Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.45, 2016

The Effect of Preservatives and Freezing on Museum Saved Fish Samples

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Abstract
Two means used for saving fish samples, namely Freezing and Preservatives represented by Alcohol and Formalin. The Freezing was used in saving samples collected newly, in addition to use Alcohol and Formalin with different concentrations 70% of Alcohol and 10% of Formalin. The concentrations of some heavy metal elements were examined, such as Potassium, Phosphorus, Calcium, Manganese, Magnesium, Zinc, Iron, Copper and Boron in samples saved in Formalin and Alcohol and frozen at different durations. The concentration of some elements has been changed during the saving duration. The study was performed on the concentration of heavy elements in the ***Liza abu*** muscles of saved and frozen fish.

Introduction
The change in the concentration of heavy metal elements gives an evidence of the interactions taking place on the bodies of fish saved for different periods extending to dozens of years. This concentration comes originally from the environment in which the fish live and the type of food they eat (1). It is noticed that fish living in environments containing high concentrations of a heavy element, this element deposits in the bodies of those fish, noting that these elements are non-dissolved (2) even if these concentrations are low (3).

Since the fish are considered at the top of water materials consumed in the diet, so we find these elements are concentrated in the fish bodies higher than in the water surrounding them or other sediments in this environment as a result of feeding on creatures in that environment (5). The existence of these heavy elements with different concentrations in the bodies of fish is a sign of pollution in the environment of fish (6).

It is possible that the concentrations of some heavy elements are changed in bodies of fish saved in Formalin over time due to its acidity and interaction of these elements over time, leading to increase concentrations of some or decrease concentrations of others and even disappearance of those elements because of changing interactions resulted from saving in Formalin for a long time. Also, the elements are vary in their concentrations over time where fish samples are saved in diluted industrial Alcohol with concentration of 70-75% and the effect of Alcohol and Formalin be clear during the early periods of saving by disappearing fish's color if these types colored clearly(7).

As for the freezing, the concentrations of heavy elements are changed slightly and may be impalpable (8), while certain elements are affected in terms of concentration when freezing temperature ranging from 3-10 ° C especially Potassium (9). Also, the freezing and freeze drying have no effect on the properties and concentrations of elements such as Carbon (C) and Nitrogen (N) in the fish muscles (10). Generally all kinds of freezing either at home, commercial or in a laboratory has no effect on the properties of some heavy metal elements nor the vital value of fish tissue freezing in these methods, on the contrary, it may kill some pathogens that are active in the body's low temperature even after the death of the fish (11).

Materials and Methods of Work
Liza abu fish were chosen for this study, (10) fish were determined for each experiment after preparing preservatives of Formalin and Alcohol according to the proposed concentrations to conduct this study. (10) fish were frozen at home, then the concentration of Formalin was diluted to 10% and Alcohol to 70%.Fish were distributed equally to each solution. A piece of meat from the forehead area was taken for each experiment about 0.5 cm^2 over the proposed time of the study.

Elements were estimated in the pieces of meat for each experiment to see the change in the concentrations of these elements, as follows:

Total phosphorus
Total phosphorus has been estimated according to the method described in (12). (10) ml of material digested has been mixed by using Caldal method into a beaker , adding (0.1) g of Ascorbic acid and (4) ml of Ammonium molybdate solution (10 g Ammonium molybdate with 150 ml of concentrated Sulfuric acid and the volume complete to liter with distilled water), the mixture is placed on the heater for one minute until the color of the solution becomes blue, then the solution is cooled and complete the volume to (100) ml with distilled water, the optical absorption is read by Spectrophotometer, type Ultraspectronic, equipped by United States of America(LKB) company at a wavelength of (620) nanometers, this reading is recorded on the standard curve for the pure Phosphorus, the concentration of Phosphorus is calculated after multiplying the result with dilution

and dividing by the sample weight , according to the following equation:

$$\% \text{ Phosphorus} = \frac{\text{Final concentration of phosphorus} \times 50 \times 100 \times 100 \times 100}{\text{Sample weight(10)g} \times 10 \times 10000000}$$

Total Potassium, Calcium and Boron

Total ratios of Potassium, Calcium and Boron were estimated by taking (1) g of sample and putting it in a tubus of digestorius by using Semi - micro kjeldal method (12), adding (1) g of catalyst CuSo4, then (5) ml of concentrated Sulfuric Acid (98%) was added, the tubus of digestorius were heated for the purpose of sample digestion. After the mixture was being very clear, the samples were cooled and the solutions were diluted to (100) ml with distilled water, the estimation of Potassium, Calcium and Boron concentrations by a Flame photometer -type PGI 2000 Automatic flame photometer English origin which showed the concentrations of Potassium, Calcium and Boron directly after recording the reading on the standard curve of Potassium mechanically. Then total concentration was multiplied with the final dilution ratio and dividing the result by the dry sample weight.

Estimation of Iron element (Fe)

The diluted sulfuric acid (HCL 1: 1 distilled water) about (5 ml) has been added to the ash (0.2 g) until all elements in ash be dissolved, then the volume is completed to (100) ml, 5 mL was taken out and (5) drops of Blue bromophenol were added and titrated with (2M) NaoAc from Sodium Acetates solution until the evidence color was changed at pH 3.5, (1) ml of Hydroquinone solution and (2) ml of Phenanthroline solution were added. Then Ammonium Cetrate solution (1 mL) was added, the volume was completed to 100 ml and left for one hour until the color was developed and intensity of absorption was measured at a wavelength of (510) nm curve to measure the Iron, the intensity of the optical absorption was measured at the wavelength of (510) nm ,then iron concentration was extracted (12).

Estimation of Sulfates

It has been taken (100) ml of the solution prepared for sample, (5) ml of Conditioning Regent was added . this regent was prepared by mixing (50 ml of Glycerol and (30) ml of Hydrochloric acid with (300) ml of water and (100) ml of Alcohol and (75) g of Sodium chloride), the sample was put in mixture on a magnetic mixer with a fixed speed, after one minute from adding a full tablespoon of the crystals of Barium Chloride, a part from it has been transferred to the cuvate to measure turbidity by optical density device, then the reading of sample was recorded on the standard curve of Sulphates solution and the final value in g / l can be extracted.

Estimation of Zinc

The commercial equipment Bio assay system(Kit) was used to allocate Zinc, 200 micromill of indigestible models by (method of digestion device Caldal) and placed in a glass tube ,(8) Microleter of EDTA and 800 Microleter of reagent extract were added, after good mixing, the tubes were placed in an incubator at a temperature of 30 ° C for 30 minutes, the optical absorption was read at a wavelength of 425 nm by Spectrophotometer type LKB, US Origin, then the final concentration was extracted after recording the reading on the standard curve of the zinc element equipped by the company, or applying the equation mentioned in the attached manual of using the device.

Estimation of Copper

The same equipment mentioned above to estimate zinc was used, 250 Microliter from samples separately (after digesting by Caldal) has been put in a glass tube and its own reagent in this kit was added, mixed well and put in the incubator at a temperature of 30 ° C for 30 minutes, then the optical absorption wavelength of (359) nm was read ,this reading has been recorded on the standard curve of the Copper element or applying the equation attached with manual of using the device.

Results and Discussion:

The effect of the freezing does not clearly appear on changing concentrations of various elements in the pieces of meat or Liza abu Fish muscles during various saving periods which were distributed to a month, two months and a full year, this results agreed with (13). As (14) also noted that more samples of fish saved by freezing have no clear change from tissue (muscle tissues) and the change of the various elements' concentrations in the meat or muscles of these fish saved in this way. This is one of the reasons that makes freezing to be the best way of saving quality of meat and fish muscles as well as maintaining the nutritional value due to the change of the saved body components, but it is no longer the better way for saving fish samples for studying. The results of

saving by freezing have not been taking into consideration because they did not occur a tangible change in the saved muscle tissue.

It is well known that the freezing method is a common way to save the high-protein products especially meat including fish meat. Because the composition of meat is muscle tissue, so it is affected more than the rest of the other vital tissues in fish. Here resorting to freezing in order to maintain samples temporarily and not for long-term, while freezing is used to be a means for saving fish as the fish meat is used for human consumption. So it is very wide and pervasive method (15). The disadvantages of saving by freezing for a long time are burning by freezing, drying the samples, changing the size because of the shrinking and extroversion of tissues and even rot and rancidity (16).

Regarding the samples saved by preservatives, a change in the concentration of heavy elements occurred by changing the periods of saving. We notice from the table (1) which contains the concentrations of some heavy metals after one month of saving in both solutions Formalin 10% and Alcohol 70%. Different factors play an important role in changing the concentration of some heavy elements unlike others. One of these factors; type of fish and feeding method and type of food in addition to the temperature of saving process which is changed with the changing seasons and time at which the samples saved with the type of fish container that was made of plastic, glass or other materials that can interact with the materials of saving over time (17).

The temperature of saving plays an important role in the sustainability of saved samples in preservatives especially acidic composition such as Formalin and Alcohol, as these solutions are considered elements of low pH, so they are clearly affected with the surrounding temperature. Therefore, this effect is clearly shown in concentrations change of some elements (18), we see from the two tables (1) and (2) a difference in the concentration of both Potassium ,Phosphorus and Calcium because of high temperature surrounding the saving area or the room temperature. The concentration of these elements is significantly decreased, while the rest of the elements mentioned in tables (1) and (2) have not been affected, the mentioned elements continued in decreasing the concentration as the storage period be more than a year, as it is shown from table (3) decline in the concentrations of these elements unlike other because of the long term of storage obviously influence on the temperature and makes interactions between different components and solutions containers with low pH, and some heavy elements such as Iron, Manganese, Zinc, Copper and Boron are considered elements that have the ability to concentrate in the tissues of the fish muscles as is confirmed by (19).

Table (1) concentrations of heavy elements in the muscle tissue after one month of saving

Elements	Muscle tissue saved in Formalin PPM	Muscle tissue saved in Alcohol PPM.
Potassium	3100	2950
Phosphorus	1950	1900
Calcium	260	250
Manganese	9,9	9,2
Magnesium	445	420
Zinc	12	11
Iron	5	4
Copper	4	4
Boron	3	4

Table (2) concentrations of heavy elements in the muscle tissue after two months of saving

Elements	Muscle tissue saved in Formalin PPM	Muscle tissue saved in Alcohol PPM.
Potassium	2900	2800
Phosphorus	1900	1750
Calcium	250	250
Manganese	9,5	9
Magnesium	400	350
Zinc	10	10
Iron	4	3,5
Copper	3	3
Boron	4	4

Some of the heavy metal elements such as Iron , Zinc , Copper, Boron , and even Mercury and Lead are considered elements of high ability of deposition in tissues more than others. Also, they have dangerous influence on the consumer's overall health and the body carried concentrations of these elements even if they are low. Their resistance to the conditions of saving is higher than others because they are complex composition and increased in their concentrations due to the objects acquired them, especially bodies swimming in the water environment polluted and rich by these concentrations (20). The tables show that these elements have not been significantly affected with concentrations depending on Preservatives represented by Formalin 10% and Alcohol

70%. In addition, these elements have no effect when the temperatures change but, on the contrary, concentrations increased when increasing the duration of conservation as in the table (3), and these results are consistent with what both explained by (21) and (22).

Table (3) concentrations of heavy elements after a year of saving

Elements	Muscle tissue saved in Formalin PPM	Muscle tissue saved in Alcohol PPM.
Potassium	3320	3180
Phosphorus	2010	1960
Calcium	290	280
Manganese	10,5	9,8
Magnesium	460	435
Zinc	14	12
Iron	6	6
Copper	5	5
Boron	7	7

In general, saving to a period for not less than one month in solutions such as Formalin 10% and Alcohol 70% leads to a decrease in the concentration of some elements, as we noticed in the tables above, such as Potassium and Phosphorus, Calcium, Manganese and Magnesium, while when this period exceeds six months and up to a year, an increase will happen in some other heavy metals concentrations such as Zinc, Iron, Copper and Boron. Results of this study agreed with what was said by (23).

From this point, we must know the differences occurring in saving fish between diluted Formalin concentration of 10% and diluted Alcohol concentration of 70%. We notice that most of the fish samples saved in Alcohol remain freshness somewhat more than Formalin which gives objects hardness up to high rubber, in addition to pale coloration in the samples saved in Alcohol less than in Formalin in which the fish sample loses its color and the eye gleaming membrane be unclear (24). Heavy elements were not affected significantly in the different saving periods by Alcohol, while changed clearly in Formalin. We find a clear difference in both solutions Alcohol and Formalin where the first one is less resistance to the surrounding environmental conditions of the sample especially when the temperature is changed, in addition to the rapid evaporation in Alcohol and its properties are changed according to the pH values of this diluted solution of Alcohol, while Formalin is affected by the external circumstances surrounding the sample less than Alcohol and gained a high degree of constancy, even when the temperatures are changed and risen as well as pH values are changed , knowing that the process of Formalin evaporation is less than of Alcohol (25) and (26).

References

1- Canli; M. And Kalay, Ay. M.(1998). Levels of Heavy Metals(Cd, Pb, Cu, Cr, and Ni in Tissue of *cyprinus carpio, Barbuscapito,* and *chondrostoma regium* from seyhan River, Turkey. Tr. J.Of Zoology, 22: 149-157.

2- Gulfraz, M.; Ahmad , T. And Afzal, H. (2001). ConcentrationLevels of Heavy and Trace Metals in the Fish and Relevent Water from Rawal and Mangla Lakes. Online Journal of Biological Science, 1(5): 414-416.

3- Vanden Broek, J. I.; Gledhill, K.S. And Morgan, D. G. (2002). Heavy Metal Concentration in the Mosquito Fish *Gambusia holbrooki* in the Manly Lagoon Gatchment. In: UTS , Freshwater Ecology Report 2002 Depertment of Environmental science,University of Technology ,Sydney.

4- Dallinger, R.;Prosi,F. ;Segner,H.and Black,H. (1987).Contamination Food and Uptake of Heavy Metal by Rainbow Trout(*Salmo gairdneri)* A field Study. Oecologia,73:91-98.

5- Olaifa, F. E.; Olaifa, A. K.; Adelaja, A. A.; Owolabi, A. G.(2004). Heavy Metal Concentration of *Clarias gariepinus* from A lake and Fish from in Ibadan, Nigeria. African Journal of Biomedical Research, Vol.7: 145-148.

6- Jassem M. Salman, Fekret M. Hussan & Mason M. Saleh.(2007). Concentrations of Nine heavy elements in the fish muscles Hamri ***Barbus grypus*** and Shilluk ***Aspius vorax*** Heckel and Silver carp ***Hypophthalmicthyes molotrix*** Collected from the Euphrates River, Environmental Research and Sustainable Development Magazine,Vol. 10: 5-20.

7- Kelvin K. P. Lim & N. Sivasothi.(1994). A guide to methods of preserving animal specimens in liquid preservatives. Originally prepared for the Zoo Hons '94 field course.

8- Arannilewa, S.O. Salawu, A.A. Sorungbe and B.B. Ola-Salawu.(2005). Effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish (*Sarotherodun galiaenus*). African Journal of Biotechnology Vol. 4 (8), pp. 852-855.

9- Ke Liang B. Chang ,* Jejia Chang , Chyuan-Yuan Shiau , and Bonnie S. Pan.(1998). Biochemical, Microbiological, and Sensory Changes of Sea Bass (***Lateolabrax japonicus***) under Partial Freezing and Refrigerated Storage, Department of Marine Food Science, National Taiwan Ocean University, Keelung 202, Taiwan, Republic of China, J. Agric. Food Chem., 1998, 46 (2), pp 682–686.

10- Keith L Bosley, Sam C Wainright.(2011). Effects of preservatives and acidification on the stable isotope ratios ($^{15}N:^{14}N$, $^{13}C:^{12}C$) of two species of marine animals, Canadian Journal of Fisheries and Aquatic Sciences, 1999, 56(11): 2181-2185, 10.1139/f 99-153.
11- Thomas L. Deardorff and Richard Thorm.(1988). Commercial blast freezing of third stage ***Anisakis simplex*** larvae encapsulated in Salmon and Rockfish, J.Parasit. 74(4): 600-603.
12- AOAC,1980. Official Methods of Analysis. 13th Ed. Association of Official Analytical Chemists. Washington , D.C.
13- J. Syväranta , A. Martino, D. Kopp, R. Céréghino and F. Santoul, **(2010)**. Freezing and chemical preservatives alter the stable isotope values of carbon and nitrogen of the Asiatic clam **(*Corbicula fluminea*)**, Hydrobiologia **,** January 2011, Volume 658, Number 1, Pages 383-388.
14- S.T. Arannilewa, S.O. Salawu, A.A. Sorungbe and B.B. Ola-Salawu **,(2005).** Effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish **(*Sarotherodun galiaenus*)**, African Journal of Biotechnology Vol. 4 (8), pp. 852-855.
15- Obuz E, Dikeman ME **(2003).** Effect of cooking beef muscle from frozen or thawed states on cooking traits palatability. Meat Sci. 65: 993-997.
16 - Kropf DH, Bowers JA (1992). Meat and meat products. In Bowers (eds.), Food Theory and applications. New York: Macmillan publishing company.pp.22-29.
17- Gulfraz, M.; Ahmad , T. And Afzal, H. (2001). Concentration Levels of Heavy and Trace Metals in the Fish and Relevent Water from Rawal and Mangla Lakes. Online Journal of Biological Science, 1(5): 414- 416.
18 - Canli; M. And Kalay, Ay. M. (1998). Levels of Heavy Metals (Cd, Pb, Cu, Cr, and Ni in Tissue of *cyprinus carpio, Barbus capito,* and *chondrostoma regium* from seyhan River, Turkey. Tr. J. Of Zoology, 22: 149-157.
19- Jorgensen, L. A. And Pederson,B. (1994). Trace Metal in Fish Used for Time Trend Analysis and as Environmontal Indicators. Envi. Bull., 28(4): 235-254.
20 - H.A. Kaoud* and A.R. El-Dahshan. 2010. Bioaccumulation and histopathological alterations of the heavy metals in *Oreochromis niloticus* fish. Nature and Science ., 8(4): 152-154.
21- Nimmo DR, Rigby RA, Bahner LH, Sheppard JM. 1978. The acute and chronic effects of cadmium on the estuarine mysid *Mysidopsis bahia*. Bulletin of Environmental Contamination and Toxicology 19: 80–85.
22 - Nevo E, Noy R, Lavie B, Beiles A, Muchtar S.1986. Genetic diversity and resistance to marine pollution. Biological Journal of the Linnean Society 29: 139-144.
23 - Gibbs RH Jr, Jarosewich E, Windom HL.1974. Heavy metal concentrations in museum fish specimens: effects of preservatives and time, Science. Apr 26;184(4135):475-7.
24- Steve W. Gotte and Robert P. Reynolds.1997. Observations on the Effects of Alcohol Vs. Formalin Storage of Amphibian Larvae, USGS Patuxent Wildlife Research Center Division Of Amphibians And Reptiles National Museum Of Natural History Washington, D. C. 20560.
25- Simmons, John E. 1991. Conservation problems of fluid-preserved collections, Texas Tech University Press, 69-89.
26- Simmons, J. E. 1994. Blythe Spirits: Problems and Potentials of Fluid Preservation. Paper presented at the 1992 ASIH Workshop on Collection Care and Management Issues in Herpetology and Ichthyology. gopher://kaw.keil.ukans.edu:70/00/curation/ichs_herps/1992_3.