Change in the fractional composition of lipids of small fish preserved in formalin solutions

by E. B. Ostyakova and L. A. Kosvina

Original title: Izmenenie fraktsionnogo sostava lipidov melkoi ryby pri konservirovanii rastvorami formalina


Translated by the Translation Bureau (WDP)
Multilingual Services Division
Department of the Secretary of State of Canada

Department of the Environment
Fisheries and Marine Service
Halifax Laboratory
Halifax, N.S.
1977

6 pages typescript
Change in the Fractional Composition of Lipids of Small Fish
Preserved in Formalin Solutions

Izmenenie fraktsionnogo sostava lipidov melkoj ryby pri
konservirovanii rastvorami formalina

Rybnoe khozyaistvo

Fisheries

<table>
<thead>
<tr>
<th>PUBLISHER - ÉDITEUR</th>
<th>DATE OF PUBLICATION</th>
<th>PAGE NUMBERS IN ORIGINAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not given</td>
<td></td>
<td>81-83</td>
</tr>
<tr>
<td></td>
<td>YEAR</td>
<td>VOLUME</td>
</tr>
<tr>
<td>Moscow, USSR</td>
<td>1975</td>
<td>-</td>
</tr>
</tbody>
</table>

REQUESTING DEPARTMENT: Environment
BRANCH OR DIVISION: Fisheries/Scientific Info. & Publications Branch
PERSON REQUESTING: Dr. R. G. Ackman, Halifax Lab.
YOUR NUMBER: 5053-1
DATE OF REQUEST: 28 February 1977

TRANSLATION BUREAU NO.: 1089219
TRANSLATOR (INITIALS): WDP
UNEDITED TRANSLATION
For Information Only
Information seulement
### Change in the Fractional Composition of Lipids of Small Fish Preserved in Formalin Solutions

By E. B. Ostyakova and L. A. Kosvina, Astrakhan Technical Institute of Fisheries and the Fishing Industry

Formaldehyde has often been recommended for preserving small fish for fodder purposes, but the effect of this preservative on the constituent substances of the fish, and in particular on the lipids in its tissues, has not been studied.

In experiments that we carried out with small frozen fish – the anchovy kilka (Clupeonella engrauliformis) and tiulka (distinction uncertain) – we preserved the fish immediately after it had been thawed (in the air) by immersing it in formalin solutions diluted to 1:2 and 1:1 for 1 and 5 minutes [respectively]. Here the content of free formaldehyde in the tissues of the preserved fish represented 1.3 to 2% of the mass of the fish.

---

*Numbers in the right-hand margin indicate the corresponding pages in the original.*
We extracted the lipids from the original fresh (thawed) and preserved fish using Folch's method and separated them into fractions in a thin layer of KSK [possible expansion potassium sulfate] silica gel in a hexane-ethyl ether-glacial acetic acid (73:25:2) system.

In order to estimate the Rf of the individual fractions of the lipids we developed the chromatograms by successive treatment with iodine fumes, a 10% alcohol solution of phosphomolybdic acid and carbonization with a chromium mixture. For quantitative analyses [we performed the same operation] with an aqueous solution of rhodamine 6Zhch [expansion not available]; in this case we localized the fractions in ultraviolet light.

We identified the different fractions of lipids using markers (cholesterol, oleic acid and trioleate)* and qualitative reactions to phosphorus, glycerin, choline, and the aldehyde and ketone groups.

We divided the most labile fraction of the lipids - the phospholipids - into classes in a chloroform-methanol-glacial acetic acid-water (65:25:4:2) system. We calculated the Rf of each class of phospholipids after treating the plates with iodine fumes and a solution of phosphomolybdic acid and carbonizing with a chromium mixture.

Our observations showed that when kilka is preserved with formalin solutions, the quantity of total lipids decreases, but the relative content of protein lipid complexes at the same time increases (from 21.6 to 37.6% of the total amount of all lipids).

As a result of qualitative TSKh [believed to expand to "thin-layer chromatography"] of tiulka and kilka lipids before and after preservation (where an identical amount of lipids was applied to the plate) we found that

*Translator's note: Taken directly from the original Russian triolet, for which no other more suitable equivalent is available.
formaldehyde promotes a decrease in the fractions of neutral fat and esters of sterols and at the same time the appearance of new, unidentified fractions with Rf = 0.057, 0.177 and 0.435. Of these, the first two yield a reaction with paraaminodiphenylhydrazine, i.e., they contain aldehyde and ketone groups and, to judge from this, they are probably products of the hydrolysis and oxidation of lipids. A third fraction with Rf = 0.435 contains phosphorus and glycerin and possibly represents phosphoglycerin (a product of the hydrolysis of phospholipids), originating as a result of the action of formaldehyde on glyceridephosphorylcholin (Rf = 0.57 to 0.585), contained in the tissues of fresh, unpreserved fish. Here cholin, which [literally] leaves with the front line, is separated.

Table 1 shows data from quantitative thin-layer chromatography of the lipids of kilka, from which it may be seen that the amount of neutral fat and esters of sterols decreases considerably after preservation of the fish. At the start of the chromatograms of the lipids of preserved kilka we find compounds, colored bright yellow, which, after being divided in a system containing glacial acetic acid, are not eluted from the silica gel by solvents. These compounds constitute about 31% of the total quality [sic] of all the lipids.

The eluates of the spot at the start yield a very bright color in a reaction with benzidine; hence we may assume that the substances forming these spots represent the products of the hydrolysis of aldehydogenic plasmalogen phospholipids (plasmal), but it may be that the reaction is provided by the formaldehyde liberated upon the action of glacial acetic acid from colored compounds of the type of Schiff's bases.

Not only triglycerides and esters of sterols but also phospholipids are subjected to intensive hydrolysis under the influence of formaldehyde.
Table 1

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>11. Пятно на</td>
<td>0,014—0,250</td>
<td>35,5</td>
<td>8,9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12. Неидентифицированная &amp; 0,378</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13. Стерины &amp; 0,378</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14. Жировые кислоты &amp; 0,46-0,49</td>
<td>16,3</td>
<td>4,2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15. Нейтральный жир &amp; 0,465</td>
<td>20,8</td>
<td>5,2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16. Эфиры стеринов</td>
<td>0,435</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17. Окрашенные соединения, не eluted from silica gel</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18. Выход липидов с силикагела</td>
<td>0,570-0,585</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19. Примечание. В числителе—показатели для мороженой кильки, в знаменателе—для консервированной. +—реакция положительная, —отрицательная.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key:

1 - Fractions of lipids;
2 - Content;
3 - Percentage of total lipids;
4 - Percentage of dry mass of fish;
5 - Qualitative reactions;
6 - To phosphorus;
7 - To choline;
8 - To aldehydes with benzidine;
9 - To aldehydes and ketones;
10 - Content of P2O5, in mg% of lipids;
11 - Spot at start;
12 - Unidentified;
13 - Sterols;
14 - Fatty acids;
15 - Neutral fat;
16 - Esters of sterols;
17 - Colored compounds not eluted from silica gel;
18 - Yield of lipids from silica gel;
19 - Note: The numerator gives the figures for frozen kilka;
The denominator gives those for preserved kilka;
+ is a positive reaction;
- is a negative reaction;
The presence of phosphorus in the liberated fraction of sterol esters indicates the appearance of intermediate products of the breakdown of the phospholipids in the form of unstable phosphatidic acids. The data given in Table 2 from qualitative thin-layer chromatography of phospholipids liberated from *kilka* and *tiulka* before and after preservation show that lysolecithin, sphingomyelin, phosphatidylserine and cephalin undergo considerable hydrolytic division, whereas the amount of lecithin noticeably increases owing to the fact that it is given off from protein-lipid complexes.

The hydrolysis of phospholipids proceeds both as far as intermediate products and with a loss of phosphorus: the phosphorus content in eluates of the fractions of lipids released from the fish after it has been preserved, proved to be half of the corresponding value in lipids derived from the original fresh fish.

When we stored fish that had been preserved in formalin, we noted a rapid oxidation of lipids. Qualitative thin-layer chromatography of the lipids of preserved *kilka* kept for a week at a temperature of 2 to 3°C showed that as a result of the storage of the fish there was a noticeable intensification in the color of the spot at the start, although the content of free formaldehyde in the fish had decreased by this time. At the same time, there was a decrease in the area of the spot of the fraction of neutral fat and a simultaneous increase in the area and intensity of coloring of the spot [equating to] the fraction of sterols (the sterols probably accumulated not only because of the the breakdown of their esters, but also as a result of their having been liberated from the complexes together with the proteins).

Our research shows that the preservation of fish in formalin solutions substantially affects the properties of its lipids. Formaldehyde
tends to accelerate the hydrolytic division of fish lipids and the liberation of phospholipids, in particular lecithin, from complexes together with proteins, and also causes the formation of colored compounds of the type of Schiff's bases.

Table 2

<table>
<thead>
<tr>
<th>1. Объект исследования</th>
<th>2. ( R_f ) классов фосфолипидов</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. Килька</td>
<td></td>
</tr>
<tr>
<td>12. Мороженая</td>
<td></td>
</tr>
<tr>
<td>13. Консервированная раствором формалина 1:1 (контакт 5 мин)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.07</td>
<td>0.17</td>
<td>0.385</td>
<td>0.44</td>
<td>0.57</td>
<td>0.61</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>12. Мороженая</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Консервированная раствором формалина 1:1 (контакт 5 мин)</td>
<td></td>
<td>0.17</td>
<td>0.385</td>
<td>0.49</td>
<td>0.57</td>
<td>0.61</td>
<td>0.67</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Key:

1 - Research item;
2 - \( R_f \) of classes of phospholipids;
3 - Amino acids and choline;
4 - Lysolecithin;
5 - Lysocephalin;
6 - Phosphatidylserine;
7 - Sphingomyelin;
8 - Inositol-phosphatides;
9 - Lecithin;
10 - Cephalin;
11 - Kılık;
12 - Frozen;
13 - Preserved in a 1:1 solution of formalin (contact 5 minutes).