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Refractometer Evaluation of Eye's Nile Tilapia in Correlation to their Sensory and Bacteriological Qualities

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Abstract | Advances in the efficiency of fish freshness are crucial for optimizing quality assessment, and enhancing consumer safety. This has created the need for developing a novel method which can easily monitor fish quality and ensure trade safety in the fish markets. Therefore, the aim of the present study was to investigate the application of a refractometer device in the freshness and quality assessment of the Nile tilapia by measuring the fish eye's fluid salinity and its correlation to sensory and bacteriological parameters. A total of 150 Nile tilapia samples were sensory evaluated by the quality index method, then bacteriologically evaluated for total bacterial, enterobacteriacae counts, and for the incidence of *E. coli* and Salmonella. In addition, the fish eye's fluid salinity was measured by a portable refractometer. In the experimental part, 80 fresh tilapia fish were stored in ice for 8 days and periodically evaluated at 24-houres intervals. Results of the current study revealed that the mean value of the sensory score of tilapia was 3.68 demerit points (maximum 21 with the lowest quality). It was obvious that 52% of samples were graded symbol A with excellent quality, 32.6% were graded symbol B with good quality, and 15.3% were graded symbol C with acceptable quality, while none of the examined samples were rejected. The mean values of total aerobic counts and enterobacteriacea counts were 3.20 $x10^4$ and 5.69 x 10^1, respectively. The incidence of *E. coli* and *Salmonella* was 38% (n=57) and 0.67% (n=1), respectively. Serological identification of isolates revealed that the *E. coli* were *O78:k80, O158:k-, O26:k60* and *O114:k90,* while the only *Salmonella* isolate was *S. entertidis*. Moreover, the mean value of the eye's fluid salinity was 6.61%. There were significant positive correlations ($p<0.05$) between the eye's fluid salinity on one and sensory and bacteriological results. Furthermore, the obtained results showed a significant increase ($p<0.05$) in fish eye's salinity along the storage period which strongly correlated with changes in total bacterial and enterobacteriacae counts. In conclusion, fish eye's salinity can be used as a novel quality criterion for Tilapia freshness using a refractometer. It is a valid support for managing perishable fish commodities and preventing possible consumer risk as well.

Keywords: Quality, Eye salinity, Nile tilapia, Refractometer, Sensory, Bacteriological

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INTRODUCTION

Fish is an excellent source of nutrients, offering good biological value proteins, fat-soluble vitamins, micronutrients and highly unsaturated fatty acids. It also contains various lipids with diverse chemical compositions, including triglycerides, diglycerides, free fatty acids, and phospholipids (Madhubhashini *et al*., 2024). Nile tilapia, *Oreochromis niloticus*, is one of the most widely distributed fish species, both in capture fisheries and aquaculture. It is highly commercialized due to its fast growth rate, economical price, and consumer preferences (Fouzi *et al*., 2023).

Fish is highly perishable food due to its high nutrient content, which promotes microbial growth and rapid spoilage. As a result, without good hygienic practices, storage, and transportation, it can deteriorate quickly, leading to a loss of quality (Bernardo *et al*., 2020). Moreover, fish consumption may subject consumers to foodborne pathogens from contaminated fish, posing serious public health risks. Foodborne diseases are among the most widespread global health concerns, with numerous reports identifying pathogenic *E. coli* and *Salmonella* species as the most significant pathogens (Saad *et al*., 2020).

The fish quality evaluation comprises the combined use of different methodologies, the most common methods are sensory evaluation, microbial inspection, chemical measurements of moisture content, volatile compounds, protein changes, lipid oxidation, and adenosine triphosphate, physical measurements, and foreign material contamination (Duarte *et al*., 2020). Sensory evaluation is a crucial factor in assessing the quality and freshness of fish and other aquatic related inspections. Proper use of sensory evaluation gives reliable and distinct information about fish (Atanda *et al*., 2023). The Quality Index Method (QIM) is one of the most commonly used sensory techniques for assessing fish quality. It is a nondestructive fish sensory evaluation tool widely applied in fish research and industry (Bernardo *et al*., 2020). QIMs generally employ a scoring system to assign numerical values to each attribute assessed, these scores can be weighted according to the significance of each attribute in assessing overall quality (Esteves and Aníbal, 2021).

It is crucial to develop QIM programs tailored to each fish species (species specificity) due to the inherent differences among them, which seems to be a disadvantage, since it restricts the procedure to qualify the species and does not allow generalization of the results (Bernardo *et al*., 2020). In addition, it needs trained and experienced assessors. Human panels tend to be costly and time-consuming, and there may be considerable variability in assessments both between and among individuals. As a result, subjective evaluations may often contradict findings from bacteriological and chemical analyses (Prabhakar *et al*., 2020).

Furthermore, studying the microbiological quality of fish is of great importance to public health, as it is directly linked to fish spoilage and food poisoning. It also offers an assessment of the fish's hygienic quality, taking into account temperature violations and hygiene practices during handling and processing (Sarkar *et al*., 2020). Standard microbiological analysis methods are frequently used to assess fish freshness. Despite the importance of these standard methods, they are expensive and time-consuming and require expert handling, often leading to significant variations in results (Atanda *et al*., 2023). Hence, new technologies have been developed to overcome these challenges and provide simpler solutions for evaluating fish freshness (Madhubhashini *et al*., 2024).

Food investigators worldwide are striving to develop fast and reliable techniques for assessing fish quality, with the goals of enhancing market value, extending shelf life, and safeguarding consumer health (Atanda *et al*., 2023). Refractometers are one of the popular analytical devices for measuring the refraction angle at the liquid-solid phase interface (refraction index). The refraction angle, which varies based on the solution's composition, enables refractometers to quickly assess the concentration of dissolved substances (Ugwu *et al*., 2018).

Only limited studies have been done on fish quality using the eye fluid refractive index (Gokoglu and Yerlikaya, 2004; Diez *et al*., 2021). Therefore, the overall goal of this study was an attempt to apply and validate the efficacy of a portable refractometer in fish quality assessment by measuring fish eye fluid salinity and correlating the results with standard laboratory analyses (sensory and microbiological). Additionally, the study monitored changes in Nile tilapia quality during 8 days of ice storage, with 24-hour interval examinations, to confirm that the refractometer serves as a fast and reliable screening tool for determining fish quality for food business operators.

MATERIALS AND METHODS

Collection of samples

Sample sizes were calculated based on the sample size calculator according to Calculator Net (2024). A total of 150 iced Nile tilapia samples, weight averaged 160±20g, were randomly collected from various local fish markets in Ismailia city. The fish samples were separately placed in clean sterile plastic bags and rapidly transferred in an icebox to the meat hygiene laboratory at the Animal Health Research Institute Ismailia branch under complete aseptic conditions. All collected fish samples were subjected to sensory, microbiological, and eye's fluid examination.

Sensory evaluation

Sensory evaluation was performed by five trained panelists by means of the quality index method (QIM) according to Fouzi *et al*. (2023), as shown in Table 1. These characteristics are assessed using a scoring system ranging from 0 to 3 demerit points. The sum of all parameters gives a total sensory score known as quality index (QI). Then fish quality was graded from A-D. Grade A (≤ 2 Demerit points) was excellent quality, grade B (>2-7 Demerit points) was good quality, grade C (>7 - 14 Demerit points) was acceptable quality, while grade D (> 14-21 Demerit points) was rejected quality.

Table 1: Quality index method (QIM) scheme for Nile tilapia (*Oreochromis niloticus*).

	Quality parameter	Scoring description	Demerit points
Appearance	Skin	Shiny grey	0
		Dull grey	1
		Discolored near gill and abdomen	2
		Discolored all over the body	3
	Scales	firm	0
		loose	1
Texture	Backside	In-rigor (firm & elastic)	0
		Finger mark disappear rapidly	1
		Finger mark leaves 1-2sec	2
		Finger mark leaves over 3 sec	3
	Belly	Firm	0
		Soft	1
Eyes	Cornea	Clear & transparent	0
		Slightly opaque	1
		Opaque	2
	Pupil	Clear black	0
		Grey	1
	Form	convex	0
		Flat	1
		Concave/ sunken	2
Gills	Colour	Blood red	0
		Pale red	1
		brown	2
	Mucus	Transparent	0
		Cloudy	1
		Milky	2
		Brownish Red	3
Odour		Fresh	0
		Neutral	1
		Sour	2
		Strongly sour	3
Quality index			0 - 21

Bacteriological evaluation

Sample preparation (ISO, 7218:2007)

From each fish sample, 25g of meat were aseptically taken and placed in 225mL of sterilized 0.1% buffer peptone water, then homogenized for 2-4 minutes to create a homogenate of the first serial dilution (10^{-1}). Then, the homogenate sample was serially diluted in tubes containing 9mL of 0.1% sterilized buffer peptone water to achieve 10-fold serial dilutions up to 10^{-6}.

Determination of total aerobic counts

The total aerobic counts of previously prepared samples were performed by using the 3M Petrifilm Rapid Aerobic Count Plate (RAC) as performed by 3M (2022a). They were placed on a flat surface, and the film of each was lifted. Using a sterile pipette, 1 mL of each dilution was dispensed perpendicularly onto the center of the bottom film. The film was then rolled back down. The inoculum was evenly spread across the growth area using a 3M Petrifilm flat spreader before the gel formed. The plates were left undisturbed for at least 1 minute to allow the gel to set, and then incubated at 35 ±1ºC for 24h in a horizontal position with the clear side facing up. All colonies, regardless of size, color or intensity, were counted and expressed as CFU/g.

Determination of enterobacteriaceae counts

The Enterobacteriacea counts in previously prepared samples were determined by using the 3M petrifilm Enterobacteriacea count (EB) plates as performed by 3M (2022b).

Detection of *Escherichia coli*

The original homogenate 10^{-1} was incubated at 35 ±2ºC for 18±2 hours. Detection of *E. coli* was carried out by 3M™ Petrifilm™ *E. coli*/Coliform Count Plate 3M (2022c). *E. coli* suspected isolates were serologically identified according to Kok *et al.* (1996) by slide agglutination technique using standard polyvalent and monovalent *Escherichia coli* antisera (Sifin Diagnostics Gmbh Berlin, Germany).

Detection of *Salmonellae*

The original sample in peptone water was incubated at 37˚C ±1˚C for 18 ±2 hours. One mL of this pre-enrichment sample were transferred into 10 mL rappaport vassiliadis broth, and incubated for 24 ±3 hours at 42ºC. Detection of salmonella was carried out by 3M™ Petrifilm™ Salmonella Express System 3M (2022d). Then serological identification was performed according to ISO 6579-3 (2014). In this guidance document reference is made to the "White-Kauffmann-Le Minor scheme', as described by Grimont and Weill (2007).

Fish eye's fluid salinity measurement

The salinity of fish eye fluid was determined using a hand held refractometer scaled for salinity, which ranged from 0 % to 100% as described by Gokoglu and Yerlikaya (2004). The equipment was calibrated with distilled water before measurements, by placing 3-5 drops on the refractometer. After calibration, eye fluid from each fish was carefully extracted from both eyes using a syringe. Then, 3-5 drops of the fluid were placed on the refractometer prism using a dropper, and the sample was covered with the built-in plate. The salinity was read from the built-in scale through the eyepiece at the opposite end of the cylindrical tube and recorded in parts per thousand (%).

Experimental design

A total of 80 fresh tilapia fish samples, 4-5 fish per kilogram, were brought from fishermen in Ismailia city. The samples were divided into 8 equal groups and alternately stored within crushed ice at a ratio of 1:3/4 (fish to ice) under refrigeration for 8 days, with continuous monitoring for changing the ice. A random group. of 10 fish, was daily picked up to analyze the changes of the following parameters during storage in ice at 4°C, starting from the arrival in the laboratory (1st day) and, subsequently, every 24 hours until the 8th day.

Sensory evaluation

A group of 5 panelists determined the changes of the sensory parameters of stored fish by means of the Quality Index Method (QIM) using the scheme (Table 1) proposed by Fouzi *et al.* (2023).

Bacteriological evaluation

The total aerobic counts of stored fish samples were performed according to the standard procedures of the 3M Petrifilm Rapid Aerobic Count Plates (RAC) 3M (2022a). While, the Enterobacteriaceae counts of stored fish samples were performed by using the 3M petrifilm Enterobacteriacea count (EB) plates according to 3M (2022b). Data obtained from the bacterial counts were expressed in CFU/g.

Fish eyes fluid salinity measurements

The changes in fish eyes fluid salinity were measured according to the standard procedures of refractometer according to Gokoglu and Yerlikaya (2004).

Statistical analysis

All values were presented as means ± standard error. The statistical analysis was performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Data subjected to one-way analysis of variance (ANOVA). Any significant differences were analyzed by the multiple comparisons procedure of LSD (least significant differences), using a level of significance of 0.05. Pearson's correlations were performed between all measured parameters.

RESULTS AND DISCUSSION

Consumers perceive fish as a healthy food, especially in comparison to animal meat, which serves as the primary substitute source of good quality protein. However, it is more perishable when compared to other food (Castrica *et al.*, 2021). Fish tend to spoil rapidly after harvesting during handling, transportation, and storage, requiring constant intervention to preserve quality (Atanda *et al.*, 2023). Therefore, safety and quality are the primary concerns for both fish consumers and processors (Rafafi and Sela, 2023).

The results in Table 2 revealed the sensory evaluation of examined tilapia samples in fish markets. The quality index scores of tilapia represented the sum of means of fish appearance, texture, eyes, gills, and odour evaluations. The mean value of quality index scores was 3.68 represented by 0.61 for appearance, 0.72 for texture, 0.88 for gills, 0.25 for odour, and 1.22 for eyes. The quality index score of zero represents the highest fish freshness quality, which as increased, the deterioration sets up to 21 as a maximum score. Additionally, the eye sensory evaluation showed that the mean score values of fish eye cornea, form, and pupil were 0.53, 0.63, and 0.07, respectively. By grading the examined samples according to quality index, it was obvious that 52% of samples were graded A with excellent quality (≤ 2 demerit points), 32.6% were graded B with good quality (> 2-7 demerit points), 15.3% were graded C with acceptable quality (>7-14 demerit points), while no samples were rejected (> 14-21 demerit points). Similarly, sensory results for tilapia fish evaluation were recorded by Prabhakar *et al.* (2020), Fouzi *et al.* (2023) and Hasan *et al.* (2023).

Table 2: Mean values of sensory evaluation and eyes fluid salinity of retail tilapia in fish markets (n=150).

	Min.	Max.	Mean±Standard error
Appearance (0-4 Demerit points)	0	3	0.61 ± 0.06
Texture (0-4 Demerit points)	0	3	0.72 ± 0.07
Gills (0-5 Demerit points)	0	2	0.88 ± 0.07
Odour (0-3 Demerit points)	0	1	0.25 ± 0.04
Eyes Cornea (0-2 Demerit points)	0	2	0.53 ± 0.04
Form (0-2 Demerit points)	0	2	0.63 ± 0.04
Pupil (0-1 Demerit points)	0	1	0.07 ± 0.02
Quality index* (0-21 Demerit points)	0	14	3.68 ± 0.31
Eye fluid salinity (‰)	5	11	6.61 ± 0.12

Quality index* is the summation of all quality parameters gives a total sensory score. As the number increases, the quality decrease.

Sensory assessment of fish's external appearance is the most effective and reliable method for determining its freshness. Implementation of the quality index method in the fish industry offers valuable insights on fish quality, playing a crucial role in ensuring efficient quality control and process management for producing high-quality fish products (Lauteri *et al.*, 2023). Additionally, it enables the indirect establishment of criteria for rejecting fish when necessary, whether for consumption, further transportation, or processing (Gutiérrez *et al*., 2015). For many years, consumers and traders have relied on the visual appearance of fish eyes to assess freshness, due to the significant changes that occur shortly after the fish is caught (Anisur, 2015).

The results in Table 2 showed the mean eye's fluid salinity values of examined tilapia measured by a refractometer. The mean eye's salinity value was 6.6%, with minimum 5% and maximum 11%. It has long been known that there is a strong correlation between the fish freshness and the fish eyes fluid refractive index (Diez *et al*., 2021). Similar previous research has reported the possibility of the evaluation of fish freshness by using the refractive index of eye fluid as Yapar and Yetim (1998), Gokoglu and Yerlikaya (2004) and Diez *et al*. (2021).

Measuring the light refraction properties of fish eye fluid provides a quick estimate of fish quality by correlating it with the levels of solutes present in the eyes. After a fish dies, surface moisture loss leads to drying and wrinkling of the eyes. Typically, fresh fish eye fluid is bright and transparent; however, this clarity diminishes over time due to drying (Murakoshi *et al*., 2013). This loss of water, along with increased membrane permeability, leads to a reduction of the intraocular pressure in the fish eye, resulting in blurred eyes and changes in light transmission properties. This affects the light refraction characteristics due to the increased concentration of solubles (Anisur, 2015). From other aspects, several previous studies have evaluated the freshness of fish by analyzing other eye characteristics (Lalabadi *et al*., 2020; Rafafi and Sela, 2023; Yildiz *et al*., 2024).

The results demonstrated in Table 3 declared that the mean value of total aerobic counts of examined tilapia were $3.20x10^4$ $\pm 5.19x10^3$. All samples fit with the permissible limits of the Egyptian Standards (ES, 3494/2005). These results were nearly similar to those recorded by Hassan and Elbahy (2022) who reported 6.73×10^4 as total aerobic counts of tilapia. While, higher results were reported by Sarkar *et al*. (2020), Ali *et al*. (2021), Ogunleye *et al*. (2021), Tsafack *et al*. (2021), Abd El-Maksod *et al*. (2023) and Hasan *et al*. (2023).

Generally, total aerobic counts in fish are linked to food safety hazards and can serve as indicators of quality and shelf life (Abd El-Maksod *et al*., 2023). Moreover, these counts reflect the hygienic conditions of the fish rearing environment, as well as the handling, transportation, and storage (Hassan and Elbahy, 2022). Microorganisms play a key role in fish spoilage, as their growth and metabolism lead to the production of various compounds, including biogenic amines, organic acids, sulfides, alcohols, aldehydes, and ketones, which contribute to the development of unpleasant and unacceptable off-flavors (Kim and Cho, 2011).

Table 3 showed that the mean value of enterobacteriaceae counts in the examined tilapia were 5.69×10^1 ± 3.22. Increased numbers of enterobacteriaceae can be linked to unsatisfactory storage and handling conditions at retail markets (Eizenberga *et al*., 2015). Enterobacteriaceae, particularly the food-poisoning strains, represent a significant group of microorganisms due to their frequent presence and activities that can negatively affect fish quality (Rawash *et al*., 2019). Additionally, several bacteria from this group are known to cause infections and mortality in both fish and humans (Hastein *et al*., 2006). Higher previous results were reported by El-Sherief (2015) who reported an *e*nterobacteriaceae count of $7.64x10^2$ cfu\g in examined tilapia samples. While, Saad *et al*. (2018) revealed that the mean value of enterobacteriaceae counts was 1.9 $x10^4$ cfu/g for *Oreochromis niloticus*. Additionally, Rawash *et al*. (2019) showed that enterobacteriaceae were detected a rate of 94% of the examined tilapia with a mean value of 2.65×10^3.

Results in Table 4 showed that the incidence of *E. coli* was 38% in examined retail tilapia in fish markets, which exceeded the maximum permissible limits of the Egyptian standards (ES, 889-1, 2009) which stipulated that fish should be free of *E coli*. Moreover, the serotyping of *E. coli* showed O78:k80 in 12%, O158:k- in 8%, O26:k60 in 10.67%, and O114:k90 in 7.33% of samples.

Escherichia coli has been involved in a number of gastroenteric diseases such as diarrhea (traveler's disease), vomiting, dysentery, fever, colitis, and hemolytic uremic syndrome with renal failure (Danba *et al*., 2011). Pathogenic *E. coli* is divided into two main categories, which are the extra intestinal *E. coli* and diarrheagenic *E. coli*. Moreover, there are currently six categories of diarrheagenic *E. coli*, including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli*

Table 3: Total aerobic and enterobacteriacae counts (cfu/g) of retail tilapia in fish markets (n=150).

	Positive samples		Negative samples		Minimum	Maximum	Mean ± Standard Error
	No	%	No	%			
Total aerobic counts	150	100	0	0	1.2×10^3	3.5×10^5	$3.20 \times 10^4 \pm 5.19 \times 10^3$
Enterobacteriacae counts	144	96	6	4	<10	1.9×10^2	$5.69 \times 10^1 \pm 3.22$

Table 4: Incidence and Serotyping of isolated *Escherichia coli* and *Salmonella* in retail fresh tilapia in fish markets (n=150).

	MPL *	Positive	Negative	Serotypes	No. of samples	% of samples
		No (%)	No (%)			
Escherichia coli	should not found	57 (38%)	93 (62%)	Polyvalent 111 O78:k80	18	12
				Polyvalent 1 O158:k-	12	8
				Polyvalent 1 O26:k60	16	10.67
				Polyvalent 1 O114:k90	11	7.33
Salmonella	should not found in 25g	1 (0.67%)	149 (99.33%)	*S.* entertidis	1	0.67

MPL* is maximum permissible limits stipulated by Egyptian standards (5021/ 2005) and (889-/2009). Percentage in relation to total number of samples (150).

(ETEC), entero-invasive *E. coli* (EIEC), entero-aggregate *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enterohaemoragic *E. coli* (EHEC) (Xiaodong, 2010). The isolation of highly pathogenic organisms like *E. coli* from fish indicates fecal contamination and environmental pollution of the fish habitat (Hassan and Elbahy, 2022). Consuming infected fish can transmit these pathogens to humans. Therefore, proper sanitary handling and correct processing practices are essential before consuming fish products (Ogunleye *et al*., 2021).

The results nearly agreed with Hassan and Elbahy (2022) and Marijani (2022), who reported that *E. coli* represented 36.6% and 39% of the examined fish, respectively. While, the results were lower than those of Hassan (2013), Valenzuela-Armenta *et al*. (2018), and Saad *et al*. (2020), who isolated *E. coli* from 57.1%, 81.6%, and 70% of the examined Nile Tilapia (*Oreochromis niloticus*) samples. However, the results were higher than Rawash *et al*. (2019), Ogunleye *et al*. (2021), Ali *et al*. (2021), and Abd El-Maksod *et al*. (2023), who reported that the incidence of *E. coli* in examined samples was 13%, 8%, 8%, and 24%, respectively.

Additionally, the incidence of *Salmonella* shown in Table 4 was 0.67% in examined retail tilapia in fish markets. *Salmonella* was absent in all fish samples except for one contaminated sample that exceeded the maximum permissible limits set by the Egyptian standards (ES 3494/2005), which recommended that fish should be free of *Salmonella* in 25g of sample. The isolated *Salmonella* serotype from the contaminated fish was *S.* enteritidis. These *Salmonella species*, which are highly pathogenic bacteria, are the leading cause of enteric diseases in both animals and humans, responsible for millions of illnesses globally, and are primarily linked to foodborne diseases as zoonotic agents (Van *et al*., 2012). The presence of *Salmonella spp*. indicates fecal contamination of the water where the fish were harvested (Abd El-Maksod *et al*., 2023). Alternatively, the fish may become contaminated with *Salmonella* after being caught. Therefore, a significant presence of *Salmonella species* suggests inadequate hygienic practices during the catching and distribution processes (Saad *et al*., 2018).

Similarly, several previous studies reported that *Salmonella* spp., were isolated from tilapia fish as Saad *et al*. (2018) recorded *Salmonella* in 24 %, Ahmed (2019) and Ogunleye *et al*. (2021) recorded *Salmonella* spp. in 2%, while, Hassan and Elbahy (2022) recorded *Salmonella* spp. in 33.3%, Marijani (2022) reported *Salmonella* spp. in 16%, and Abd El-Maksod *et al*. (2023) reported *Salmonella* species in 4%. While other studies reported that none of the examined fish samples contained *Salmonella* spp., as Eizenberga *et al*. (2015), Valenzuela-Armenta *et al*. (2018), Rawash *et al*. (2019), Sarkar *et al*. (2020) and Saad *et al*. (2020).

Table 5: Correlation coefficients of eye fluid salinity with sensory and bacteriological evaluation of retail tilapia in fish markets (n=150).

		Eyes fluid salinity	
		R^2	P-value
Sensory evaluation	Total quality index	0.967**	0.000
	Eye sensory evaluation	0.889**	0.000
Bacteriological evaluation	Total bacterial counts	0.742**	0.000
	Enterobacteriacae counts	0.864**	0.000

R^2= Pearson correlation of eye salinity with sensory and bacteriological evaluation. ** Correlation is significant at the 0.01 level (2-tailed).

Moreover, Table 5 revealed the correlation coefficients of tilapia eye's fluid salinity with sensory and bacteriological evaluations of retail tilapia in fish markets. The results showed that there were strong positive correlations, which were 0.967, 0.889, 0.742, and 0.864 with quality index score, eye sensory evaluation, total aerobic counts, and Enterobacteriacae counts, respectively. Similarly, Yapar and Yetim (1998) and, Gokoglu and Yerlikaya (2004) confirmed the correlation between fish freshness and the eye's fluid refractive index value.

In regard to the experiment results, the sensory evaluations of stored fish represented in Table 6 revealed that the quality index scores significantly increased ($p<0.05$) as storage time progressed, reflecting a decline in quality and a higher likelihood of fish rejection as storage time increased. The initial quality index score of zero demerit points reached up to a maximum demerit point of 20.2 at the 8th day represented as 3.9 related to appearance, 3.9 related to texture, 4.4 related to the gills, 3.0 related to odour, and 5.0 related to the eyes.

The stored fish initially displayed bright skin and a grayish color, which gradually faded to a dull and dark gray as the samples neared the point of unacceptability. Changing of the natural color of fresh fish during storage, leading to discoloration may have been due to pigment oxidation or other factors. It is known that lipids, when oxidized, convert into peroxides, aldehydes, ketones, and lower aliphatic acids (Wąsowicz *et al*., 2004). Regarding the initial firmness, it may be attributed to the natural stiffening of the muscle during the rigor mortis process, which decreases over the storage period. This reduction could result from proteolysis, causing the muscle to soften. The firmness of the fish flesh was assessed by applying finger pressure to the muscle, which returned to its original shape immediately after the pressure was released (Atanda *et al*., 2023). While concerning odor and gills color, they were significant characteristics that underwent considerable changes during the storage period. The gill color, which were initially bright red and free of mucus, transitioned to a red-brown color with white patches and thick mucus. Furthermore, the gill odor experienced significant changes throughout storage, shifting from a metallic smell to a blood-like odor, and eventually developing a rancid smell, which is deemed undesirable. Similar studies were performed by Gutiérrez *et al*. (2015), Rodrigues *et al*. (2016), Goliat *et al*. (2016), Sánchez *et al*. (2019), Atanda *et al*. (2023), Fouzi *et al*. (2023), and Lauteri *et al*. (2023).

By grading the tilapia samples according to quality index, it was obvious that fish at the first day were graded A with excellent quality (≤ 2 demerit points), while, fish stored till second and third day were graded B with good quality (> 2-7 demerit points). However, fish stored until the fourth to sixth day were graded C with acceptable quality (>7-14 demerit points). Finally, fish stored until the seventh to eighth day were graded D with rejected quality (> 14-21 demerit points). Similar grading of tilapia fish was performed by Fouzi *et al*. (2023). Significantly strong positive correlations were observed between the storage period and fish appearance (R=0.891), texture (R=0.882), gills (R=0.980), odour (R=0.923), eyes (R=0.887) and quality index score (R=0.932). The longer the fish is stored, the lower its quality becomes, as a higher quality index score indicates poorer quality (Atanda *et al*., 2023).

According to results in Table 7 and Figure 1, it was interestingly seen that eye sensory evaluations of stored fish represented by cornea, pupil, and form significantly changed along the storage time ($p<0.05$). The results revealed significant strong positive correlation between the storage period and eye quality parameters; cornea (R^2=0.878), pupil (R^2=0.709), and form (R^2=0.880). On the first storage day, the fish were still very fresh, with a shiny bright pupil and a clear cornea, and the eyes remained convex. By the second day, changes in the fish eyeballs began to appear. On the third and fourth days, signs of quality deterioration emerged, with the pupil turning grayish and the cornea becoming cloudy. From the fifth to seventh days, more noticeable quality decline occurred, with the eyeballs starting to appear slightly sunken. By the eighth day, dramatic changes were observed as eyes became deeply sunken, the pupil turned milky white and the cornea became cloudy and yellow. These changes were primarily due to the activity of microbes and endogenous enzymes in the fish eyes (Kunjulakshmi *et al*., 2020). Similar studies were performed by Rodrigues *et al*. (2016), Shi *et al*. (2018), Kunjulakshmi *et al*. (2020), and Fouzi *et al*. (2023).

Table 6: Changes in sensory evaluations of tilapia fish samples stored under ice at 4°C at daily examination for 8 days.

	Appearance 0-4 Demerit points	Texture 0-4 Demerit points	Gills 0-5 Demerit points	Odour 0-3 Demerit points	Eyes 0-5 Demerit points	Quality index* 0-21 Demerit points	Grading**
1st Day	0.0±0.00^{f}	0.0±0.10^{g}	0.0±0.00^{f}	0.0±0.00^{f}	0.0±0.00f	0.0±0.10^{f}	A
2nd Day	0.2±0.13^{f}	1.0±0.00^{f}	0.3±0.15^{f}	0.3±0.15^{f}	0.2±0.20^{ef}	2.0±0.56^{f}	B
3rd Day	0.8±0.20^{e}	1.3±0.21^{ef}	1.0±0.33^{e}	1.1±0.10^{e}	0.9±0.40^{e}	5.1±1.07^{e}	B
4th Day	1.5±0.26^{d}	1.8±0.29^{de}	1.9±0.18^{d}	1.3±0.15^{de}	2.1±0.38^{d}	8.6±1.09^{d}	C
5th Day	2.1±0.31^{c}	2.3±0.30^{cd}	2.2±0.13^{cd}	1.6±0.16^{cd}	3.5±0.34^{c}	11.7±1.13^{c}	C
6th Day	2.4±0.27^{bc}	2.7±0.21^{bc}	2.6±0.22^{c}	1.9±0.10^{c}	3.9±0.38^{bc}	13.5±0.99^{c}	C
7th Day	2.9±0.18^{b}	3.2±0.20^{b}	3.7±0.15^{b}	2.4±0.16^{b}	4.4±0.30^{ab}	16.6±0.78^{b}	D
8th Day	3.9±0.10^{a}	3.9±0.10^{a}	4.4±0.22^{a}	3.0±0.00^{a}	5.0±0.00^{a}	20.2±0.39^{a}	D
R^2	0.891**	0.882**	0.980**	0.923**	0.887**	0.932**	

Results represented as means ± standard errors. Means of the same column with different letters are significantly different ($p \le 0.05$). R^2= Pearson correlation with storage time, ** significant at the 0.01 level (2-tailed). Quality index* is the summation of all quality parameters gives a total sensory score. As the number increases, the quality decrease. Grading**: Grade A (< 2 Demerit points) was excellent quality, grade B (>2 - 7 Demerit points) was good quality, grade C (>7 - 14 Demerit points) was acceptable quality, while grade D (> 14 - 21 Demerit points) was rejected quality.

Table 7: Changes in total aerobic counts, Enterobacteriaceae counts, eye fluid salinity and eye sensory evaluation of tilapia fish samples stored under ice at 4°C at daily examination for 8 days.

	Total aerobic counts	Enterobacteriaceae counts	Eye fluid salinity	Eye sensory evaluations		
				Cornea (0-2 Demerit points)	Pupil (0-1 Demerit points)	Form (0-2 Demerit points)
1st Day	$1.58x10^3 \pm 9.28x10^{1\ f}$	$2.05x10^1 \pm 1.28^{\ g}$	$6.3 \pm 0.36h$	0.0 ± 0.00^e	0.0 ± 0.00^d	0.0 ± 0.00^f
2nd Day	$9.32 \times 10^3 \pm 1.67 \times 10^{2\ e}$	$4.25 \times 10^1 \pm 2.77^{\ f}$	$9.6 \pm 0.16g$	0.1 ± 0.10^{de}	0.0 ± 0.00^d	0.1 ± 0.10^f
3rd Day	$7.60 \times 10^4 \pm 2.54 \times 10^{3\ d}$	$5.98 \times 10^1 \pm 1.85^{\ f}$	12.8 ± 0.42^f	0.3 ± 0.15^{cd}	0.4 ± 0.16^c	0.4 ± 0.13^e
4th Day	$9.58 \times 10^4 \pm 9.97 \times 10^{2\ d}$	$8.46 \times 10^1 \pm 2.71^e$	17.1 ± 0.28^e	0.6 ± 0.16^c	0.6 ± 0.16^{bc}	0.9 ± 0.10^d
5th Day	$3.38 \times 10^5 \pm 1.90 \times 10^{4\ c}$	$1.52 \times 10^2 \pm 8.61^d$	20.0 ± 0.40^d	1.5 ± 0.16^b	0.8 ± 0.13^{ab}	1.2 ± 0.13^{cd}
6th Day	$5.45 \times 10^5 \pm 3.01 \times 10^{4\ bc}$	$2.36 \times 10^2 \pm 6.48^c$	$23.5 \pm 0.59^{\ c}$	1.7 ± 0.15^{ab}	0.8 ± 0.13^{ab}	1.4 ± 0.16^{bc}
7th Day	$8.47 \times 10^5 \pm 3.04 \times 10^{4\ b}$	$3.45 \times 10^2 \pm 8.61^b$	$32.8 \pm 0.40^{\ b}$	1.9 ± 0.10^a	0.9 ± 0.10^a	1.6 ± 0.16^b
8th Day	$2.61 \times 10^6 \pm 1.95 \times 10^{5\ a}$	$4.08x10^2 \pm 12.48^{\ a}$	40.8 ± 0.59^a	2.0 ± 0.00^a	1.0 ± 0.00^a	2.0 ± 0.00^a
R^2	0.785**	0.950**	0.964**	0.878**	0.709**	0.880**

Results represented as means ± standard errors. Means within column with different letters are significantly different ($P < 0.05$). R^2= Pearson correlation with storage time. ** = Correlation is significant at the 0.01 level (2-tailed).

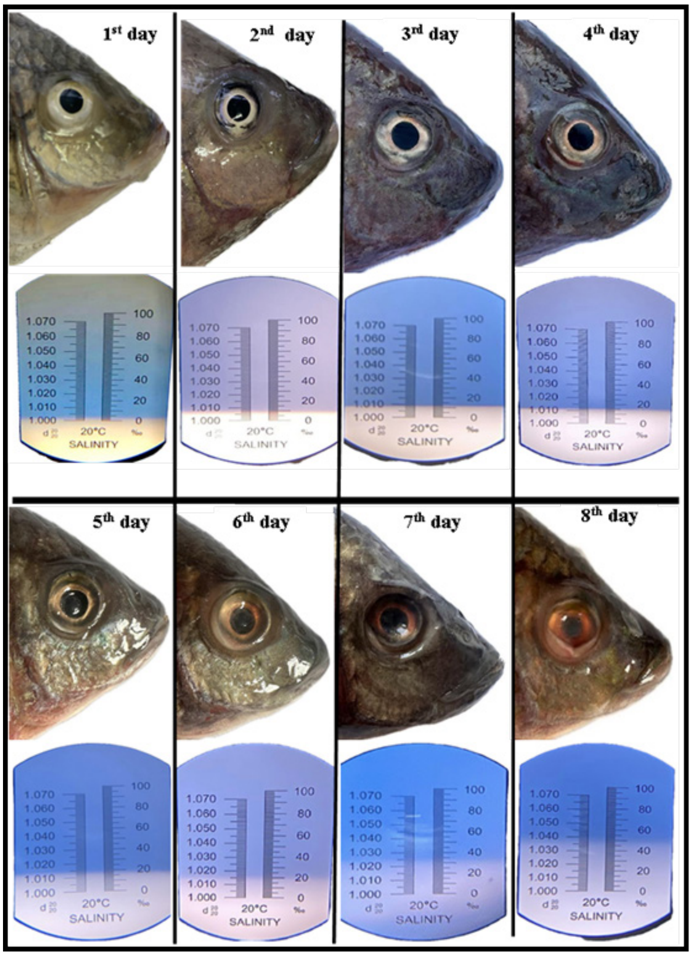

Figure 1: Changes in eye fluid salinity and eye sensory changes of tilapia fish samples stored under ice at 4°C at daily examination for 8 days.

The changes in tilapia eye's fluid salinity of stored fish measured by a refractometer. It was obvious that eye salinity values significantly increased ($p<0.05$) within storage. The initial value was 6.3±0.36% at the first day, indicating that the fish was still fresh. After 8 days of storage, the value reached 40.8±0.59 %. There was a strong positive correlation between eye fluid salinity and storage time (R^2=0.964). These results were in agreement with Yapar and Yetim (1998), Gokoglu and Yerlikaya (2004), and Ghattas *et al.* (2017), who reported that the refractive index values increased in refrigerated fish within days of storage. The glossiness of fish eyes diminishes over time, leading to a loss of wetness and brightness (Murakoshi *et al.*, 2013). This decrease in brightness and transparency during storage is linked to a change in viscosity, as compounds from the surrounding eye tissues transfer into the eye fluid, altering its composition. Consequently, the refractive index and relative viscosity of the fish eye fluid can be used as indicators to monitor the decline in freshness during storage (Liao *et al.*, 2016).

Additionally, the initial total aerobic count value of the stored fish was $1.58x10^3 \pm 9.28x10^1$ CFU/g, which significantly increased ($P<0.05$) with increasing storage time and finally reached $2.61x10^6 \pm 1.95x10^5$ CFU/g at the 8th day. There was a strong positive correlation between total aerobic counts and storage time (R^2 = 0.785). The acceptability limit for the total aerobic counts of fish should not exceed 10^6 as stipulated by the Egyptian Standards (ES 3494, 2005). According to this threshold, the samples became unacceptable after 7 days of cold storage. The longer the fish is stored, the lower its quality, even when kept at chilling temperatures. This occurs because the chilling temperature (2-4°C) is insufficient to completely inhibit bacterial growth (Hidayat, 2016). Similar results were previously reported by Ghattas *et al.* (2017), Sánchez *et al.* (2019) and Lauteri *et al.* (2023).

The fish storage had a noticeable effect on the increase of enterobacteriacae counts. The initial value was $2.05x10^1 \pm 1.28$ CFU/g. During storage, this value was increased to

4.08x10^2 ±12.48 at the 8th day. There was a strong positive correlation between enterobacteriacae counts and storage time (R^2= 0.950). Similar studies were previously reported by Ucar *et al.* (2020), and Mohamed and Ammar (2021), where significant increases in enterobacteriaceae counts were observed during the fish storage.

As shown in Table 8, the results revealed that there were statistically significant strong positive correlations between eyes fluid salinity and quality index scores, eye sensory scores, total bacterial counts and Enterobacteriace counts of stored fish. The correlations for these treatments were R^2=0.917, 0.852, 0.865, and 0.956, respectively. These correlation studies indicated that eye fluid salinity is a good freshness indicator and a good index of spoilage for tilapia under refrigerated storage. Similar results were previously reported by Gokoglu and Yerlikaya (2004) where a strong correlation between refractive index values and sensory scores was observed at storage.

Table 8: Correlation coefficients of changes in eye fluid salinity with sensory and bacteriological evaluation of tilapia fish samples stored under ice at 4°C at daily examination for 8 days.

Quality parameters		Eye salinity	
		R^2	P - value
Sensory evaluation	Quality index scores	0.917**	0.000
	Eye sensory evaluation	0.852**	0.000
Bacteriological evaluation	Total bacterial counts	0.865**	0.000
	Enterobacteriace counts	0.956**	0.000

R^2 = Pearson correlation of eye fluid refractive index with sensory and bacteriological evaluation. ** Correlation is significant at the 0.01 level (2-tailed).

Results in Figures 2 and 3 showed that the eye fluid salinity reached 32.8 on the seventh day of storage and marked the threshold for spoilage. Beyond this value, the total bacterial count would exceed permissible limits, and the quality index score of sensory evaluation would reach its maximum, indicating fish spoilage. Thus, it could be concluded that fish eye fluid salinity should remain below 32.8.

Fish represents a matrix of high nutritional value, with its production and consumption experiencing significant growth in recent years. Enhancements in the effective assessment of freshness are crucial for optimizing quality evaluation, enhancing consumer safety, and minimizing losses of raw materials. The current study, through the light on using the refractometer as simple, quick, and novel methods for reflecting the sensory and bacteriological quality of the tilapia fish based on their eye's fluid salinity. More studies still needed for trials using of refractometer on other fish species or processed fish.

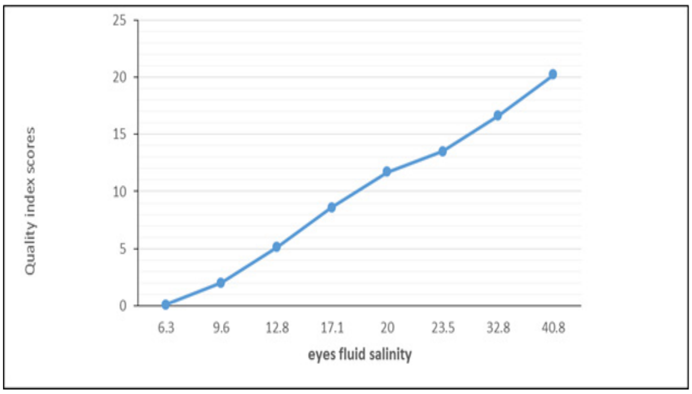

Figure 2: Relations between eyes fluid salinity and quality index scores of sensory evaluations of tilapia fish samples stored under ice at 4°C at daily examination for 8 days.

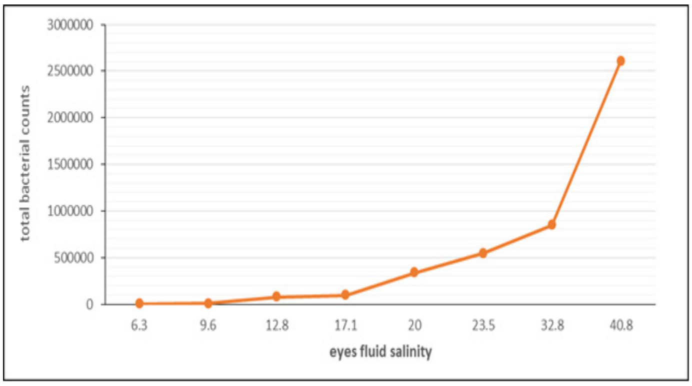

Figure 3: Relations between eyes fluid salinity and total bacterial counts of tilapia fish samples stored under ice at 4°C at daily examination for 8 days.

CONCLUSIONS AND RECOMMENDATIONS

The portable refractometer can be effectively used as a simple, rapid, novel method to assess and monitor the freshness, sensory and, bacteriological quality of Nile tilapia by measuring its salinity of eye fluid. This method is easy to use, and readily accessible, requires minimal trained employees, less costs, or analysis time, as well as provides reliable results. It can be adopted by authorities, producer associations or consumers as a dependable procedure when necessary. This approach could provide valuable support for the fish industry and retail in managing perishable tilapia fish at markets.

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NOVELTY STATEMENT

The novelty of our work entitled "*Refractometer Evaluation* *of Eye's Nile Tilapia in Correlation to their Sensory and Bacteriological Qualities*" can be summarized a The portable refractometer can be effectively used as a simple, rapid, novel method to assess and monitor the freshness, sensory and, bacteriological quality of Nile tilapia by measuring its salinity of eye fluid.

AUTHOR'S CONTRIBUTION

Idea, research design and revising the final version done by Ali Meawad Ahmed, methodology, chemical analysis, data curation, statistical analysis and writing the original draft done by Asmaa I.A. Morsy, Rehab E.M. Gaafar, Nada Ibrahim H. Ahmed. All authors. All authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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