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Evaluation of the Quality of Fish Storage and Isolation, Identification of the Bacteria *Escherichia coli* and *Aeromonas hydrophila*

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ABSTRACT

The study aimed to evaluate the quality of fish storage and isolation, identification of bacteria *Escherichia coli* and *Aeromonas hydrophila*, because these bacteria are significantly contributor to foodborne disease and infection disease in humans. The totals of fish used in this study were 90 samples collection randomly from differently markets in Al-Shirqat during the period of study from first March to end May 2025. The results were increased significant of the pH value association with increase of storage period fish meat at 4 ºC, the pH value of fish meat increase during at 72 hours storage in 4 ºC (6.7±0.03), and lowest pH value in first periods 0 hours of storage (5.6±0.04). The Thiobarbituric acid value increase gradually with increased of storage period, the higher Thiobarbituric acid value record in 72 hours (1.18±0.004) during storage and the lowest Thiobarbituric acid value in first periods 0 hours (0.82±0.002) of storage. Cooking loss percentage significant differently ($P \leq 0.05$) between periods of storage. In 72 hours of storage, the highest cooking loss (16.7±0.03) percentage recorded and the lowest cooking loss (12.5±0.02) percentage recorded 0 hours. The results of water holding capacity was (29.5±0.02) decreased significant ($P \leq 0.05$) during storage fish meat to 72 hours at 4 ºC comparative with (36.3±0.05) in first periods 0 hours of storage. In conclusion the bacterial risks are closely related to food safety. The Vitek II used in this study because is one of the best devices to identify all types of pathogenic bacteria very accurately and within a short period, learn about their classification. The present study concluded that *Aeromonas hydrophila* and *Escherichia coli* were the most frequent bacterial species that have isolated from examined fish frozen samples. These bacterial could cause foodborne illness among the consumers. Also the pH and Thiobarbituric acid were increased significantly associated with increases of storage period of storage fish meat samples stored at 4 ºC. The cooking loss and water-holding capacity were lowest with advance of storage period.

INTRODUCTION

Fish is source of important protein for muscular health and was higher biologically value, meaning the contains all the essentially fatty acids, amino acids required, minerals and vitamins to supported of body function **(Noreen *et al*., 2025)**.

Fish are good source to human foods that promotes growthly and protections of body from diseases such as prevent ricket and mental diseases in children, coronary heart disease and cardiovascular **(Luo *et al*., 2022; Bashar *et al*., 2025)**. The fish is demand increase significant with the increasing in the world population because of effective feeds conversion, favourable taste and higher commercially value **(Tavares *et al*., 2021)**. Red and white meat can become contamination at any stage of production process, of skinning,plucking, cutting, washing and even frozen or refrigerated storage **(Abboud *et al*., 1999; Masoumbeigi *et al*., 2017)**. The eating imported fish and frozen is risk, especial when they are repeated frozen, when the ice melts from frozen fish which it provide good media to growthly of microbial. The bacterial activity are after several days of freeze and are pathogenic and harmful, leads to the rotten and decomposition of fish meats that become unsuitable to consumption **(Kim *et al*., 2017; Ajena, 2021; Al-Bayati *et al*., 2024)**. This raw meat is an ideal breeding ground for microorganism making it susceptible not only to spoilage but also to contamination with foodborne pathogen such as *Listeria*, *Staphylococcus aureus*, *E.coli*, *Salmonella* or *Campylobacter* which cause illness and death worldwide, especialized in development countries **(Petternel *et al*., 2014; Mercer *et al*., 2015)**. The toxins production by *E. coli*, especially Shiga toxin, causes food poisoning in the consumer causes eat fish contamination with bacterial or toxin **(Khan *et al*., 2013; Kim *et al*., 2020; Jasim *et al*., 2021)**. Pathogenics strain, such as Shiga toxin product by *E. coli*, can lead to significant foodborne illnesses, moreover *E. coli* is considered as problematic particularly when it is present in ready- to-eat food **(Essa *et al*., 2020; Zhang *et al*., 2025)**. The *Aeromonas hydrophila* is common found in freshwater environment. It is recognized as a major pathogenically in aquaculture, causing significant morbidity and mortality among various fish species **(Semwal *et al*., 2023**; **Shakir *et al*., 2024)**. This bacterium has significantly effect on the welfare and productivity of various fish species, especially carp **(***Cyprinus carpio L*.**)**, and is therefore considered one of the main causes of disease in fish farms **(Pereira, 2023; Jumma, 2024; Sabah *et al*., 2024)**. Also the fish may potentially be a cause of foodborne *Aeromonas* species, which have been identified as emerging foodborne bacteria causing a severe hazard to public fitness **(Batra *et al*., 2016; Alhtheal *et al*., 2024))**. **Igbinosa *et al*., (2012)** who reported *Aeromonas spp.* are related to food poisoning and many human sicknesses, including extra-intestinal and gastrointestinal infections, skin and soft tissue diseases, traumatic diseases, and respiratory-urinary tract diseases. The important of *Aeromonas spp.* in human gastrointestinal disorders are *A. sobria*, *A. caviae*, and *A. hydrophila* **(Stratev *et al*., 2012; Ammar *et al*., 2019)**. The aimed of this study to evaluation of the quality the fish meat storage and isolation, identification of bacteria *Escherichia coli* and *Aeromonas hydrophila*, because these bacteria are significantly contributors to infectious diseases and foodborne illnesses in humans.

MATERIALS AND METHODS

Fish study

The totals of fish used in this study were 90 samples collected randomly from different markets in Al-Shirqat, during ten times **(**9 fish in one time**)** through the period of study from first March to end June 2025.

Evaluation of the quality of fish frozen

Chemical tests

The pH Measurement

The pH meter **(**Metter Toledo Delta320, Switzerland**)** used to measure of pH value for fish meat samples, according to the procedure **(Lee *et al*., 2016; Kim *et al*., 2023)**.

Take 5g from each fish meat sample was homogenizations with 20mL of distil water, and then filtration by paper filter Whatman and the pH of suspensions was measure by used pH meter. The stander buffer at 4.00 and 7.00 was applying to probe of pH meter for Calibration method.

Thiobarbituric acid (TBA) Measurement

The direct extraction method, suited for fish meat, begins by mixing 10 grams of minced fish with 25 milliliters of a cold 7.5% trichloroacetic acid solution. Following filtration of extract, a portion of the filtrate is combined with TBA and heated in a boiling water bath to 35 min. After cooling, the resulting solution is measured against a reagent blank and comparative to standard prepared using 1,1,3,3 tetraethoxypropane. The calibration curve is then created by plotting micrograms of malondialdehyde against absorbance values. Using this plot, TBARS are calculated and expresses as milligrams of malondialdehyde per kilogram of sample **(Sun *et al*., 2001; Irwin and Hedges, 2004)**.

Physical tests

Cooking loss

The placing 25g of each sample, in closed polyethylene bags, then put in the water bath in 75 ^{o}C to 50 minute, after cooking fish meat samples were dried by filter paper. The cooking loss was determined in each sample as a percentage ratio between before and after cooking weight **(Honikel, 1998)**.

Water holding capacity (WHC)

The samples of meat are cutting from fish and immediate weight. A sample weight of 25g and place in the net and then suspended at an inflated bag, ensuring that samples does not make contact with bag and sealed. After storage period **(**24, 48 and 72

hrs.**)** at cold temperature **(**4 ^{o}C**)**, and again samples weight **(Honikel and Hamm, 1994; Honikel, 1998)**.

Meat Samples for Isolation and identification of bacterial strains

The collection of samples from stored fish meat were immediately placed in individually labeled tubes and sent to the microbiology laboratory for cultivating and isolating bacteria using primary culture media on the same day, then identification of bacteria by using the Vitek II system.

Isolation and identification of bacterial strains

The meat samples preparation: take 30g from all fish meat and sliced to small pieces and then put in a vial that was labeled and filled with peptone water and buffered peptone water. The little fragments were standardized. The peptone water was streaked over mannitol salt, MacConkey, chocolate, and blood agars using a sterile loop. The plates with streaks were subjected to incubation under both anaerobic and aerobic condition at temperature of 37°C to duration 24 hrs. Subsequently, the VITEK II system as used to validate the identity of every purified biochemical test colony. Using its card system, the VitekII Gram-negative automated system analyzer usually used to identify most Gram-Negative bacteria. The system performs inhibition and resistance, 64 biochemically tests measure carbon source utilization and enzymatic activity.

Statistical analysis

The analysis of data was by used **(**SAS, Version 9.1.**)**. The least significantly difference **(**LSD**)** post hoc test was used to assess significantly difference between means using two methods: ANOVA and the least significantly difference post hoc test. Statistically significantly is defined as probability level at **(**$P \leq 0.05$**)**.

RESULTS AND DISCUSSION

The results were significant increase ($P \leq 0.05$) in pH value association with advance period of storage for fish meat in 4 ^{o}C. The pH value of fish meat samples were significantly (6.7±0.03) during at 72 hours storage in 4 ^{o}C, comparative with other time stored, and the lowest PH value recorded in first periods 0 hours (5.6±0.04) of storage (Table 1). The impact of storage period was significantly ($P \leq 0.05$), the TBA concentration increase gradual with the advance period of storage, the higher TBA value showed at 72 hrs (1.18±0.004) during storage, and the lowest TBA value recorded in first periods 0 hours (0.82±0.002) of storage in all groups (Table 1). The results revealed that the cooking loss percentage significant differently ($P \leq 0.05$) between periods of storage. In 72 hours of storage, the highest cooking loss (16.7±0.03) percentage recorded and the lowest cooking loss (12.5±0.02) percentage recorded 0 hours (Table 1). The results obtained from the study revealed that the effect of the period was (29.5±0.02) decreased significant ($P \leq 0.05$) during storage fish meat to 72 hours at 4 ^{o}C. Table (1) showed lower of water-holding capacity percentage with advance of period storage. The results of

present study were identified 70 strains by using Vitek II system. The identification of bacterial including: *Aeromonas hydrophila* (20) (Fig. 1), *Escherichia coli* (13), *Aeromonas veronii* (8), *Aeromonas sobria* (7), *Klebsiella oxytoca* (7), *Citrobacter freundii* (4), *Citrobacter amalonaticus* (4), *Citrobacter braakii* (3), *Enterobacter cloacae* (2) and *Enterobacter aerogenes* (2). The present study reported the differently types of bacterial during isolations of samples from frozen fish and *Aeromonas hydrophila* and *Escherichia coli* constituting highest proportion of isolations during identification of bacteria (Table 2). The bacteria prevalent types in frozen fish were *Aeromonas hydrophila* (28.57%) and *Escherichia coli* (18.57%), accounting for 50% of the isolates.

Table 1. The percentage of chemically and physically tests of fish meat samples during period storage at 4 ^{o}C

Hours storage	Chemical tests		Physical tests	
	pH	Thiobarbituric acid	Cooking loss %	Water holding capacity
0 hour	5.6±0.04 b	0.82±0.002 c	12.5±0.02 c	36.3±0.05 b
24 hours	6.2±0.02 c	0.87±0.001 c	12.8±0.02 c	33.1±0.03 c
48 hours	6.4±0.01 c	0.92±0.002 b	13.3±0.02 b	32.2±0.03 c
72 hours	6.7±0.03 a	1.18±0.004 a	16.7±0.03 a	29.5±0.02 a
LSD	0.82	0.21	0.96	0.932

The small letter in same column is significant differently **(***P*≤0.05**)**.

Table 2. Identification types of Gram-negative bacterial by using the VitekII system

Bacteria strains	Number of strains Correctly indented	Diffusion rates of bacterial %
Aeromonas hydrophila	**20**	**28.57**
Escherichia coli	**15**	**21.43**
Aeromonas veronii	**8**	**11.43**
Aeromonas sobria	**6**	**8.57**
Klebsiella oxytoca	**7**	**10**
Citrobacter freundii	**4**	**5.71**
Citrobacter amalonaticus	**4**	**5.71**
Citrobacter braakii	**3**	**4.29**
Enterobacter cloacae	**2**	**2.86**
Enterobacter aerogenes	**1**	**1.43**
Total	**70**	**100**

Identification Information	Analysis Time	4.55 hours	Status:	Final
Selected Organism	97% Probability	Aeromonas hydrophila		
	Bionumber:	1625613551500251		
ID Analysis Messages				

Biochemical Details																
2	APPA	+	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAIap	-
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATK	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GLyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Fig. 1. Identification of the *Aeromonas hydrophila* by used Vitek II system

The results were highest significant difference ($P \leq 0.05$) in pH of the fish meat stored after 72 hrs this result agreement with **Gómez-Limia *et al*. (2021)** who showed increase pH during storage because growth of acidophilic bacteria or fungi that produce organic acids as byproducts during the degradation of nitrogenous or carbohydrates in fish. The differences in pH values of fish are attributed to the different storage periods, the longer the period of storage, the greater the production of nitrogenous substances, such as ammonia, that are produced by microbes or enzymes that are autolyzed in the muscle **(Goulas & Kontominas, 2005; Li *et al*., 2012)**. The pH change noted can be attributing to partial release of amino acids and carbonyl compounds due to protein destruction **(Ab-aziz *et al*., 2020)**. **Alizadeh-Sani *et al*. (2020)** who reported the higher of pH was because the enzymes activity, such as protease and lipase enzymes which results in highest volatile components, during the period of storage. Also, **Elnaggar *et al*. (2023)** who showed highest of pH number during cold stored in 4±1°C. The study findings do not align with the practice of storing meat and fish at room temperature. It was observed that pH value of fish meat gradually decreased over the period of storage. The first day, of pH value was recorded at 5.6, and this continued to decline, reaching 5.3 by the end of three-day storage period for the fish samples. These results are consistent with similar studies in the field **(Indiarto *et al*., 2023)**, the evaporation of water during storage causes to lower in moisture contents of above samples.

The result of this study showed higher significantly of Thiobarbituric acid **(**TBA**)** during increased storage time of fish. Lipid oxidation of meat product is primarily attributed to the non-enzymatic lipid oxidation. Thiobarbituric acid levels are employed as a lipid oxidation index in several foods **(Papuc *et al*., 2017; Jalloob *et al*., 2022)**. Throughout the entire storage time, the TBA value of the fish increased, indicating a secondary lipid oxidation **(Ayirezang *et al*., 2023)**. The result of Thiobarbituric acid values were gradually increased with the time of storage. These results were similarity with result reported by **(Hernández *et al*., 2009; Berizi *et al*., 2018)**. **Elnaggar *et al*. (2023)** who reported the highest of Thiobarbituric acid **(**TBA**)** values during cold stored at 4±1°C. **Fernández *et al*. (2012)** showed non-significantly difference in TBA

concentrations in salmon frozen in 18°C although there was a slight increased during freeze to sixty days for differently freeze period. **Fan *et al*. (2009)** noted that TBA level did not increased during the first 3 months of freezing stored in -20 °C for silver carp, while TBA concentrations increase with advance stored duration, from 1.06mg malonaldehyde/kg fish at the end of the third month to 1.52mg malonaldehyde/kg fish after 12 months of freezing. The results of cooking losses were significantly different with frozen storage period progressed. The cooking of meat causes decreases in the weight because of water loss during cooking. The compound water is not only lost during cooking and also several components such as salt, collagen, sarcoplasmic proteins, lipids, myofibrillar, flavor and compounds of polyphosphates that are also lost with the water **(Gerber *et al*., 2009; Sanchez *et al*., 2012)**. It was noticed that during period of frozen, the cooking loss levels increase and vice versa, cooking yield decrease **(Gehan *et al*., 2022)**. The higher in cooking loss during fish stored causes of muscle protein disintegration, subsequently denaturation. The protein is main substance that binding meat to water **(Dey, 2023)**. **Kassem and Emara (2010)** who reported the cooking causes the weight loss because moisture evaporation and dripping of melted fat. **Shi *et al*. (2015)** reported the cooking loss in silver carp storage up to 3 days in ice prior to thermal treatment tend to increases with the time of storage. Water holding capacity is the capacity of fish to maintain of original volume or increase it, the capacity is dependent on the method of handling the fish and the condition of the meat **(**fresh, chilled or cooked**) (Ruilova-Duval, 2009)**. The WHC is the capability of meat to holding all or part of its water and is one of the most important characteristics of meat quality **(Watanabe *et al*., 2018)**. The oxidization of proteins in processed meat products results in a lower in their water content WHC and capacity to form texture **(Xiong *et al*., 2000)**. Changes in structure during froze stored were directed relation to proteins denaturation and the sensory properties of muscles fish **(Lorentzen *et al*., 2020)**. Water-holding capacity is often associated with post-mortem structural changes, including the degradation of the muscle matrix and myosin protein denaturation **(Dawson *et al*., 2018; Xie *et al*., 2022)**. The decline in WHC during frozen storage may be attributed to moisture loss from the meat's surface. Several pre- and post-harvest factors, such as stress, feeding conditions, and rigor mortis, can influence WHC values **(Rotabakk *et al*., 2018; Abd *et al*., 2025)**. **Zhang *et al*. (2019)** showed a decrease in WHC values due to the growth of ice crystal during froze stored, which significantly contributes to protein denaturation. Changes in WHC in frozen fish muscles after thawing are clearly affected by freezing storage and are linked to alterations in myofibrillar proteins **(Tan *et al*., 2018)**. The results identified 70 strains by using Vitek II system. The identification of bacterial including: *Aeromonas hydrophila* (20), *Escherichia coli* (13), *Aeromonas veronii* (8), *Aeromonas sobria* (7), *Klebsiella oxytoca* (7), *Citrobacter freundii* (4), *Citrobacter amalonaticus* (4), *Citrobacter braakii* (3), *Enterobacter cloacae* (2) and *Enterobacter aerogenes* (2). The results of this study reported differently types of bacterial during isolations from frozen

fish and *Aeromonas hydrophila* and *Escherichia coli* were constitute highest proportion of isolations during identification of bacteria (Table 2). The bacteria prevalent types in frozen fish were *Aeromonas hydrophila* (28.57%) and *Escherichia coli* (18.57%), accounting for 50% of the isolates. The current study results agreement with **Castro-Escarpulli *et al*. (2003)** who reported increased significantly in *Aeromonas* species isolation from sample of frozen fish. Alsso **Asmaa *et al*. (2023)** who reported the examined fish contamination by *Aeromonas spp.* was detected 12.2%, While **Castro-Escarpulli *et al*. (2003)** recorded a prevelance of 32.8% of *Aeromonas spp*. isolated from frozen fish. The Isolation *Aeromonas hydrophila* from frozen fish was 53.3% reported by **Zainab (2011)**. The results of this study were similarity with result reported higher contamination of frozen fish by *Aeromonas hydrophila* **(Nuha *et al*., 2021)**. The result study agreement with research by **Alttai *et al*. (2023)** showed increased of *E. coli* isolated from frozen fish in local markets. **Nuha *et al*. (2021)** who showed increase significantly of *E. coli* during isolations bacteria from frozen fish, this result agreement with findings this study. The result of *E. coli* bacteria was isolated from frozen fish was 35.3 percentages in study **Safana *et al*. (2025),** this result agreement with findings in present study. The existence of *E. coli* in meat frozen may pose risk to consumers, and this is consistent with **Abuelhassan *et al*. (2014)** who explained that the presence of pathogenic *E.coli* bacteria is a potential risk to human health because the ability of *E.coli* to survive in frozen meat. **Neshtiman *et al*. (2025)** showing the greatest isolation rates of *E. coli* was 20% in fish meat and 18.57% in gills isolation from fish frozen.

CONCLUSION

In conclusion the bacterial risks are closely related to food safety. The Vitek II used in this study because is one of the best devices to identify all types of pathogenic bacteria very accurately and within a short period, learn about their classification. The present study concluded that *Aeromonas hydrophila* and *E. coli* were the most frequent bacterial species that have isolated from examined fish meat samples. These bacterial could cause foodborne illness among the consumers. Also, the pH and TBA were increased significantly associated with increases of storage period of storage fish meat samples stored at 4 ºC. The cooking loss and water-holding capacity were lowest with advance of storage period.

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