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Chemical, Microbial and Organoleptic Properties of Egyptian Fesikh Produced by Traditional and Artificial Fermentation Techniques

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ABSTRACT

Fesikh is a traditional salted fermented whole fish, high nutrition, unique flavor and is a popular product in Egypt. The aim of this study was to reduce and control in salting fermentation process during preparing the salted fermented Tobara fish (Mugil capita). Estimation safety and quality of this product showed that, an aerobic bacterial load was <1 log CFU/g, lactic acid bacteria count was 5 log CFU/g, values of total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), and non-protein nitrogen (NPN) were ranged from79 to 120.1 mg N/100g, 1.96 to 0.9 mg MDA/kg, and 0.2 to 0.48%, respectively. Increasing lactic acid bacteria count after 6 hours of fermentation might inhibit the growth of coliform and reduced total bacterial load. All Tobara Fesikh products prepared in this study were accepted organoleptically. Fesikh produced under artificial fermentation was superior in colour and texture but that fermented under ambient conditions was superior in odour. Based on these results, the trails in this study change to a safe and high quality of fesikh Tobara fish product can be produced in short time.

1. Introduction

Traditional preserved fish products are still having wide acceptance around the world due to their accustomed taste, aroma closely related to the food habits. Fermentation is one of the most important ways of fish preservation. During fermentation, fish has undergone degradative changes through enzymatic or microbiological activity either in the presence or absence of salt. The enzymes for the fermentation process come either from the fish digestive system and /or from the naturally bacteria in the fish (Essuman et al., 1992).

From the nutritional point of view, salted fermented fish is a source of protein, essential amino acids, vitamins, and minerals (Rabie et al., 2009). It is characterized by a strong odour due to the natural and microbial of enzymatic action in the fish muscle (Campbell-Platt, 1987). Lactic acid bacteria, micrococcus, yeast, and other microorganisms were detected in the salted fermented fish (Zaitsev et al. 1969). Wood (1997) and Tian et al. (2011) isolated and identified dominant lactic acid bacteria in traditional pickled fish. It was mainly Lactobacillus casei, and Lactobacillus spp.

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Wu et al. (2014) added five kinds of lactic acid bacteria during fish salting. They found that such bacteria not only caused shortening the curing time, but also enhanced the flavor, prevented the amine production, improved product quality and safety. CorbiereMorot-Bizot et al. (2006) attributed the above changes to ability of lactic acid bacteria to secrete protease, lipase, nitrate reductase, amino acid deaminase, and amino acid decarboxylase. All these enzymes play an important role in quality, color, and flavor of salted products.

Most salted fermented fish products are made from rich or semi fatty fish. During salting process fat subjected to oxidation (Saisithi, 1967) and enzymatic (Lipases) hydrolyzing producing products reacting with other decomposed especially amines (Lovern, 1962, Bal and Dominova, 1967) forming coloured and odour substances (Jones, 1966). Such changes are dependent on the salt content and salting period (Amano, 1962). Thiobarbituric acid (TBA) value is one of the criteria that indicate fat oxidation. It must be <3 in a very good, >5 in a good and between 7 and 8 in an acceptable salted fish product (Varlik et al., 1993; Sinhuber and Yu, 1958). Also Total volatile basic nitrogen (TVB-N) is an important parameter for the assessment of fish quality (Amegovu et al., 2012, Wu

and Bechtel, 2008). It includes group of biogenic amines forming during preparing the salted fermented fish (Horsfall et al., 2006).

In Egypt, Fesikh is a traditional name for the salted-fermented Bouri fish (Mugil cephlus). It is popular and used as the main dish in some Egyptian feasts (El-Sebaiy and Metwalli, 1989). The main problems of the Fesikh production are their long processing time, unhomogeneous quality, hazard health risks due to the unhygienic conditions used during production and it's consumption in raw form without cooking. In this study, the aim was to use either solar energy or controlled heating during fermentation to accelerate salting processes. The quality and safety of the Tobar fish, Fesikh during fermentation and salting processes were investigated by estimating some biochemical parameters such as TBA, TVN, and NPN, as well as the microbiological analysis.

2. Materials and methods

2.1. Materials:

2.1.1. Fish: Fresh farmed Tobar fish (Mugil capita) with an average of 25 ±2 cm in length and 165 ±8.8 g in weight were purchased from Suez local fish market, Suez, Egypt during October 2015.

2.1.2. Other materials: Refined coarse salt was brought from El-Nasr Salines Company and polyethylene bags were obtained from Suez commercial market, Egypt.

2.2. Methods:

2.2.1. Technological process:

As showed in Fig (1), fresh Tobar fish were washed and every 6 to 7 fish (about1 Kg) put in two plastic bags and closed tightly after released air by hand. Bags distributed to fermentation for 8 hours, some hangs and fermented at ambient temperature (30 ±5°C) and others put in an incubator at 33 ±2°C. Fermented fish was dry salted (10% coarse salt, w/w) for 6 days in plastic bags.

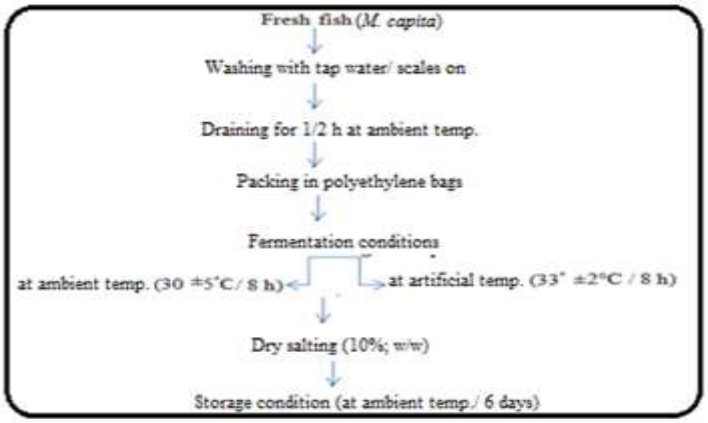


Fig. 1: Flow sheet for salted fermented tobar fish production

2.2.2. Analytical methods:

Samples of six fermented and salted fish were filleted, minced and subjected for each of the following analyses;

2.2.2.1. Biochemical analysis:

Non Protein Nitrogen (NPN) was carried out according to AOAC (1990). Total Volatile Basic Nitrogen (TVB-N) was done following the procedures of Pearson (1970). Thiobarbituric acid (TBA) was determined according to the method described by Tarladgis et al. (1960). Samples (5g) were homogenized in 25 ml of TBARS solution (0.375% TBA, 15% TCA, and 0.25M HCl), and the mixture was heated for 10 min in boiling water (95-100 □C) to obtain a pink color. After being cooled with running water, the mixture was centrifuged at 5500 g for 25 min. Finally, the absorbance of the supernatant was determined at 538 nm using a spectrophotometer. The results were expressed as mg Malonaldehyde/kg sample.

2.2.2.2. Microbiological analysis:

Samples (10 g) were taken aseptically, transferred to aseptic plastic bags and homogenized manually for 2 minutes with 90 mL 0.1% peptone water (LAB M, UK). Appropriate decimal dilutions of the samples were made using the same diluents, and 1ml of each dilution was inoculated in different growth media to estimate microbial counts. Total viable count was determined using Plate Count Agar (PCA) (LAB M, UK) incubated at 37□C for 24h. Lactic acid bacteria (LAB) were grown on DeMan Rogosa Sharpe (MRS) (LAB M, UK) agar at 30□C for two days. Compact dry ready-to-use dry films (R-Biopharm) were used for Coliform enumeration and incubated at 37□C for 24h. The results are reported as logs of colony-forming units per gram (cfu/g) (Santo et al., 2008; Al-Harbi and Uddin, 2005).

2.2.3. Sensory methods:

The end point of fish fermentation was identified according to the following apparent signs; high flexibility body muscles when it pressed by fingers, good swelling appearance of the abdominal cavity and there were not any objectionable odours. Colour, taste, odour, texture and overall acceptability of salted fermented tobar products were determined using ten trained panelists on a hedonic scale ranging from (1) to (9) as mentioned by Rangana (1977).

2.2.4. Statistical Analysis

The standard deviation was calculated using the method described by Sendecor and Cochran (1967). The means of the obtained results were analyzed using T-test using SPSS version 17.0 at a $p < 0.05$ Significance.

3. Results and discussion

3.1. Microbiological quality:

3.1.1. Effect of fermentation at artificial conditions:

Results of the microbiological load of aerobic bacteria, lactic acid bacteria, and total coliform in fermented tobara products were shown in Table 1. Before starting fermentation process, microbiological loads were tested and represented by sample zero. Total bacterial count (TBC) recorded 6.6 logs CFU/g and no detectable count neither for lactic acid bacteria nor for coliform. Fishes were incubated at artificial and ambient conditions, and then samples were taken every 2 hours for analysis for a total of 8 hours. Highest

bacterial load was recorded on PCA, MRS, and Coliform plates after 6 hours of fermentation in incubator (6.9, 4.4, 1.8 log CFU/g) and at ambient conditions (6.6, 4.2, 1.8 logs CFU/g) respectively. Load in the TBC, LAB, and Coliform on the 3 previous mentioned media declined in both trails methods investigated after 8 hours to reach to the count of bacteria in fish treated under ambient conditions (4.4, 4.5, and <1 log CFU/g) compared to other treatment (6.6, 4.8, and <1 logs CFU/g), respectively. Sunlight has slight more inhibition effect on bacterial growth and this might be due to UV radiation, which effect on bacterial genetic materials primarily responsible for microbial inactivation (Gayan et al., 2014; Ahmed and Amin, 2019).

Growth of lactic acid bacteria after 6 hours of fermentation in fishes might compete or inhibit the growth of coliform from 1.8 to <1.0 log CFU/g, and reduced total bacterial load from 6.6 to 4.4 log CFU/g, in case of fermentation at ambient temperature and little reducing effect in fermentation at artificial conditions (Table, 1). These growth inhibition effects might be attributed to the actions of bacteriocins and adequate acidification in agreement with Zeng et al. (2013).

3.1.2. Effect of salting:

After 2 days of salting, growth of lactic acid bacteria was also associated with reduction of total bacterial load in fish fermented at artificial conditions from 6.5 to 4.5 logs CFU/g, and from 5.6 to 4.6 log CFU/g in fish fermented under ambient conditions (Table, 2). According to Achinewhu and Oboh (2002), salt concentration is likely to have a pronounced influence through decrease available water essential for the microbial growth and rate of fermentation. Therefore, in the present work, the growth of TBC was inhibited by the high salt concentrations. Growth of lactic acid bacteria in addition to semi-anaerobic conditions and salt concentration might reduce the growth of pathogenic and spoilage bacteria in fermented fish products, so it can prolong salted fermented fish preservation time and safety (Dai et al., 2013). Also, Ndaw et al. (2008) concluded that with addition of 5% NaCl to the Moroccan sardines, and artificial inoculation Lactobacillus delbrueckii subsp. Delbrueckii, under 30°C fermentation, Escherichia coli, S. aureus and Salmonella were not detected, the sulfite-reducing Clostridia, and the total number of bacteria were also inhibited.

After salt had been added to fermented fish reduction of total microbial has started after 3 days, from application to be 4.5log CFU/g in salted fish fermented at artificial conditions and 4.6 logs CFU/g in salted fish fermented in ambient. Best reduction with complete estimated reduction of total bacteria count to be less than 1.0log CFU/g recorded after 4 days in fish fermented at artificial conditions and after only 3 days in fishes fermented at ambient conditions with no significant differences ($p > 0.05$). This reduction might be due to the bacteriostatic and bactericidal action of common salt (Zaitsev et al., 1969; Lee and Kaletunç, 2015), which also had reported to improve the taste and quality of fermented fish (Pyrgotou et al., 2010).

3.2. Chemical analysis:

Results of chemical analysis of Tobara Fesikh during ambient and artificial fermentation; and after salting for protein, non-protein nitrogen (NPN), TVN, and TBA of fresh Tobara fish samples were 17.86%, 0.12%, 16.8mg N/100g, and 0.56 mg MAD/Kg at zero time, respectively (Fig.2, 3, 4). The previous data agreed with Pearson (1970) who suggested that for white-fleshed fish, TVN levels below 20mg N/100g indicate that the fish is fresh, whereas the fish would be rejected for human consumption when the TVN level exceeds approximately 50mg N/100g (Silva et al., 1998). These previous parameters increase after ambient and artificial fermentation process and after salting. Where, the NPN, TVN, and TBA reached to 0.16%, 20.6mg N/100g and 3.79 after 6hr of ambient fermentation, and 0.24%, 21.0 mg N/100g and 0.75 mg/kg after 6hr of artificial fermentation, respectively. Then TVN increased after 8 hours to 26.6mg N/100g in ambient fermentation and to 61.6mg N/100 g in artificial fermentation (Fig, 2).

Change in NPN compounds is due to the role of gut content in case of whole fish during the fermentation process (Fig.4). This was supported by Oetterer (2003) in which the treatment of enzymes present in the digestive system which increased the NPN during fermentation. The value was also attributed to the proteolytic activities of the fermenting microorganisms (Babu et al., 2005). The gradual increase of TVN at the fermentation stage is probably due to autolysis by the enzymatic and bacterial action of the fish (Majumdar et al., 2005). But using artificial fermentation at a temperature, 33°C had more effect than fermentation at ambient temperature as the elevation of temperature and subsequent microbiology and biochemical changes in the fish muscles. TBA reached to >3 during fermentation at ambient temperature due to lipid hydrolysis with the effect of sunlight and microbial action but no rancid odour was noticed (Fig., 3).

After salting of ambient and artificial fermented fish, TVN was tripled and doubled and reached to 79.0 and 120.1 mg/100g after 6 days, respectively. Also, data showed increase in NPN especially in salted Fesikh fermented at artificial coditions (0.48%). Obvious decrease in TBA was pronounced in salted Fesikh fermented ambient temperature (1.96 mg/kg). Due to salting, some liquid released from the fermented fish and the breakdown of proteins with the action of microbes caused the continuous production of volatile bases (Babu et al., 2005 and Majumdar et al., 2005). Some of lipid hydrolysis got rid from flesh during salting, so TBA became in the acceptable limit <3 for a very good product.

3.3. Sensory evaluation:

The apparent signs for the good end of ambient and artificial fermentation processes were appeared at the same time after 8 hours. According to the sensory results (Table, 3), the ambient and artificial salted fermented Tobara products; Tobara Fesikh prepared in this study were accepted organoleptically. The results revealed that Tobara Fesikh products had odour similarly rated product odour i.e. had the desirable Fesikh odour free of any objectionable odour. Salted fermented product at ambient coditions was the superior in odour than other one. Taste panel results suggest that using artificial temperature during fermentation improved both colour and texture of the control Tobara Fesikh and had high quality attributes.

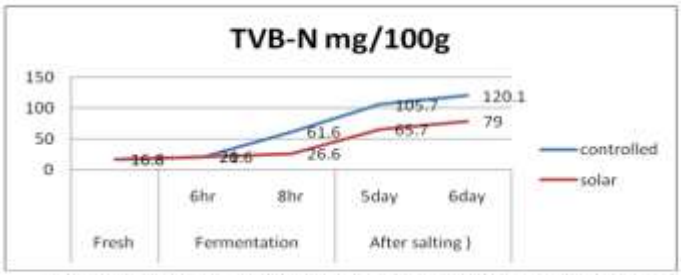


Fig. 2: Effect of fermentation and salting periods on TVB-N of Tobara fish

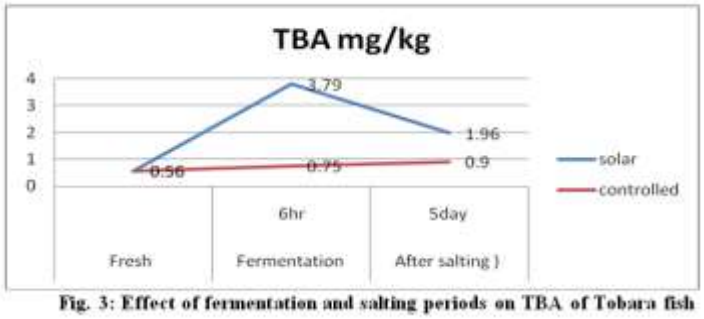
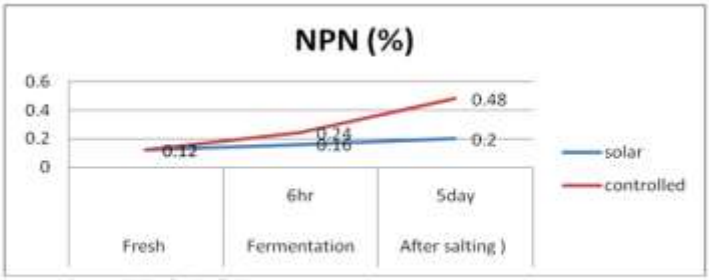


Fig. 3: Effect of fermentation and salting periods on TBA of Tobara fish



*Protein in fresh fish was 17.86%

Fig. 4: Effect of fermentation and salting periods on NPN of Tobara fish

Table 1: Effect of time of fermentation technique on total bacterial count (TBC), lactic acid bacteria (LAB) and Coliform of Tobara fish.

Fermentation Time	Artificial Fermentation Log (CFU/ g)			Ambient Fermentation Log (CFU/ g)		
(hours)	TBC	LAB	Coliform	TBC	LAB	Coliform
zero	6.5	<1 EST [a]	<1 EST	6.5	<1 EST	<1 EST
2	6.1	<1 EST	<1 EST	5.1	<1 EST	<1 EST
4	6.4	<1 EST	1.6	6.4	<1 EST	1.6
6	6.9	4.4	1.8	6.6	4.2	1.8
8	6.6	4.8	<1 EST	4.4	4.5	<1 EST

[a] :estimated to be less than 10 colonies

Table 2: Effect of salting period on total viable count (PCA), and lactic acid bacteria (LAB) of Tobara fish.

Salting period (days)	Artificial Fermentation Log (CFU/ g)		Ambient Fermentation Log (CFU/ g)	
	PCA	LAB	PCA	LAB
0	6.6	4.8	4.4	<1 EST
1	4.9	<1 EST [a]	4.8	<1 EST
2	6.5	5.8	5.6	4.4
3	4.5	<1 EST	4.6	<1 EST
4	5.6	<1 EST	<1 EST	<1 EST
5	<1 EST	<1 EST	<1 EST	5.4
6	<1 EST	5.5	<1 EST	<1 EST

[a] :estimated to be less than 10 colonies

Table 3: Sensory evaluation of Fesikh Tobara products (9 point scale)

Quality attributes	Characteristics	Tobara Fesikh products:	
		Ambient Fermentation	Artificial Fermentation
Odour	Appetizing odour, free of any objectionable odours.	8.7±0.1	8.5±0.2
Colour	pink, homogenous	9.2±0.3	9.5±0.3
Taste	lightly salted, free of any objectionable taste	9.0±0.2	9.2±0.3
Texture	firm, not chewy	8.9±0.4	9.3±0.2
Overall quality	High quality products, collected served quality attributes	8.9±0.3	9.1±0.3

4. Conclusion:

From the previous results, it is observed that all data were according the allowance limits. It could be concluded that Tobara Fesikh samples prepared by using short time fermentation method were safe for human consumption. This will improve the economic situation of the processors who adopt improved technologies for fermented fish processing in addition to a healthy product can be obtained.

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