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Chemical evaluation of the quality of farmed fish in Kafr El-Sheikh city in Egypt

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ABSTRACT

The present study was conducted in farmed fish in Kafr El-Sheikh city Egypt to find out the chemical evaluation of the quality of most famous types of fish in Kafr El-Sheikh city in Egypt to determine the nutritional value of these cultured freshwater fishes. Quality indices were evaluated in 90 samples from three different fish species collected randomly from farms of Kafr El-Sheikh city (*Mugil cephalus*, *tilapia niloticus* and *Clarias gariepinus*) 30 of each were collected from different farms in Kafr El-Sheikh city, Egypt. The results of pH, total volatile nitrogen (TVN), Trimethylamine (TMA), Thiobarbituric acid (TBA) and Histamine were 5.20±0.154, 28.55±3.09, 4.09±0.24, 3.50±0.144 and 15.49±2.05 for *Tilapia niloticus*, While were 5.25±0.112, 21.68±1.53, 3.31±0.351, 2.21±0.252 and 11.39±1.43 for *Mugil cephalus*. In addition, they were 9.20±1.74, 25.98±1.91, 4.36±0.543, 2.02±0.276 and 17.18±2.24 in *Clarias gariepinus*. This study can show the nutritional quality and freshness of *Tilapia niloticus*, *Mugil cephalus* and *Clarias gariepinus*.

1. INTRODUCTION

In Egypt, fish farming is a cash crop with higher benefits and net revenue than other agricultural fields (Abdel-Wahed et al., 2018). Aquaculture plays a critical part in development. The aquaculture sector grew around the world, including in Egypt, to suit human consumption needs (Asaad, 2008). Egypt produces roughly 73.8% of Africa's total cultured fish and ranks the ninth in the globe, producing about one million tons and accounting for 1.54% over all cultured fish (FAO, 2012).

Tilapia fishes have become an economically important fish in aquaculture around the world (Charo-Karisa et al., 2005). In Egypt Mullet fish (*Mugil cephalus*) are commercially important fish because they have high market value and have been successfully cultivated (Bahnasawy et al., 2009). Due to its big body weight, rapid growth and rich protein in excellent boneless flesh, the African catfish, or (*clarias gariepinus*) is regarded a viable species for fish cultivation in Egypt (Shourbela et al., 2014). Freshness is strongly tied to quality; it is one of the most significant parameters for the fish sector. Determination of post-mortem pH provides indications of physical properties changes occurring in fish muscle during storage period (Izumi, 2012). Volatile amines (TVN, TMA) are responsible for odour and flavour of fish that linger for several days after being caught and they are used to assess the quality of fish (Etienne et al., 2005). Estimation of (TBA) Thiobarbituric acid was used to evaluate the buildup of secondary oxidation products (Pegg et al., 2004). Fish is highly susceptible to biogenic amine formation, especially histamine, putrescine, cadaverine and

tyramine (Bunka et al., 2013). Blood pressure control, allergic response, cellular development and synaptic transmission are all important physiological actions of histamine in humans (Comas-Basté, 2019).

This study was carried out for evaluation the quality assessment of three types of fish species (*Mugil cephalus*, *Tilapia niloticus* and *Clarias gariepinus*) through measurement of general parameters (pH), total volatile nitrogen (TVN), trimethylamine (TMA), thiobarbituric acid (TBA) and histamine.

2. MATERIAL AND METHODS

2.1. Collection of samples:

Ninety random samples of farmed fishes represented by *Tilapia niloticus*, *Mugil cephalus* and *clarias gariepinus* (30 of each) were collected from the different fish farms. The weight of samples ranged from 200 g (*Tilapia niloticus*, *Mugil cephalus* and *Clarias gariepinus*). Each sample was kept in a separated plastic bag and preserved in an ice box then transferred to the laboratory for examination.

2.2. Determination of pH (Pearson, 2006):

Approximately 10 g of material was blended with 10ml of neutralized distilled water in blender. The homogenate was shaken continuously for 10 minutes at room temperature. The pH value (Bye model 6020, USA). The pH meter Calibration utilizing two buffer solutions with known pH (alkaline pH 7.01, acidic pH 4.01). Therefore, pH electrode was washed with neutralized water and then introduced into the homogenate after the temperature correction system was adjusted.

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2.3. Determination of Total Volatile Nitrogen (TVN) (FAO,1980):

10g of the sample was added to 30 ml of distilled water in a clean dry beaker and thoroughly mixed for 2 minutes with a blender. To achieve a pH of 5.2, two drops of 0.02M HCl were applied. The homogenate was heated to 70 degrees Celsius, then cooled to room temperature before being filtered. The outer ring was precisely filled with 2ml sample extract and 1ml saturated potassium carbonate (KCO₃). The Conway unit was rotated as gently as possible and the dish was covered and incubated at 36°C for 2 hours, HCl in the inner ring was titrated against 0.01M NaOH by using methyl red indicator (T1 ml).

$$\text{TVN}/100\text{g} = 26.88 \times (2-T1)$$

Where:

T1 = volume of NaOH consumed in the titration.

2.4. Determination of Trimethylamine (TMA):

The Conway test was used in the approach established by the FAO (1980). The interior compartment of the Conway dish was filled with 2ml of 0.05 M H₂SO₄, but the outer ring of the dish was filled with 2 ml of the sample extract and 1ml of saturated potassium carbonate (KCO₃). In addition, the dish was covered and incubated at 36°C for 2 hrs. As a result, the inner ring's H₂SO₄ was titrated against 0.01M NaOH by using methyl red indicator (T2 ml).

$$\text{TMA}/100\text{g} = 26.88 \times (2-T2)$$

Where:

T2 = volume of NaOH consumed in the titration.

2.5. Determination of Thiobarbituric Acid Number (TBA):

The method adopted for estimation of TBA by Pikul *et al.*, (1989) was applied as follow: -TBA test which depends on determination of malonaldehyde (MD) as an end product of lipid peroxidation. The extent of oxidative rancidity is normally reported as TBA number or values and expressed as milligrams of malonaldehyde equivalents per kilogram of the samples.

2.6. Determination of histamine by ELISA: RIDASCREEN® Histamine (Art. No.: R1604):

The purpose of this enzyme immunoassay was to determine the amount of histamine in plasma and urine. The test was used to determine histamine release in heparinized whole blood when used in conjunction with a supplementary kit (available for purchase separately, cat. no. BA E-1100). Histamine was first acylated quantitatively. A microliter plate format was used in the next competitive ELISA kit. The antigen was bound to the solid phase of the microliter plate the results were statistically evaluated by application of (ANOVA) test (Feldman *et al.*, 2003).

3. RESULTS

Table 1 Analysis of PH, histamine, TVN, TMA and TBA in different fish types

Variables	Source of Variance	Degree of freedom	Sum of squares	Mean square	F	P value
PH	Between groups	2	315.936	157.968	7.651	.00**
	Within groups	87	1796.155	20.645		
TVN	Between groups	2	724.311	362.155	2.318	.105
	Within groups	87	13593.316	156.245		
TMA	Between groups	2	17.929	8.965	1.881	.159
	Within groups	87	414.607	4.766		
TBA	Between groups	2	531.31	265.65	2.34	.102
	Within groups	87	9843.78	113.14		

** indicate P< 0.01 and significant at 1% level.

Table 2 Minimum, maximum, mean and standard error (SE) of PH for different fish types

Fish	Min.	Max.	Mean ± SE
<i>Tilapia niloticus</i>	2.17	6.21	5.20 b ± 0.154
<i>Mugil cephalus</i>	3.62	6.30	5.25 b ± 0.112
<i>Clarias gariepinus</i>	3.23	35.21	9.20 a ± 1.42

The means of each factor designated by the same letter are not significantly different at the 5% level using Duncan's MRT.

Table 3 Minimum, maximum, mean and standard error (SE) of TVN mg % for different fish types

Fish	Min.	Max.	Mean ± SE
<i>Tilapia niloticus</i>	11.00	73.60	28.55 a ± 3.09
<i>Mugil cephalus</i>	11.90	40.50	21.68 a ± 1.53
<i>Clarias gariepinus</i>	12.50	64.40	25.98 a ± 1.91

The means of each factor designated by the same letter are not significantly different at the 5% level using Duncan's MRT.

Table 4 Minimum, maximum, mean and standard error (SE) of TMA mg% for different fish types

Fish	Min.	Max.	Mean ± SE
<i>Tilapia niloticus</i>	1.57	6.21	4.09 a ± 0.24
<i>Mugil cephalus</i>	1.18	7.11	3.31 a ± 0.351
<i>Clarias gariepinus</i>	1.02	13.02	4.36 a ± 0.543

The means of each factor designated by the same letter are not significantly different at the 5% level using Duncan's MRT

Table 5 Minimum, maximum, mean and standard error (SE) of TBAmg/Kg for different fish types

Fish	Min.	Max.	Mean ± SE
<i>Tilapia niloticus</i>	2.43	4.70	3.50 a ± 0.144
<i>Mugil cephalus</i>	0.20	5.84	2.21 b ± 0.252
<i>Clarias gariepinus</i>	0.19	5.20	2.02 b ± 0.276

The means of each factor designated by the same letter are not significantly different at the 5% level using Duncan's MRT.

Table 6 Minimum, maximum, mean and standard error (SE) of histamine for different fish types

Fish	Min.	Max.	Mean ± SE
<i>Tilapia niloticus</i>	1.14	42.25	15.49 a ± 2.05
<i>Mugil cephalus</i>	1.27	29.73	11.39 a ± 1.43
<i>Clarias gariepinus</i>	1.40	47.10	17.18 a ± 2.24

The means of each factor designated by the same letter are not significantly different at the 5% level using Duncan's MRT.

Table 7 Number (NO), percentage (%) and Chi square between fish types and acceptability

Variables	Permissible limit	<i>T. niloticus</i>		<i>M. cephalus</i>		<i>C. gariepinus</i>		χ^2	P value
		NO	%	NO	%	NO	%		
PH	≤6.5	30	100	30	100	20	66.67	22.5	0.00**
	> 6.5	0	0	0	0	10	33.33		
TV N	≤30	22	73.3	26	86.7	25	83.30	1.88	0.39
	> 30	8	26.7	4	13.3	5	16.70		
TMA	≤10	30	100	30	100	27	90.00	6.2	0.04 *
	> 10	0	0	0	0	3	10.00		
TBA	≤4.5	25	83.3	28	93.3	28	93.30	2.22	0.329
	> 4.5	5	16.7	2	6.7	2	6.70		
Histamine	≤20	22	73.3	25	83.3	19	63.30	3.06	0.216
	> 20	8	26.7	5	16.7	11	36.70		

4. DISCUSSION

Chemical indices such as (pH, TVN, TMA, TBA and histamine) are the most widely used parameters to evaluate fish quality and freshness. This search showed three types of farmed fish (*T. niloticus*, *M. cephalus*, and *C. gariepinus*) which examined chemically to determine their keeping quality and safety for human consumption. Concerning pH, the obtained results were nearly closed to those of Ibrahim (2017) who examined *M. cephalus* and *T. niloticus* and lower than those obtained by Sathivel (2005). Fish species differ in their optimal PH range, the pH range from 6.5 to 8.5 is generally recommended for freshwater fish, the pH of more than 11 or less than 4 become lethal to most fish species. Many freshwater fishes come from poorly buffered waters that are high in organic acids and thus do best in neutral to slightly acidic conditions, while marine fishes require a stable alkaline pH. pH values changed during each 24 hours, depending upon the temperature, quantity of plants (algae and others), and the size of the pond, so try to take the measurements at about the same time of day. The pH values were a suitable index for freshness assessment, and it can be useful as a guideline for quality of fish (Ruiz Capillas and Moral, 2001). According to (Table 1 and Table 2) the differences between species were significant between *clarias* and other two types but there is no significant between (*Tilapia* and *mugil*). Egyptian Organization for Standardization and Quality (EOSQ, 2005) had reported the critical limits of pH for chilled fish portion which should not be more than 6.5. 100% of samples accepted in *T. niloticus* and *M. cephalus* while, 66.67% of samples were accepted and 33.33% were not accepted in *C. gariepinus*.

Regarded to TVN, the protein degradative processes, occurring during fish perishing, produce an increase of typical substances, such as ammonia and volatile nitrogen compounds, and the concentrations of which are index of the deterioration status. The TVBN (Total Volatile Basic

Nitrogen) is a method of analysis that quantifies the presence of nitrogenous compounds (ammonia and dimethyl and tri methyl amine) in fish from the sea or from river, revealing the degree of freshness. Generally, TVN is synthesized by reaction from protein mainly as a result of microbial activity (Gamal *et al.*, 2011). Thus, TVN levels are affected by the method of catch, postmortem treatment and storage temperature as well as it differs according to fish species (Nazemroaya *et al.*, 2011). Liu *et al.*, (2011) mentioned that the TVB-N content in meat samples was an important indicator for estimating meat freshness. During the storage, the changes of TVB-N content in four kinds of meat samples were increased and the samples stored for less than 7 days in decompression room. After 10 days, the rapid increase of TVB-N content suggested that the quality of all samples depressed significantly.

It was clear that in tables (1, 3) obtained results were higher than those of Ibrahim (2017) and Mahmoud (1990). There were no significant differences between species. In Table 7, according to EOS (2005) TVN permissible limit (30) so, in *T. niloticus*, 37.3% accepted and 26.7% not accepted, in *M. cephalus* 86.7% and 13.3% and in *C. gariepinus* 83.3% accepted and 16.7 not accepted.

Ali *et al.* (2010) reported that the spoilage was the result of whole series of complicated deteriorative changes brought about in dead fish tissue by its own enzyme, by bacteria and by chemical action. The early reaction of spoilage is autolytic and bacterial enzymes become progressively the more active in the later storage. After catching the fish, the oxygen supply in the tissue ceases due to disruption of the circulatory system. In short time of post-mortem, the mitochondrial system ceases to function. Adenosine triphosphate (ATP) is gradually depleted through the action of various ATPase. After residual supplies of creatine phosphate have been depleted, anaerobic glycolysis continues to regenerate some ATP with the end product, lactate accumulation. Trimethylamine

Oxide Degradation by enzymes Trimethylamine oxide (TMAO) is found largely in most marine fish; in contrast, its presence is negligible or nil in freshwater fish. Two major enzymes including oxidoreductase and TMAO demethylase reduce TMAO to trimethylamine (TMA) during postmortem changes and associated biochemical and microbial reactions in fish muscle. TMAO is further degraded into dimethylamine and formaldehyde by enzymatic action and the odorless (TMAO) is reduced to tri methylamine, a volatile base. TMA regarded as a good index of quality for many fish species by Baixas-Nogueras *et al.* (2002). TMA differs according to species and also it is affected by storage time and catching season. The results in Table (4) were nearly similar to those of Mahmoud (1990) and Ibrahim (2017) and there is no significance between species. In Table (7) differences between samples within group were significant ($P < 0.05$). According to EOSQ (2005), the permissible limit of TMA was 10. Hence, all the examined samples were accepted in (*Tilapia*, *Mugil*) but in *Clarias* 90% accepted and 10% not accepted.

TBA is a significant quality index for fatty fish (Lynch and Frci, 1993). The higher lipids content of fish the higher TBA value in fish (Caponia *et al.*, 2004). The TBA factor is responsible for a rancid flavor, off odor, colors well as texture deterioration (Olafsdottir *et al.*, 1997). In Table (1,5), the differences among examined fish were highly significant ($P < 0.01$). In Table (7), according to Egyptian Organization for Standardization EOS (2005), the permissible limit of TBA was 4.5.

In *Tilapia* 83.3% accepted and 16.7 % not accepted, in *Mugil* and *clarias* 93.3% accepted and 6.7% not accepted.

Riebroy *et al.* (2004) mentioned that histamine has been used as a quality indicator for fresh fish Mohamed *et al.* (2011) mentioned that formation of high levels of biogenic amines especially histamine in fish product may be rapid, and their development depend on the number of microorganisms present. Histamine is a naturally occurring endogenous substance in the human body which is derived from the decarboxylation of the amino acid histidine. Histamine may also be present in certain foods containing free histidine and is generated by certain bacteria during spoilage and fermentation of fish. Histamine-rich foods may cause food intolerance in sensitive individuals and histamine contamination in fish and fish products.

The results recorded in Table (6) show that histamine levels "mg %" in the examined samples of farm fishes. In Table (7) according to EOSQ (2005) permissible limit (20), in *tilapia* 73.3% accepted, 26.7% not accepted in *Mugil* 83.3% accepted 16.7% not accepted and in *clarias* 63.3% accepted, 36.7 not accepted.

5. CONCLUSION

In conclusion, the present study indicated that chemical indices as (pH, TVN, TMA, TBA and histamine) are the most effective indicators for quality, freshness as well as nutritive value of fish. TVN and TMA appear as reliable indicators for determination of proteolytic activity; however, TBA is a good index for demonstration of fat rancidity. In contrast, pH is a poor indicator of fish quality as it is widely range of and freshness.

6. REFERENCES

1. Abdel-wahed, R.K.; Shaker, I, M.; Elnady, M.A. and Soliman, M.A.M. (2018): Impact of fish – farming management of water quality, plankton abundance and growth performance of fish in earthen ponds, Egyptian J. Of Aquatic Biology & fisheries, 22(1): 49; 63.
2. Ali, M. Y.; Sharif, M.D. I.; Adhikari, R.K. and Faruque, O. (2010): Postmortem variation in Total Volatile Base Nitrogen and Trimethylamine Nitrogen between Galda (*Macrobrachium rosenbergii*) and Bagda (*Penaeus monodon*). Univ.j. Zool. Raishahi. Univ. 28: 07 – 10.
3. Asaad, T.M.A. (2008): Bacteriological studies on *Aeromonas Hydrophila* in fish in Kafr El-Sheikh Governorate. In faculty of veterinary Medicine, Kafrelsheikh University.
4. Bahnasawy, M.; Khidr, A. and Dheina, N. (2009): Assessment of heavy metals concentration in water, plankton and fish of lake Manzla, Egypt, EJABF., 2036.
5. Baixas-Nogueras S., Bover-Cid, S., Veciana –Nogue, T. and Vidal-Carou, M.C. (2002): Chemical and sensory changes in Mediterranean hake (*Merlucciusmerluccius*) under refrigeration (6-8oC) and stored in ice. Journal of Agriculture and Food Chemistry 50, 6504-6510.
6. Bunka, F., Budinsky, P., Zimakova, B., Merhaut, M., Flasarova, R., Pachlova, V., Kuban, V. and Bunkova, L. (2013): Biogenic amines occurrence in fish meat sampled from restaurants in region of Czech Republic, Food Control 31, 49-52.
7. Caponio, F., A. Lestingi, C. Summo, M. T. Bilancia, and V. Laudadio (2004): Chemical characteristics and lipid fraction quality of sardines (*Sardina pilchardus* W.) influence of sex and length. Journal of Applied Ichthyology 20, 630-635.
8. Charo-Karisa, H.; Rezk, M.A.; Bovenhuis, H. and Komen, H. (2005): Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. Food chemistry, 99:83-91.
9. Comas-Basté, O.; Latorre – Moratalla, Maria L.; Sánchez- Pérez, Sónia; Veciana-Nogués, Maria T. and Vidal –Carou, Maria del Carmen (2019): Histamine and other Biogenic Amines in food. From Scombroid poisoning to Histamine Intolerance, Intechopen, 8:4333.
10. Dalgaard J., E., Ochenschlager, P., Jensen, B., Undeland, I., Mack, I. Egyptian Organization for Standardization and Quality Control (2005): Reports related to No. 3439, 2005 for chilled fish. Egyptian Standards, Ministry of Industry, Egypt.
11. Etienne, M.; Ifremer and Nantes (2005): Methods for chemical quality assessment volatile amines as criteria for chemical quality assessment sea food plus Traceability Valid, 16-22.
12. FAO (1980): Manual of Food Quality Control. FAO, United Nation, Rome, Italy.
13. FAO/WHO "Food and Agriculture Organization and "World Health Organization (2012): Public Health Risks of Histamine and other Biogenic Amines from Fish and Fishery Products, FAO headquarters, Rome Italy.
14. Feldman, D., Ganon, J., Haffman, R. and Simpson, J. (2003): The solution for data analysis and presentation graphics, second Edn., Abacus Lancrpts, Inc., Berkeley, USA.
15. Gamal, F. M., Eman, M. Hegazy and Abdelatef, M. (2011): Physicochemical properties and mycotoxins contents of *Tilapia* fish fillet after solar drying and storage. Global Veterinaria 7,138-148.
16. Ibrahim, M. S. A. (2017): Chemical indices for different types of fish and shellfish spoilage. Thesis, Master of Veterinary Medicine, Benha University, Egypt.

17. Izumi S. (2012): Spectral changes in fillet of Atlantic salmon as affect by freshness loss and spoilage during cold storage. A dissertation for degree of philosophiae doctor, Faculty of Bioscience, Fisheries and economics, Department of Norwegian College of fishery science.
18. Liu, J.; Guan, X.; Shen, Y. and Zhang, P. (2011): Identification of Meat Freshness Based on Particle Swarm Optimization and Support Vector Machine, 2nd International Conference on Biotechnology and Food Science, IPCBEE, (7): IACSIT Press, Singapore.
19. Lynch, S. M. and Frci, B. (1993): Mechanism of copper and iron- dependent oxidative modification of human low-density lipoprotein. *Journal of Lipid Research* 34, 1745-1753.
20. Mahmoud, Y. E. (1990): Studies on the sanitary condition of some Nile fish marketed in Kalyobia governorate. Thesis, Master of Veterinary Medicine. University of Benha, Egypt.
21. Mohamed, A. R.; Soher El-Saidy; A. A. El- Badawy; Hassan, S. and Xavier, F.M. (2011): Biogenic amine content in selected fermented foods as determined by Ion – exchange, Chromatography. *Journal of food protection*, 74(4): 681- 685.
22. Nazemroaya, S., Sahari, M. A. and Rezaei, M. (2011): Identification of fatty acid inMackerel and shark fillets and their change during six months of frozen storage. *Agriculture Science Technology Journal* 13, 553-566.
23. Olafsdottir, G., Martinsdottir, E., Ochlenschlager, J., Dalgaard, P., Jensen, B., Undeland, I., Mack, I. M., Henehan, G., Nielsen, J. and Nielsen, H. (1997): Methods to evaluate fish freshness in rear and industry. *Trends in Food Science and Technology* 8, 258-265.
24. Pearson, D. (2006): *Chemical Analysis of Foods*. 11th Ed, Publishing Co., Churchill Livingstone, Edinburgh, London, United Kingdom.
25. Pegg, R.B.; Wrolstad, R.E.; Acree, T.E.; Decker, E.A.; Penner, M.H.; Reid, D.S.; Schwartz, S.J.; Shoemaker, C.F.; Smith, D. M. and Sporns, P. (2004): Spectrophotometric measurement of secondary lipid oxidation products. *Handbook of food Analytical chemistry*. John Wiley and sons Inc.; Hoboken, NJ, USA: PP.547-564.
26. Pikul, J.; Leszezynski, D.E. and Kummerow, F. (1989): Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *Journal Agri, Food chemistry*, 37: 1309-1313.
27. Riebroy, S.S.; Benjakul, W.; Visessanguan, k.; Kijrongrojana; and Tanaka, M. (2004): Some characteristics of commercial som-fug produced in Thailand. *Food Chemistry*. 88: 527-535.
28. Ruiz-Capillas, C. and Moral, A. (2001): Correlation between biochemical and sensory quality indices in hake stored in ice. *Food research international*.34, 441-447.
29. Sathivel, S. (2005): Chitosan and protein coating affect yield, moisture loss, and lipid oxidation of apple pink salmon fillets during frozen storage. *Journal of Food Science* 70, 445-459.
30. Shourbela, R. M.; Abd El- Latif A. M. and Abd El-Azem, M. A. (2014): Induced spawning of african cat fish, *clarias gariepinus* using (gnrha) combined with dopamine antagonists, *Benha veterinary medical journal*, 27(1):25-35.