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Enhancing the Value Added and Quality Characteristics of Fermented Mullet Fish (Feseekh) by Microbial Inoculation with *Lactobacillus plantarum* and *Saccharomyces cerevisiae*

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ABSTRACT

This study explored the use of roe-free mullet fish in producing microbial fermented mullet products (L, S, and M) by inoculating them with *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, or a combination of both. We compared the chemical compositions, amino acid profiles, quality and microbiological indices, and sensory properties of these products with natural fermented whole fish (T) and kippered flesh (C). Microbial fermentation products (L, S, and M) exhibited elevated levels of moisture, protein, and fat (ranging from 63.50 to 66.76%, 63.18 to 64.17%, and 20.80 to 22.94%, respectively) compared to the T product. The essential to non-essential amino acid ratios in L, S, and M products ranged from 0.96 to 1.06. These products showed higher umami taste, calculated protein efficiency ratio (C-PER) (1.94 to 2.22), and calculated biological value (C-BV) (84.02 to 86.55) compared to the T product. Caloric values for L, S, and M products (ranging from 149.67 kcal to 166.88 kcal per 100g) were similar to the T product (157.19 kcal per 100g). Furthermore, the levels of thiobarbituric acid reactive substances (less than 1mg malonaldehyde/ kg), total volatile basic nitrogen (ranging from 77.11mg to 118.65mg per 100g sample), and salt content (ranging from 3.56 to 3.75%) in L, S, and M products were significantly lower than in the T product. Microbial quality indicators indicated the absence of *Salmonella*, *Shigella*, *E. coli*, and *Clostridium botulinum*, with the presence of *Staphylococcus aureus* and *Proteus* spp. Sensory evaluations demonstrated that microbial fermentation products (L, S, and M) received the highest ratings, ranging from extremely favorable to very favorable. These findings indicate the potential of microbial fermented mullet products as nutritious and palatable food options.

INTRODUCTION

Fermentation is the biotechnological process based on hydrolysis of food components through microorganisms or enzymes activities. In general, the microbial population in food can either cause spoilage of food making it putrid or produce a fermented food product that is edible with improved safety and delicacy **(Steinkraus, 2004)**. Among the various types of fermented food products, fermented fish is one of the most well-liked. In several communities around the world, the use of fermented fish as a meal dates back to ancient times **(Prajapati & Nair, 2013)**.

Since different nations employ various techniques for fermenting fish, each kind of fermented fish has a unique flavor and nutritional value. The fish species, the catch location, and the fermentation methods all affect the flavor and ability to digest the fish muscles **(Tamang& Samuel, 2010)**. Fermentation is carried out under strictly controlled circumstances in order to preserve primary and secondary microbial metabolites in the final product. Various microorganisms and enzymes, endogenous or exogenous, induced favorable biochemical changes that led to a distinctive flavor of fermented products. Proteases from fish and halophilic bacteria hydrolyze fish-derived proteins into peptides or single amino acids during the process of proteolysis **(Du *et al*., 2019)**. Bitter, umami, or sweet tastes can be sensed by taste-active peptides, amino acids and amino acid derivatives **(Zhao *et al*., 2016)**.

Microorganisms and enzymes soften fish through acidification, gelation of myofibrillar and degraded muscle proteins and lipids. Acidification during fermentation prevents spoiling and contamination of fermented fish generates, as well as extending their shelf life through the generating of antimicrobial compounds. Protein muscle gelation led to a reduction of elasticity, cohesiveness and hardness (**Waisundara*et al*., 2016**).

Fish fermentation may involve challenging procedures including washing, degutting, salting and drying, which have a considerable impact on the flavor, texture and color of the end products as well as their nutritional content **(Panda *et al*., 2011; Fadda*et al*., 2010)**. Salt and carbohydrate sources such as rice, flour and sugar can be added to enhance and control the flavor of the finished products **(Zeng *et al*., 2015)**. Carbohydrates accelerate the fermentation process and absorb extra moisture **(Lee *et al*., 1997)**. Additionally, salt helps lessen the impacts of moisture by preventing the growth of bacteria that cause fish to spoil and by promoting the development of halophilic and salt-tolerant bacteria during fermentation. The fermented fish products are classified according to their salt level to no-salt, low-salt (3-8%), and high-salt (more than 20%).

In Egypt, feseekh is mainly a salted fermented whole mullet fish (*Mugilcephlus*) served as a main appetizer dish at feasts (**Rabie*et al*., 2009; Zang*et al*., 2020**). Feseekh with a low salt level matures in 15- 20 days, but a variety with a high salt content matures in 2-3 months depending on ambient temperature **(Rabie*et al*., 2009)**. The final feseekh product has a distinct sensory profile and is packaged appropriately **(Ibrahim *etal*., 2021; Pandey & Upadhyay, 2022)**. For a controlled fermentation process, it is important to check the levels of aerobic and anaerobic bacteria, the absence of pathogenic bacteria, the contents of moisture, protein and salt, as well as the TVB-N and TBA levels in samples of salted fermented fish **(Gassem, 2019)**.

Fish fermentation depends primarily on the cultural heritage of each society. Therefore, the presence of poor hygienic conditions during the processing, transportation and sale of fermented fish affects the safety of the product **(Novoslavskij*et al*., 2016)**. Therefore, pathogens transmitted through food fermented in unsafe ways may pose a threat to the health of consumers. Previous studies have shown that the presence of a mixture of competing microbial strains in fermentation has a beneficial effect on preventing harmful microbes through secondary metabolic products, which ensures the safety of the fermented products **(Zheng *et al*., 2017; Liao *et al*., 2019; Zhang *et al*., 2022)**.

Lactic acid bacteria are considered one of the initiators used in fish fermentation processes, as they lower the pH and prevent the activity of spoilage bacteria **(Lyhs*et al*., 2001)**. In addition, it secretes proteolytic enzymes and creates distinctive flavors in these fermented products. Previous studies have shown that these bacteria are considered probiotics in fermented foods **(Mahulette*et al*., 2018)**. On the other hand, yeast is considered one of the probiotics that is not widely used in fish products since it may cause a change in the color and flavor of fermented products as a result of its microbial activity **(Cappa & Cocconcelli, 2001)**. Yet, using a mixture of lactic acid bacteria and yeast may help the yeast to grow and show its effective activity in the product as a result of reducing the pH of the product. It may also help in the release of vitamins and other growth factors.

Mullet fish are harvested in Egypt between October and December in order to obtain the roe for processing, and their flesh is fast perishable with a low economic value. Concequently, the purpose of this study was to increase the added value of the flesh of this type of kippered mullet fish through fermenting it by microbial inoculation with *Lactobacillus plantarum* or *Saccharomyces cerevisiae*, or a mixture of them, while providing appropriate conditions for their activity in the presence of a low percentage of salt and sugar. Subsequently, the quality characteristics of microbial inoculated kippered mullet products were assessed in relation to whole fish naturally fermented in the presence of a high percentage of salt and to kippered fish naturally fermented without inoculation in the presence of a low percentage of salt and sugar.

MATERIALS AND METHODS

1. Materials

1.1. Biological raw materials

1.1.1 Fish. Almost fifty kilograms of fresh mullet (Bouri) fish (*Mugilcephalus*) from the Mugilidae family were purchased in November 2022 from an aquaculture farm in Ismailia Governorate, Egypt. The average length and weight of the fish ranged from 43 to 45cm and 0.65 to 0.75kg, respectively. All fish samples were transported with ice (1:1 w:w) to the laboratory of the Faculty of Fish Resources, Suez University, Egypt.

1.1.2 Bacteria and yeast. The inocula of *Lactobacillus plantarum ss. plantarum* (oral-Jensen 1919, DSM 20174) and *Saccharomyces cerevisiae* (ATCC 9763) were obtained as broth from the Cairo Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain-Shams University, Egypt.

1.1.3 Chemicals. Chemicals such as HCl, NaOH, boric acid, glacial acetic acid, methanol, glucose, hydrogen peroxide (3%), sterile paraffin oil, phenolphthalein, potassium iodide and brilliant green were purchased from Diachem Chemicals. While, sodium hydroxide (NaOH) was obtained from El-Gomhouria Co. for Chemicals and Drugs. For coarse and fine salt, they were purchased from Ismailia Crystal Company in north of Sinai. All chemicals used in this study were analytical grade types.

1.1.4 Media. Peptone water (DM185D, MAST, UK), plate count agar (Lab M, UK) , anaerobic egg yolk agar (TPC, India), buffered peptone water (BPW) broth (1.07228.0500EMD Millipore, Germany), xylose-lysine-deoxycholate agar (XLD, ACUMEDIA 7166A ,USA), baird parker agar base (BPA, 1.05406.0500 EMD

Millipore, Germeny), egg yolk emulsion potassium Tellurite (42111605, Italy) and salmonella-shigella agar (S.S, OXOID LTD, ENGLAND) were used in the present study.
1.1.5 Other materials. Polyethylene (PE) bags, transparent stretch wrap film and aluminum foil (AL–F) were purchased from the local market in Suez Governorate, Egypt.

2. Methods

2.1. Technological methods

2.1.1. Preparation of bacteria and yeast starter cultures. *Lactobacillus plantarum* starter culture was prepared by transferring it into 5ml nutrient broth and incubating at 37°C for 48h. Just 2ml of incubated bacteria culture was transferred into 100ml sterile MRS broth and incubated at 37°C for 48h to obtain the inoculum count (10^8CFU/ml) that was used in the subsequent fermentation procedure.

Saccharomyces cerevisiae starter culture was prepared by transferring it into 5ml yeast mold broth and incubating at 30°C for 72h. Exactly, 2ml of incubated yeast culture was transferred into 100ml sterile yeast mold broth and incubated at 30°C for 72h to get the inoculum count (10^8CFU/ml) that was ready for use in the following fermentation process.

2.1.2. Preparation of fermented salted fish. Fig. (1) depicits the preparation of fermented salted mullet (Bouri) fish was carried out through natural procedure or microbial inoculation way. In the natural fermentation method, fresh mullet fish were washed to remove any clay, especially in the gills, and the fish were left at ambient air temperature until the fish surface became dry (approximately 20 minutes). Subsequently, every 6 fish individuals were packed into two polyethylene bags and wrapped with stretch wrap film and Al-F to close tightly. All fish bags were incubated at (30±2°C) for 12h during the fermentation period. Fermented fish were removed from bags and dry salted with 15% coarse salt (w/w) for 5 days at room temperature in polyethylene bags.

In the microbial inoculation method, after rinsing the fish with cold water, they were manually opened into a kippered shape using a knife. Then, each sample was gutted, removing the backbone and head. Moreover, the kippered fish sample was rinsed again with cold water and treated with one of the following four treatments:

1- 2% *Lactobacillus plantarum* inoculum (10^8 CFU/ml) + 4% fine salt + 2% maltose.
2- 2% *Saccharomyces cerevisiae* inoculum (10^8 CFU/ml) + 4% fine salt + 2% maltose.
3- 2% mixed (1% *Lactobacillus plantarum*+ 1% *Saccharomyces cerevisiae*) inoculum + 4% fine salt + 2% maltose.
4- 4% fine salt + 2% maltose (without inoculum).

The treated kippered fish were packed into two polyethylene bags and tightly sealed with stretch wrap film and aluminum foil. Fermentation was carried out at 30 ± 2°C for 12 hours, and complete curing was achieved after 5 days at room temperature.

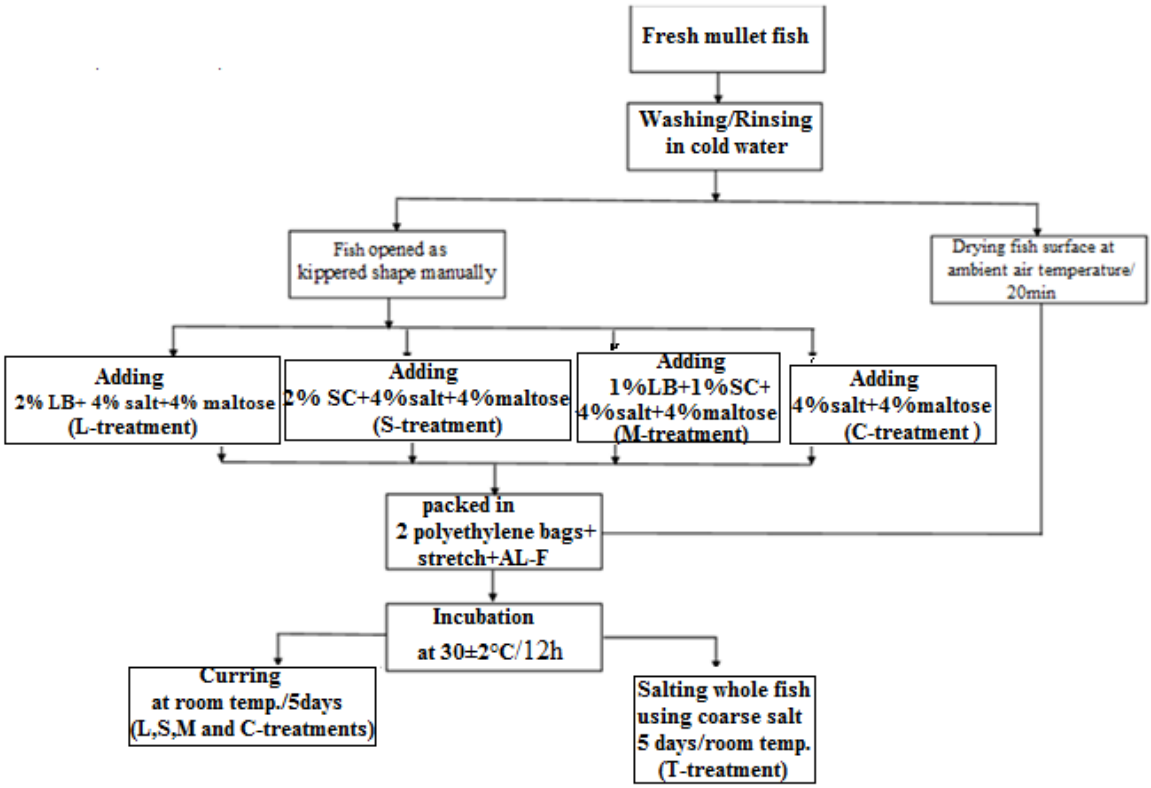

Fig. 1. Preparation steps for whole and kippered fermented salted mullet fish by natural and microbial inoculation methods

3. Biochemical analysis

Moisture, ash and protein contents beside sodium chloride percentage (NaCl) were analyzed according to standard procedures of **AOAC (2000)**. Fat content was extracted and calculated using mixture solvent of chloroform: methanol (2:1 v/v) according to **Bligh *et al*. (1959)**. The rest component (Carbohydrate) was computerized by difference.

Thiobarbituric acid reactive substances (TBARS) value was determined following the method described by **Pearson (1976)**. The distilled samples versus blank were heated in the water bath for 35min with TBARS reagent. After cooling, the optical density (OD) of the sample against the blank was recorded with spectrophotometer (T60 UV-Visible Spectrophotometer) at 537nm, and the TBARS value was estimated in the following manner:

TBARS *value* (*mg*malonaldehyde / *kg* sample) = 7.8 × OD

In addition, total volatile basic nitrogen (TVB-N) was performed using modified distillation following the method of **Antonacopoulos (1968)**. The distillated sample containing tashiro indicator was neutralized by titrating with 0.1 N HCl. The TVBN value was calculated by applying the following equation:

TVBN (*mg*/*100g*) = (*ml* 0.1 N HCl × *1.4* × *100*) / *weight of sample*

The pH value was determined by homogenizing 10g flesh sample in 90ml distilled water and recording the results with a calibrated pH meter (*OHAUS STARTER* 2100 Bench pH meter, *OHAUS* Instruments, USA).

Amino acid profile was revealed by protein hydrolysis with 12M HCl at 100°C for 24h. Finally, the hydrolyzed protein was filtered, and 1.0ml of it was injected into HPLC following the method described by **Serb *et al*. (2013)**.

The percentage of amino acids contributing to fermentation tastes was calculated based on the weighed calculation of each amino acid's taste profile according the following equations:

Σ TUAA = Aspartic (ASP) +Glutamic (GLU) +Glycine +Alanine.

Σ TSAA= Threonine + Valine + Cystine + Methionine + Lysine + Arginine + Serine + Glycine + Alanine + Proline

Σ TABB = Valine + Histidine + Cystine + Methionine + Phenylalanine + Isoleucine + Leucine + Lysine + Tyrosine + Arginine + Proline.

Umami taste % = Σ TUAA (g) / Σ AA contributed in tastes (g)

Sweet taste % = Σ TSAA (g) / Σ AA contributed in tastes (g)

Bitter taste % = Σ TABB (g) / Σ AA contributed in tastes (g)

Where, Σ TUAA (g) means the sum of total amino acids in grams that contributed to give umami taste; Σ TSAA (g) stands for the sum of total amino acids in grams that contributed to give sweet taste; Σ TABB (g) represents the sum of total amino acids in grams that contributed to give bitter taste; Σ AA contributed in tastes (g) means the sum of all amino acids in grams that give umami, sweet and bitter tastes.

Nutritional value was followed based on the caloric value and calculated both protein efficiency ratio and biological value. The caloric value of fermented fish flesh was computerized using the formula developed by **Falch *et al*. (2010)**.

[*Caloric value* (kcal/100g) = (% *lipid*×9) + (% *protein*×4) + (% *carbohydrate*×4)]

Protein Efficiency Ratio (C-PER) and Biological Value (C-BV) were computed according to **FAO/WHO (1985)**.

C-PER was computerized using the three equations listed below;

PER*a*= -(*0.684*) + *0.456* Leucine – *0.047* Proline

PER*b* = -(*0.468*) + *0.456* Leucine + *0.105* Tyrosine

PER*c* = -(*1.816*) + *0.435* Methionine + *0.780* Leucine + *0.211* Histidine – (0.944) Tyrosine

C-BV was calculated as follows:

C-BV= *39.55* + (*8.89* Lysine as g/100g *protein*).

4. Bacterial analysis

4.1. Sampling

Samples of fermented fish from the six different treatments were collected in triplicate at the end of the fermentation process for bacterial analysis. Samples were transferred to the laboratory of the faculty of Fish Resources, Suez University for immediate examination.

4.2. Aerobic plate count (APC)

Samples (5g) were diluted with 45ml 0.1% peptone water (DM185D, MAST, UK), and serially diluted for up to five 1-10 dilutions. According to **Buck and Cleverdon (1960)**, each dilution was spread platted on plate count agar (Lab M, UK) and incubated at 35±2°C for 24 hours. Bacterial populations were measured as the number of colony-forming units (Log CFU/g) per gram of sample following the guidelines outlined by **APHA (2001)**.

4.3. Anaerobic bacteria count

Aseptically, 10g of sample were weighed and added into sterile 90mL water blank and homogenized. Each dilution (1.0ml) was pour-platted in anaerobic egg yolk agar (TPC, India), and then it was incubated for 48 hours at 35°C in anaerobic jars, following the guidelines outlined by **FDA (2001)**. The bacterial count was expressed as Log CFU/g. **(Vanderzant & Splittstoesser, 1992)**.

4.5. Isolation and identification of pathogenic bacteria

4.5.1 *Salmonella* spp. *Salmonella* spp. were identified from samples by enrichment in buffered peptone water (BPW) broth, followed by culturing in tetrathionate selective broth (UNE-EN ISO 6579-1:2017). After incubation at 37°C for 24h, cultures were plated on xylose lysine deoxycholate agar (XLD, Acumedia, USA) and incubated **(Boukharouba, 2022)**. Selected colonies were biochemically examined and validated with IMVC, including indol (I), methyl red (MR), vogusproskauer (VP) and citrate (C). Additionally, triple sugar iron (TSI) was tested and confirmed using API 20E (BioMérieux, France) **(Sugiartha*et al*., 2018; Boukharouba, 2022)**.

4.5.2 *E. coli.* *E. coli* was isolated and identified from the sample of products using *E. coli* broth (EC) and incubated at 37°C for 24h. Cultures were streaked on the selective medium MacConkey agar (MAC). After incubation, the characteristic colonies of *E.coli* were examined biochemically by IMVC test and confirmed using API 20E (BioMérieux, France) **(Bolton *et al*., 2007; Sugiartha *et al*., 2018)**.

4.5.3 *Shigella.* The isolation *Shigella* from samples was performed using tryptic soy broth with yeast extract added (TSYE) as enrichment media. Incubated at 37°C for 24h and streaked on the selective medium *Salmonella-Shigella* Agar (S.S, OXOID LTD, ENGLAND). Selected colonies were biochemically confirmed by IMVC (**Taylor *et al*., 1968; FDA, 2013)**.

4.5.4 *Staphylococcus* spp. According to UNE-EN ISO 6888-1:2000 protocol, samples enrichment in a BPW and platted on Baird Parker agar base with the egg yolk potassium tellurite emulsion (EMD Millipore, Germany). After incubation at 37°C for 24h, suspected colonies of *S. aureus* were subjected to IMVC biochemical tests, catalase test, and glucose fermentation. Additionaly, regrowth was performed on mannitol salt agar selective plates **(Boukharouba*et al*., 2022)**.

6. PCR and 16S rRNA gene sequencing

6.1. DNA extraction and PCR

This technique was performed to confirm results and identify unknown colonies. DNA was extracted by bacterial DNA preparation kit (Jena Bioscience, Germany). The universal oligonucleotides primers F:5′-GAGTTTGATCCTGGCTTAG-3′ and R: 5′GTTACCTTGTTACGACTT-3′ were used to amplify partial 16S DNA **(Azwai1 *et al*., 2016**).

6.2. DNA sequencing

Purification of the PCR products was performed using QIA quick Kit (Qiagen, Germany). The second PCR was completed using big-dye terminator V3.1 cycle sequencing kit. Additional purification was achieved with CENTRI-SEP columns (Princeton Separations, NJ). Genetic Analyzer-3500 (Applied Biosystems, Massachusetts, USA) was used for DNA sequencing and then subjected to BLAST search through the Mega program (7.0.20) **(Iwatsuki*et al*., 2021)**.

7. Sensory evaluation

The appearance, texture, color, odor, flavor, taste, and overall acceptability of fermented fish flesh samples were evaluated by 20 trained panelists belonging to Fish Processing Technology Department of Faculty of Fish Resources at Suez University, Egypt. A 9-point hedonic scale was used, with "9" denoting extremely like, "8" denoting very much like, "7" denoting moderately like, "6" denoting slightly like, "5" denoting neither like nor dislike, "4" denoting slightly dislike, "3" denoting moderately dislike, "2" denoting very much dislike, and "1" denoting extremely dislike (**Amerine*et al*., 1965**). The mean value and standard deviation of each criterion were used to express each sensory attribute score.

8. Statistical analysis

All survey variables, including means, standard deviations (SD), ANOVA one-way test analysis and frequency percentages (%) received descriptive analysis using IBM SPSS Statistics 25 (IBM Corporation, New York, USA). Samples were compared using an independent sample t-test, and the results were presented as means ± SD. To compare mean differences, Duncan's multiple range tests **(Duncan, 1955)** were used. A probability value ($P< 0.05$) was used to indicate differences for statistical significance.

RESULTS AND DISCUSSION

1. Proximate composition

The chemical contents of fresh and fermented salted mullet in natural and microbial ways are represented in Table (1). The results in Table (1) show that the moisture, protein and fat contents in fresh mullet were 70.14±1.16%, 69.39±0.54%, and 24.51±0.37%, which decreased significantly ($P<0.05$) by fermentation and salting process. The highest significant ($P< 0.05$) reduction in moisture content (58.79±1.05%) was noticed with natural fermentation of whole fish (T), while moisture contents in all microbial fermentation ranged between 63.50±1.99% in kippered fish fermented by *Lactobacillus plantarum* (L) and 66.76±1.20% in kippered fish fermented by mixture of *L. plantarum* and *Saccharomyces cerevisiae* (M). The highest protein content on dry basis was significantly ($P< 0.05$) showed in kippered fish fermented without any inoculation (C) (65.65±0.76%), which may be due to protein denaturation on flesh surface by salt and reduction of protein hydrolysis related to fermentation based on natural flesh enzymes without bacterial or yeast inoculation. The microbial fermented treatments (L, S, and M) had protein and fat contents ranging from 63.18 ±0.81 to 64.17 ±0.68% and 20.80 ±0.42 to 22.94±0.26%, respectively, which means retaining less hydrolysate proteins and more fat than C treatment. On the other hand, treatment T showed the lowest protein content (54.91±0.90%) on dry basis ($P< 0.05$), where fermentation was firstly carried out before salting, which led to the release of more hydrolysate proteins that dissolved in water by osmotic pressure. The ash content of T treatment was significantly ($P< 0.05$) the highest content (26.70±0.31%) due to the highest reduction in moisture content and high salt added during the salting process, compared to all the microbial fermented treatments (L, S and M), which ranged from 11.89 ±0.14 to 13.57±0.15%.

Table 1. Proximate composition of natural and microbial fermented mullet vs. fresh mullet fish

Treatment	Moisture (%)	% On dry weight basis			
		Protein	Fat	Ash	Carbohydrate
F	70.14±1.16[a]	69.39±0.54[a]	24.51±0.37[a]	4.92±0.07[e]	1.18±0.21[c]
T	58.79±1.05[d]	54.91±0.90[d]	17.63±0.40[f]	26.70±0.31[a]	0.76±0.12[d]
L	63.50±1.99[c]	63.22±0.73[c]	22.13±0.32[c]	13.38±0.11[c]	1.27±0.04[c]
S	66.11±0.96[b]	63.18±0.81[c]	22.94±0.26[b]	11.89±0.14[d]	1.99±0.06[a]
M	66.76±1.20[b]	64.17±0.68[c]	20.80±0.42[d]	13.57±0.15[c]	1.62±0.090[b]
C	64.69±0.66[bc]	65.65±0.76[b]	19.51±0.30[e]	14.12±0.10[b]	0.72±0.10[d]

[a-f]Mean ± SD in the same column having different letters are significantly different at ($P< 0.05$).
F: Fresh fish, T: Natural fermentation of whole fish, L: Fermentation using *Lactobacillus plantarum* for kippered fish, S: Fermentation using *Saccharomyces cerevisiae* for kippered fish, M: Fermentation using mix of *L. plantarum* and *S. cerevisiae* for kippered fish, C: Fermentation without inoculation for kippered fish.

The results of the current study indicated higher moisture, protein, and fat contents, while showing lower ash content, compared to those found in fermented salted fish (Hout-Kasef) by **Gassem (2019)**, where they were 47.96, 49.4, 14.26 and 37.67%, respectively. Moreover, the natural fermented mullet in this research was comparable to the salted fermented fish (Feseekh) found by **Ibrahim *et al.* (2021)**, where the moisture, protein, fat and ash contents recorded values of 60.25, 54.06, 15.92 and 19.6%, respectively.

2. Amino acids profile

Amino acids composition is an indicator of protein quality and taste characteristics of any food product. Fresh mullet fish as well as the traditional and microbial fermented mullet treatments in the current study were composed of 16 amino acids, 9 were essential and 7 were non-essential (Table 2). Fresh mullet had an essential-to-non-essential amino acid (TEA/TNEA**)** with a ratio of 1.11; while the whole natural fermented mullet had a ratio of 0.97. The TEA/TNEA ratio varied from 0.96 to 1.06 in microbial fermented kippered mullet, and it was also 1.12 in kippered fermented mullet without microbial inoculation.

The amounts of essential amino acids that appeared in the highest concentration were in kippered mullet fermented without microbial inoculation and with microbial inoculation (treatment C, L, S, and M) which ranged from 12.45 to 12.91mg/100g sample accounting for 48.98 to 52.78 % of the total amino acids, followed by natural fermented mullet (treatment T) (9.38mg/ 100g sample representing 49.27% of the total amino acids) (Table 2). The greatest essential amino acids in fresh mullet were phenylalanine, lysine, histidine, methionine and threonine (1.84, 1.82, 1.71, 1.26, and 1.12mg/100g sample, respectively). Histidine was significantly reduced during the fermentation process, particularly in treatment L (0.35mg/ 100g sample), while lysine, methionine and phenylalanine content increased in the microbial fermented treatments (L, S, and M), reaching 3.21- 3.34mg/ 100g sample, 1.82- 2.18mg/ 100g sample, and 2.25- 2.41mg/ 100g sample, respectively, while treatment C showed the highest increase in methionine (3.01mg/ 100g sample). The proportion of essential amino acids was higher in this study compared to Feseekh samples from various Egyptian Governorates, where it ranged from 30.7 to 36.9% of the total concentration of amino acids **(Ibrahim *et al.*, 2021)**. However,

Rabie *et al*. (2009) claimed that after 60 days of preservation, these essential amino acids represented 68% of the total concentration of amino acids.

Table 2. Amino acids contents (mg/100g sample) of natural and microbial fermented mullet fish

Amino acids	Taste characteristics	L	S	M	C	T	F
	Essential amino acids						
Threonine	Sweet (+)	1.14±0.10^{a}	1.18±0.07^{a}	0.83±0.04^{c}	1.15±0.09^{a}	0.99±0.03^{b}	1.12±0.07^{a}
Valine	Sweet/bitter(-)	0.35±0.030^{c}	0.32±0.04^{c}	0.25±0.06^{c}	1.15±0.09^{a}	0.33±0.05^{c}	0.49±0.07^{b}
Histidine	Bitter (-)	0.35±0.06^{d}	1.22±0.04^{c}	1.21±0.03^{c}	1.44±0.08^{b}	1.48±0.07^{b}	1.71±0.12^{a}
Cystine	Bitter/sweet/ sulfur (-)	ND	ND	ND	ND	ND	ND
Methionine	Bitter/sweet/ surfur (-)	2.18±0.06^{a}	1.82±0.03^{c}	1.98±0.05^{b}	1.12±0.06^{e}	0.98±0.07^{f}	1.26±0.05^{d}
Phenylalanine	Bitter (-)	2.25±0.16^{b}	2.41±0.18^{b}	2.32±0.21^{b}	3.01±0.28^{a}	1.76±0.17^{c}	1.84±0.23^{c}
Isoleucine	Bitter (-)	0.76±0.09^{c}	0.73±0.08^{c}	0.79±0.06^{bc}	1.06±0.070^{a}	0.66±0.08^{c}	0.90±0.03^{b}
Leucine	Bitter (-)	1.58±0.29^{a}	1.52±0.31^{a}	1.44±0.27^{a}	1.59±0.34^{a}	1.53±0.26^{a}	1.66±0.23^{a}
Lysine	Sweet/bitter(-)	3.32±0.26^{a}	3.34±0.27^{a}	3.21±0.17^{a}	1.74±0.29^{b}	1.33±0.29^{b}	1.82±0.33^{b}
Tyrosine	Bitter (-)	0.52±0.07^{bc}	0.36±0.03^{de}	0.43±.040^{cd}	0.65±0.04^{a}	0.32±0.05^{e}	0.58±0.07^{ab}
Σ EAA		12.45±1.12^{a}	12.90±1.05^{a}	12.46±0.93^{a}	12.91±1.34^{a}	9.38±1.07^{b}	11.38±1.20^{ab}
	Non-essential amino acids						
Arginine	Sweet/bitter(-)	0.95±0.07^{ab}	0.88±0.03^{b}	0.90±0.06^{ab}	1.00±0.080^{a}	0.56±0.07^{c}	0.97±0.04^{ab}
Aspartic	Umami (+)	3.32±0.13^{a}	2.94±0.05^{b}	2.93±0.08^{b}	2.10±0.11^{c}	1.85±0.08^{d}	1.12±0.11^{e}
Serine	Sweet (+)	1.39±0.02^{bc}	1.30±0.06^{c}	1.39±0.04^{bc}	1.47±0.06^{ab}	1.33±0.04^{c}	1.49 ±0.07^{a}
Glutamic	Umami (+)	4.77±0.14^{a}	4.65±0.26^{a}	4.63±0.15^{a}	3.96±0.27^{b}	3.42±0.17^{c}	3.90±0.31^{b}
Glycine	Sweet/umami(+)	1.00±0.09^{b}	0.84±0.05^{c}	1.06±0.09^{ab}	1.15±0.09^{a}	0.76±0.07^{c}	1.12±0.07^{ab}
Alanine	Sweet/umami(+)	0.63±0.04^{a}	0.60±0.04^{a}	0.60±0.07^{a}	0.72±0.09^{a}	0.70±0.08^{a}	0.70±0.11^{a}
Proline	Sweet/bitter(-)	0.91±0.06^{c}	0.92±0.05^{c}	0.90±0.04^{c}	1.15±0.06^{a}	1.04±0.09^{b}	0.93±0.05^{c}
Σ NEAA		12.97±0.55^{a}	12.13±0.54^{ab}	12.41±0.53^{ab}	11.55±0.76^{b}	9.66±0.60^{c}	10.23±0.76^{c}
Σ TAA		25.42±1.67^{a}	25.03±1.59^{a}	24.87±1.46^{a}	24.46±2.10^{ab}	19.04±1.67^{c}	21.61±1.96^{bc}
TEA/TNEA		0.96	1.06	1.00	1.12	0.97	1.11

$^{a-e}$Mean ± SD in the same row having different letters are significantly different at (P< 0.05).

The total amount of non-essential amino acids appeared close to the essential ones in fresh mullet and all treatments after fermentation. Fresh mullet had a non-essential amino acid content of 47.34%, which increased to 50.74.63% after natural fermentation of whole-mullet fish. However, the proportion after microbial fermentation appeared to range from 48.46 to 51.02% (Table 2). Glutamic, aspartic, serine and glycine were the non-essential amino acids that were present in the highest concentrations in fresh mullet, and the microbial fermented kippered mullet treatments (L, S, and M) showed considerable increase in both glutamic and aspartic acids after fermentation. The proportion of non-essential amino acids in the present investigation was lower than that in Feseekh samples obtained from different Egyptian Governorates, which made up 63.0-69.2% of the total amino acid concentration **(Ibrahim *et al*., 2021)**.

3. Fermentation tastes

The amino acids that are responsible for umami, sweet, and bitter tastes are identified in Table (2). In fresh mullet fish, the percentage of amino acids responsible for each taste relative to all amino acids that contributed to all tastes were 23.67% for umami taste, 34.26% for sweet taste, and 42.08% for bitter taste (Table 3). Microbial fermentation (L, S, and M sample) improved the umami taste (27.97, 26.76, and 27.30%, respectively) and somewhat kept or reduced the sweet taste (34.15, 33.19, and 32.93%, respectively), but induced amino acids reduced the bitter taste (37.89, 40.06, and 39.77%, respectively). Furthermore, whole mullet naturally fermented (T) appeared to improve in umami taste and a slight reduction in sweet and bitter tastes, while kippered mullet fermented without microbial inoculation (C) appeared to slightly increase in umami and bitter tastes besides a slight decrease in sweet taste as a result of natural muscle enzymes and flora activities. According to the findings of **Yang *et al*. (2022)**, the fermentation could enhance the umami flavor of mandarin fish. Numerous studies **(Nakata *et al*., 1995; Zhuang *et al*., 2016; Huang *et al*., 2019**) found that the structures of sequential umami amino acids formed umami peptides that revealed a significant umami taste. **Zhu *et al*. (2021)** found that the anchovy sauce's taste components increased over the first few months of fermentation before decreasing over time. Umami amino acids, sweet amino acids, and salty amino acids were the most common amino acids after the first year of fermentation, while bitter amino acids and sour amino acids dominated after the second year.

4. Nutritional values

The caloric values of fresh mullet as well as the natural and microbial fermented mullet fish were computed based on wet weight (Table 3). The caloric value of fresh mullet was 150.07kcal/ 100g which increased after fermentation in all samples, especially in the L sample (166.88kcal/100g), whereas the M sample showed a minor decrease (149.67kcal/ 100g) according to variation in the contents of protein, fat and carbohydrate.

The quality of fermented fish protein was evaluated by computing protein efficiency ratio (PER) and biological value (BV) based on some amino acids (g) per 100g protein (Table 3). C-PER and C-BV values obviously increased after microbial fermentation, recording values 2.12 and 86.24, 2.22 and 86.55, and 1.94 and 84.02 in L, S, and M samples, respectively. On the other hand, natural fermentation (T) and kippered fermentation without microbial fermentation (C) led to a decrease in C-PER and maintained the C-BV close to the value of fresh mullet. The current results suggest an improvement in protein quality through microbial fermentation. Specifically, one unit of microbial fermented mullet protein could potentially be translated into two units of weight gain or more. Additionally, approximately 84% or more of this microbial fermented mullet protein could be absorbed and converted into body protein. According to **Sarojnalini and Vishwanath (1995)**, the biological value and protein efficiency ratio of fermented fish products, Hentak and Ngari, were 96.94 & 97.83 and 1.8 & 1.8, respectively.

Table 3. The nutritional values and the amounts of amino acids responsible for tastes of natural and microbial fermented mullet fish

Parameter	F	T	C	L	S	M
Σ TUAA (g)	6.84±0.60^{c}	6.73±0.40^{c}	7.93 ± 0.56^{b}	9.72±0.40^{a}	9.03±0.40^{a}	9.22±0.39^{a}
Σ TSAA (g)	9.90±0.86^{b}	8.02±0.79^{c}	10.65±0.91^{ab}	11.87±0.73^{a}	11.20±0.65^{ab}	11.12± 0.62^{ab}
Σ TABB (g)	12.16±1.22^{a}	9.99±1.20^{a}	13.91±1.39^{a}	13.17±1.15^{a}	13.52±1.06^{a}	13.43±0.99^{a}
Σ AA contributed in tastes (g)	**28.90**	**24.74**	**32.49**	**34.76**	**33.75**	**33.77**
Umami taste %	23.67	27.20	24.41	27.96	26.76	27.30
Sweet taste %	34.26	32.42	32.78	34.15	33.19	32.93
Bitter taste %	42.08	40.38	42.81	37.89	40.06	39.77
Caloric value (kcal/100g)	150.07	157.19	155.73	166.88	158.25	149.67
C-PER	1.61	1.38	1.42	2.12	2.22	1.94
C-BV	62.87	61.36	63.11	86.24	86.55	84.02

Umami taste % = Σ TUAA (g) / Σ AA contributed in tastes (g), Sweet taste % = Σ TSAA (g) / Σ AA contributed in fermented taste (g), Bitter taste % = Σ TABB (g) / Σ AA contributed in tastes (g).

[a-c]Means with a different letter in the same row are statistically significant at $P<0.05$.

ΣTUAA = Aspartic (ASP) + Glutamic (GLU) + Glycine + Alanine.

ΣTSAA = Threonine + Valine + Cystine + Methionine + Lysine + Arginine + Serine + Glycine + Alanine + Proline.

ΣTABB = Valine + Histidine + Cystine + Methionine + Phenylalanine + Isoleucine + Leucine + Lysine + Tyrosine + Arginine + Proline.

5. Physicochemical properties

Table (4) reveals that the pH value of fresh mullet was 6.46, and after natural fermentation and dry salting of whole mullet fish, this value increased to 6.82, which may be due to the formation of amine compounds. On the other hand, microbial fermentation treatments (L, S, and M) showed a great reduction in pH value due to the activity of inoculated bacteria or yeast in the presence of maltose, which induced the production of some organic acids such as lactic acid, especially in L sample (5.11±0.06). Low pH values of microbial fermented mullet samples in the current investigation were comparable to market samples of rice bran-fermented sardines and lonailish from Tripora (5.32 and 5.66, respectively) (**Yatsunami &Takenaka, 1996; Majumdar & Basu, 2010**). However, the pH of the naturally fermented mullet used in this investigation was close to the range of 6.3 to 6.9 found in Feseekh samples gathered from various governorates in Egypt **(Ibrahim *etal*., 2021)**.

The salt content of fresh mullet fish was 0.24±0.06% (Table 4). At the end of the salting process after the fermentation of whole mullet fish, a dramatic increase in salt content of 10.61±0.69% was detected alinged with decreasing moisture and protein denaturation. In other microbial fermented samples, the salt contents ranged from 3.56±0.27% to 3.82±0.49% as a result of moderately decreasing the moisture in the presence of low salt (4%) during the fermentation and salting process. The salt amount in the current investigation was much lower than the 11.9% salt content discovered in Feseekh samples taken from several supermarkets in Aswan Governorate, Egypt **(Edris*et al*., 2020)**, but it was close to that of naturally fermented whole mullet.

Table 4. Quality indices of natural and microbial fermentation of mullet fish

Treatment	pH	Salt content (%)	TABRS (mg MDA/kg sample)	TVB-N (mg/100g sample)
F	6.46±0.16^{b}	0.24±0.06^{c}	0.56±0.22^{cd}	13.82±0.51^{e}
T	6.82±0.03^{a}	10.61±0.69^{a}	1.44±0.51^{a}	253.48±5.61^{a}
C	5.66±0.04^{d}	3.67±0.46^{b}	1.01±0.09^{ab}	83.85±1.49^{c}
L	5.11±0.06^{f}	3.75±0.60^{b}	0.92±0.12^{bc}	77.11±1.20^{d}
S	5.84±0.09^{c}	3.56±0.27^{b}	0.36±0.08^{d}	118.65±2.15^{b}
M	5.43±0.10^{e}	3.82±0.49^{b}	0.28±0.01^{d}	75.83±1.56^{d}

$^{a-f}$Means with a different letter in the same column are statistically significant at $P< 0.05$.
F: Fresh fish, T: Natural fermentation of whole fish, L: Fermentation using *Lactobacillus plantarum* for kippered fish, S: Fermentation using *Saccharomyces cerevisiae* for kippered fish, M: Fermentation using a mixture of *L. plantarum* and *S. cerevisiae* for kippered fish, C: Fermentation without inoculation for kippered fish.

Secondary lipid oxidation was measured depending on TBARS values of fermented mullet fish. Microbial fermented samples, L, S, and M samples were < 1mg malonaldehyde/ kg sample, while whole mullet fish that naturally fermented reached a value of 1.44 ±0.51mg malonaldehyde/kg sample (Table 4). All natural and microbial fermented mullet fish samples in this study have good quality, where the acceptable range is 1– 2mg malonaldehyde per kg of sample according to **Lakshmanan (2000)**. All-natural and microbial fermented mullet in this study had TBA concentrations that were lower than those of Feseekh samples obtained from various governorates in Egypt, which ranged between 1.36 and 2.94mg malonaldehyde/kg **(Ibrahim *et al*., 2021)**.

The TVB-N value of fresh mullet was 13.82 ±0.51mg/100g sample, where it was below 30mg N/100g flesh as the acceptable limit specified by the **EOS (2005)** for chilled fish (Table 4). Fermentation and salting process resulted in protein breakdown by endogenous enzymes and microorganisms' activities and biochemical changes in the fish muscle **(Kakati & Goswami, 2013)**. Natural fermentation of whole mullet fish exhibited the highest TVB-N value (253.48 ±5.61mg/ 100g sample) in the presence of high salt content, which caused protein denaturation that was easily decomposed by endogenous enzymes and natural microorganisms. On the other hand, microbial fermentation in the presence of low salt content reduced the intensity of protein breakdown which ranged between 75.83 ±1.56mg/ 100g in M sample to 118.65±2.15 in S sample. However, the high amount of TVBN in these microbial fermented mullet didn't show any ammonia-like odor. The findings of this investigation are consistent with those of **Anihouvi*et al*. (2012)**, who found that naturally fermented cassava fish for Lanhouin Production had high TVB-N values that fluctuated between 264.7 to 389.9mg/ 100g. Similar findings were reported by **Roy *et al*. (2015)** in Telesech-fermented fish products from Tripura State in India, where TVB-N levels were high (210.92mg/ 100g).

6. Microbiology properties

Fish treated with *Lactobacillusplantarum* (L), *Saccharomyces* (S), or their mixture (M) had an aerobic plate count (APC) that ranged from 8.08 to 8.38 log CFU/g, without any appreciable variations (P>0.05). Fermentation without the inoculation of kippered fish (C) or natural fermentation of whole fish (T) resulted in lower microorganism counts compared to the treated samples, indicating that the inoculated starter culture increased

the number of micro-organisms (Table 5).Similarly, **Zhang *et al*. (2022)** reported the same increase of APC in fermented fish inoculated with *L. plantarum* compared to uninoculated ones. At the end of fermentation, the APC values were 8.64 ±0.05 and 8.8 ±0.04 log CFU/g of *Lactobacillus* and mixed group of *Lactobacillus* and *Saccharomyces* groups, respectively.

Table 5. Aerobic plate count (APC) and anaerobic plate count (log CFU/g) in fermented fish with different treatments

Treatment	APC	Anaerobic
F	3.16 ± 0.09^{a}	2.67 ± 0.12^{f}
C	7.94 ± 0.12^{c}	5.36 ± 0.04^{a}
L	8.08 ± 0.05^{b}	3.78 ± 0.09^{ed}
S	8.21 ± 0.04^{b}	3.95 ± 0.08^{d}
M	8.38 ± 0.07^{b}	4.36 ± 0.04^{c}
T	6.22 ± 0.05^{d}	4.83 ± 0.12^{b}

[a-f]Mean ± SD in the same column having different letters are significantly different at $P < 0.05$.
F: Fresh fish; T: Natural fermentation for whole fish; L: Fermentation using *Lactobacillus plantarum* for kippered fish; S: Fermentation using *Saccharomyces cerevisiae* for kippered fish; M: Fermentation using a mix of *L. plantarum* and *S. cerevisiae* for kippered fish; C: Fermentation without inoculation for kippered fish.

Non-treated fermented fish had a value >1 log difference which fluctuated between 6.22 log CFU/g in the closed fish and 7.94 log CFU/g in the open gutted kippered fish (Table 5). In this context, **Karyantina *et al*. (2021)** found that, the APC in jambal roti closed fermented fish ranged from 1.2×10^{3} to 6.0×10^{6}CFU/g. However, the increase in gutted fish might be explained as a result of improper handling. Likewise, opening conditions for a prolonged period and unhygienic handling led to high APC count in ngari (6.65 log CFU/g) and hentaak (7.81 log CFU/g) fermented Indian fish **(Majumdar*et al*., 2015)**. The same microbial load for different fermented kippered fish products was recorded in the study of **Roy *et al*. (2014)**.

Both the APC and the anaerobic bacterial count may increase as a result of improper treatment. When compared to other treatments, kippered fermented fish had the greatest anaerobic bacterial count (5.36 log CFU/g) (Table 5), followed by traditional fermented whole fish (T), recording 4.83 log CFU/g, as it provided the perfect environmental condition for anaerobic bacterial growth. Previously, the anaerobic count of traditional fermented fish recorded 3.33 log CFU/g in two markets in Lomé **(Tidjani*et al*., 2014**), 4.63 and 7.0×10^{4} log CFU/g in dry salted Fesseekh in Egypt **(Abd-Allah *et al*., 2011; Osman *et al*., 2012; Tidjani*et al*., 2014)**.The anaerobic count in fermented fish treated with *Lactobacillus* (3.78 Log CFU/g) and *Saccharomyces* (3.95 log CFU/g) showed no significant differences ($P > 0.05$), with a combined count reaching 4.36 log CFU/g (Table 5).Anaerobic bacterial count in the inoculated group was less than in the uninoculated group, as *L. plantarum* and *Saccharomyces* might resist other anaerobic bacteria and competed for nutrients.

Salmonella was identified in fresh fish but not in any fermented fish (Table 6). *Salmonella* is not a natural resident of the aquatic environment, but diverse *Salmonella* serovars are still widely spread in fresh and marine water and seafoods **(Prabhakar *et al*., 2020)**. Seafood contamination after harvest is a result of unproper handling and low

sanitation in landing and at retails **(Salam*et al*., 2023)**. In all of the fermented fish that were analyzed, *Salmonella* growth was stifled by an anaerobic environment, acidity, high salt level and low moisture content. Likewise, *Shigella* and *E. coli* were not found. Notably, the addition of *L. plantarum* and *Saccharomyces* did not support the growth and the multiplication of anaerobic *Clostridium botulinum.* However, *L. plantarum* and *Saccharomyces*, have been shown to successfully conquer the growth of pathogenic foodborne bacteria **(Zhang *et al*., 2022)**.

Table 6. Bacteria isolated and identified from fermented fish with the different treatments

Treatment / Possible bacterium	F	C	L	S	M	T
Salmonella spp.	+ve	-ve	-ve	-ve	-ve	-ve
Shigella spp.	-ve	-ve	-ve	-ve	-ve	-ve
E. coli	-ve	-ve	-ve	-ve	-ve	-ve
Staphylococcus	+ve	+ve	+ve	+ve	+ve	+ve
Clostridium botulinum	-ve	-ve	-ve	-ve	-ve	-ve
Proteus vulgaris	nt	nt	nt	+ve	nt	Nt
Proteus mirabilis	nt	nt	nt	nt	nt	+ve (genbank ACC# OQ029382)
Proteus penneri	nt	nt	+ve (genbank ACC# OQ029383)	nt	nt	Nt

nt: Not tested

F: Fresh fish, T: Natural fermentation of whole fish, L: Fermentation using *Lactobacillus plantarum* for kippered fish, S: Fermentation using *Saccharomyces cerevisiae* for kippered fish, M: fermentation using a mix of *L. plantarum* and *S. cerevisiae* for kippered fish, C: Fermentation without inoculation for kippered fish.

On the other hand, *Staphylococcus* spp. was identified in fresh and all types of fermented fish (Table 6), indicating its presence in the environment with chances of spreading among the seafood workers. Correspondingly, *Staphylococcus* was detected in five different fermented fish local products (pedah, belacan, ngari, tungtap and shidal) in Southeast Asian cuisine **(Narzary*et al*., 2021)**. Moreover,**Guan *et al*. (2011)** detected the same *Staphylococcus* in the fermented fish product in Korea "Jeotgal". When random colonies from aerobic and anaerobic plates were selected for further identification by 16S rDNA gene sequencing, several species of *Proteus* were identified associated with L, S, and T (Table 6). *Proteus vulgaris*, *Proteus mirabilis* (genbank Accession # OQ029382), and *Proteus penneri* (genbank accession # OQ029383) belong to the Enterobacteriaceae family. They were recovered from municipal wastewater and had also been associated with foodborne illnesses in various nations **(Botschner*et al*., 2023)**.

7. Sensory properties

The sensory properties of natural and microbial fermented mullet fish were examined and tabulated (Table 7 & Fig. 2). According to the organoleptic parameters, the highest scores of appearances and color were observed with natural fermented whole

mullet (T) (8.60±0.31 and 8.55±0.54, respectively) while microbial fermented kippered mullet (S, M, and L) had the highest scores of taste, texture, flavor and acceptability. Natural fermented salted kippered mullet (C) samples showed the lowest sensory scores, where the panelists indicated that the flesh had salted characteristics than fermented. These variations in sensory parameters scores may be related to the regular functioning and activity of a dominant type of microbes, either *Lactobacillus plantarum* or *Saccharomyces cerevisiae*, which led to the formation of less TVB-N in microbial fermented samples than in natural fermented samples that depend on the activity of natural flora and internal enzymes besides its high salt content. The overall acceptability of microbial fermented mullet fish samples (S, M, and L) was graded between like extremely and like very much while T and C samples (naturally fermented) graded as like moderately.

Table 7. Sensory evaluation of natural and microbial fermentation of mullet fish

Characteristics	T	C	L	S	M
Appearance	8.60±0.31^{a}	6.87±0.08^{b}	8.42 ±0.22^{a}	8.55±0.27^{a}	8.30±0.21^{a}
Taste	8.20±0.20^{a}	7.52±0.37^{b}	8.38±0.19^{a}	8.55±0.33^{a}	8.44±0.14a
Texture	8.25±0.16^{a}	7.34±0.26^{b}	8.45±0.37^{a}	8.55±0.43^{a}	8.40±0.35^{a}
Color	8.55±0.54^{a}	7.92±0.33^{a}	8.40±0.56^{a}	8.55±0.25^{a}	8.20±0.70^{a}
Flavor	7.25±0.21^{b}	7.87±0.26^{a}	8.37±0.35^{a}	8.35±0.32^{a}	8.35±0.33^{a}
Overall acceptability	7.55±0.70^{b}	7.19±0.12^{b}	8.33±0.29^{a}	8.55±0.24^{a}	8.32±0.15^{a}

$^{a-f}$Mean ± SD in the same row having different letters are significantly different at $P< 0.05$.
F: Fresh fish, T: Natural fermentation of whole fish, L: Fermentation using *Lactobacillus plantarum* for kippered fish, S: Fermentation using *Saccharomyces cerevisiae* for kippered fish, M: fermentation using a mix of *L. plantarum* and *S. cerevisiae* for kippered fish, C: Fermentation without inoculation for kippered fish.

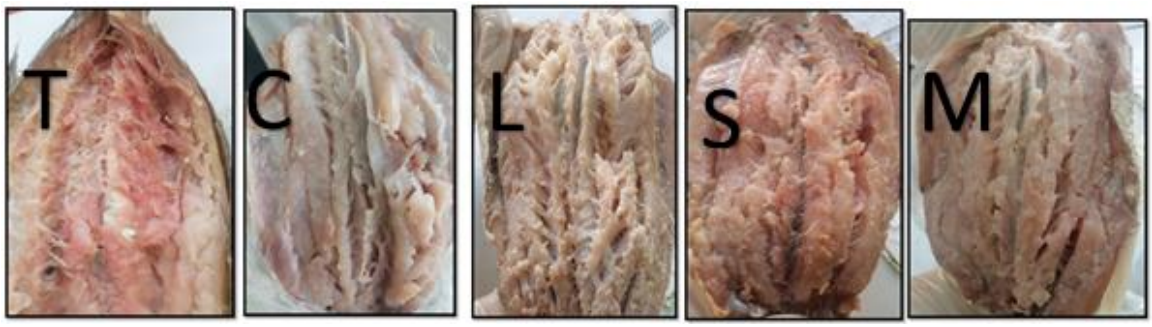

Fig. 2. Appearance of natural and microbial fermented mullet fish products showing: T: Naturalfermented whole fish, L: Microbial fermented fish using *Lactobacillus plantarum*, S: Microbial fermented fish using *Saccharomyces cerevisiae*, M: Microbial fermented fish using a mix of *L. plantarum* and *S. cerevisiae*, C: Natural fermented fish without inoculation

CONCLUSION

Microbial fermentation procedure has emerged as an effective method for producing Feseekh from roe-free mullet fish. The results also showed the possibility of increasing the weight yield after fermentation and salting as a result of the high moisture

content in the final product. Samples fermented microbial inoculation (L, S and M) appeared to have a better level of sensory, chemical and microbial quality than samples fermented naturally. This may be due to the production of some primary and secondary metabolic components during fermentation, such as organic acids, antimicrobials and antioxidants substances.

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