtained here agree better with the assumption that phosphate is bound to the myofibrillar proteins than with the hypothesis that earth alkali metals were removed from the muscle.

There are some data in the literature on the extracting effect of the standing solutions. The investigators at Torry Research Station (1962) state that loss of soluble materials from fish in salting is always small, less than one per cent. TOMLINSON et al. (1965) note that during standing various substances, such as acid-soluble phosphorus compounds, lactic acid, soluble proteins and B-vitamins are transferred to the medium. In the present experiments, loss in dry matter, phosphorus compounds and metals investigated took place, excluding the components present in the standing solution. It seemed that the extracting power of pyrophosphate was greater than that of chloride, but this effect of pyrophosphate did not come into operation when chloride was present simultaneously.

The most interesting of the present results is the effect of the standing solutions on the cation excess of the fish fillets, since at this point the presence of pyrophosphate seemed to decrease the effect of NaCl. In all cases, there was an excess of cations in the fillets as compared with the inorganic anions studied; due to standing in NaCl solution, this cation excess was greatly decreased. Immigration of NaCl caused a considerable rise in the amounts of both cations and anions, but nevertheless the cation excess was after one day's standing smaller in this than in any other treatment. In the presence of pyrophosphate, this decrease was much smaller. The fact that the cation excess in the ash is smaller may mean that fewer cations in the tissue are bound to the organic anions, such as organic acids and proteins. In the presence of pyrophosphate, the amounts of both cations and anions were even greater than in NaCl solution alone, but the cation excess was only slightly less than the original. It may be assumed that the binding of the cation excess to the tissue could cause a shift of the pH in the alkaline direction and this again would improve the solubility of the proteins. One interesting feature in the phenomenon is that the difference in the cation excess is clear-cut after one day's standing, but after 5 days' standing the difference has almost completely levelled out. A similar return is also often observable with the solubility of the proteins in the presence of phosphates or citrates (LINKO & NIKKILÄ 1961, NIKKILÄ et al. 1964): the solubility is somewhat decreased during the first day of standing, but returns to the original in the course of the following days.

Summary

The purpose of this investigation was to find out what changes take place in the quantities of the ash components of Baltic herring fillets, such as alkali and alkaline earth metals, chloride and phosphate, when the fillets are kept in chloride and phosphate solutions. It was hoped that these experiments might give information on the nature of the protective effect of phosphate, as it is capable of preventing harmful changes in the myofibrillar proteins, caused by NaCl solution.

The standing solutions were 3 % NaCl, 1 % Na pyrophosphate, and a solution containing both these salts. The fillets were investigated at the beginning and after a storage of 1 and 5 days. The total ash, Na, K, Mg, Ca, Cl, and P was analyzed in the fillets.

The original values obtained were compared with the values given for Baltic herring and herring in the literature, as far as such data were available. The Na and Cl values

of Baltic herring obtained here were somewhat lower than those reported for herring. The values obtained for K, Ca and P are somewhat high, but still the values in general agree in range with the values in the literature.

Storage in the solutions increased the values of ash, even if this increase in regard to the pyrophosphate solution was only stated if the ash values were calculated for the original weights of the fillets.

As far the metals were concerned, the amount of Na increased in all cases, as both chloride and pyrophosphate were used as Na salts. By contrast, the amount of K was considerably decreased, the amount of Mg less and that of Ca still less. The loss of K and Mg continued progressively for the whole standing period, while the amount of Ca returned towards the original value after a decrease during the first day. With all the metals the size of the change depended on the strength of the standing solution.

The amount of chloride in the fillets was increased if storage took place in solutions containing NaCl; immigration of Cl was independent of the presence of pyrophosphate. Pyrophosphate alone extracted Cl from the fillets almost completely. The amount of phosphate in the fillets was again decreased in all the solutions, the decrease being greatest when the standing solution contained no phosphate; even then, the fillets still retained approx. 23 % of the original P. When the standing solution contained phosphate, the loss of P was small and independent of the presence of chloride.

When the metals and anions investigated were calculated as equivalents, it was stated that in all cases there was an excess of cations in the fillets. During standing, changes took place in the cation excess. When standing in pyrophosphate solution alone, a small progressive increase of the cation excess was observable, while when standing in NaCl solution alone, the cation excess first decreased strongly and then partly returned towards the original value. If the solution in addition contained also pyrophosphate, the decrease was much less noticeable. In this respect the pyrophosphate has clearly inhibited the effect of NaCl, and this may be connected with the influence of pyrophosphate protecting the myofibrillar proteins.

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SELOSTUS

ALKALI- JA MAA-ALKALIMETALLIEN SEKÄ KLORIDIN JA FOSFAATTIEN MUUTOKSISTA SILAKKAFILEITÄ SUOLALIUOKSISSA SEISOTETTAESSA

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Tässä tutkimuksessa on pyritty selvittämään, mitä muutoksia tapahtuu silakkafileiden tuhkakomponenttien alkali- ja maa-alkalimetallien sekä kloridin ja fosfaatin määrässä, kun fileitä seisotetaan kloridi- ja fosfaattiliuoksissa. Näiden kokeiden avulla koetettiin saada käsitystä fosfaatin suojavaikutuksen luonteesta, kun se pystyy estämään NaCl-liuoksen aiheuttamat haitalliset muutokset kalan lihasproteiineissa.

Seisotusliuoksina oli 3 % NaCl, 1 % Na-pyrofosfaatti sekä liuos, joka sisälsi molemmat edellämainitut suolat. Näytteet tutkittiin alussa sekä 1 vrk. ja 5 vrk. seisotuksen jälkeen. Näytteistä analysoitiin kokonaistuhka, Na, K, Mg, Ca, Cl sekä P.

Saatuja alkuarvoja verrattiin kirjallisuudessa silakalle ja sillille esitettyihin arvoihin, sikäli kuin sellaisia oli löydettävissä. Silakan Na ja Cl-arvot olivat vähän sillin arvoja alhaisemmat. Saadut K, Ca ja P-arvot olivat hiukan korkeahkoja, mutta pääpiirteissään kuitenkin samaa suuruusluokkaa kuin vastaavat kirjallisuusarvot.

Suolaliuoksissa seisottaminen suurensi tuhkan arvoja, joskin pyrofosfaattiliuokseen nähden tuhkamäärän vähäinen nousu oli todettavissa vain kun arvot laskettiin fileiden alkupainoja kohti.

Metalleista Na-määrä nousi kaikissa seisotusliuoksissa, sillä sekä kloridi- että pyrofosfaattiliuoksessa oli käytetty Na-suoloja. Sensijaan fileiden K-määrä laski voimakkaasti, Mg-määrä vähemmän ja Ca-määrä vielä vähemmän. K- ja Mg-määrän aleneminen jatkui progressiivisesti koko seisotusajan, kun taas

Ca-määrä alkoi 1 vrk. kestäneen alenemisen jälkeen jälleen palautua korkeammaksi. Kaikilla k.o. metalleilla muutoksen suuruus riippui seisotusliuoksen voimakkuudesta.

Kloridin määrä nousi Cl-pitoisissa seisotusliuoksissa; kloridin sisääntunkeutuminen oli riippumaton pyrofosfaatin läsnäolosta. Pelkkä pyrofosfaattiliuos uutti fileistä kloridin jokseenkin täysin. Fileiden fosfaattimäärä taas aleni kaikissa seisotuksissa, eniten silloin kun seisotusliuos ei sisältänyt fosfaattia, mutta tällöinkin fileissä oli vielä n. 23 % jäljellä. Kun seisotusliuos sisälsi pyrofosfaattia, oli fileiden fosforimäärän aleneminen pieni ja riippumaton kloridin läsnäolosta.

Kun tutkitut metallit ja anionit laskettiin ekvivalenteiksi, todettiin että kaikissa tapauksissa fileissä oli kationeja ylimäärä. Tässä ylimäärässä tapahtui seisotuksen kuluessa muutoksia. Pelkässä pyrofosfaattiliuoksessa seisotettaessa tapahtui fileiden kationiylimäärässä pientä progressiivista nousua, kun taas pelkässä NaCl-liuoksessa seisotettaessa kationiylimäärä aluksi jyrkästi aleni ja senjälkeen osittain palautui. Kun NaCl-liuos sisälsi lisäksi pyrofosfaattia, tämä aleneminen oli huomattavasti vähäisempi. Tässä kohdin pyrofosfaatti on selvästi estänyt NaCl:n vaikutusta, mikä saattaa olla yhteydessä pyrofosfaatin proteiineja suojaavan vaikutuksen kanssa.