Quality Changes and Microbial Load of Marinated Tilapia (*Oreochromis niloticus*) Stored at Refrigeration Temperature

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Abstract

Tilapia is the main species farmed commercially in Egypt and is the most common for marketing as fresh fish. However, the usage of tilapia in marinade production is not common in Egyptian culture. The current investigation was aimed to produce tilapia (*Oreochromis niloticus*) marinade using different acetic acid concentrations with different methods, to determine its bacterial load and best sensory quality during storage at 4°C. The fish processing were headed, gutted, filleted, marinated and stored at 4°C until the end point, which was assessed by pH measuring, sensory, and microbiological analyses. Warm marinated was performed using solutions containing 2, 3% acetic acid and 3% NaCl then transferred into plastic jars, covered with 3% gelatin. Higher concentrations of acetic acid (5%) and NaCl (6%) were used for cold marinating process for 30 days then transferred into packaging solution of 2.5% acetic acid and 3% NaCl. Bacterial load has increased after 7 days with warm marinated fillets with best sensory scores in the first 3 days using 3% acetic acid solution, and best sensory scores in the first 7 days with 2% acetic acid. For cold marinated fillets, load of bacteria was reduced after 3 days from application of marinating solution until to the end of marinating process and after application of packaging solution with 2.5% acetic acid. The best sensory scores of cold marinated fillets were lower than the ones belong to warm marinated process. There is no significant change ($p > 0:05$) in pH values during storage.

KEYWORDS: Fish; marinated tilapia; *Oreochromis niloticus*; marinating, bacterial load

Introduction

Egypt has been the second largest producer of tilapia in the world for many years, with an enormous potential for further development in tilapia industry ***(MacMillan, 2014).*** One of the most common value added product is processing of fillet. Today, an important component of the growing tilapia industry is the proliferation of various product forms.

Due to the increasing consumer demand for fish products with prolonged shelf-life, many researches have focused on preservation techniques to control bacterial growth for safety purposes or for extending the shelf-life of the products ***(Sallam, 2007).*** Marinades are fish products consisting of fresh, frozen, salted fish or portions of fish processed by treatment with edible acids and salt and put up in brines, sauces, creams or oil ***(Meyer, 1965).*** Marinades are semi-preserves; acid, usually acetic acid and salt are added to the fish to retard the action of bacteria and enzymes resulting in a product with a characteristic flavor and an extended but limited shelf life ***(McLay, 1972).*** The aim of Marinating is not only to prevent microorganism growth, but also is used to tenderize or to change taste, textural and structural properties of raw material. Initial quality of raw materials, considering their freshness, microbiological load and physical damage, is an important factor which influences the quality of the end product (Fuselli *et al*, 1994). Keeping qualities depend largely upon storage temperatures. Marinades stored at cooler temperatures (4–6°C) keep a long time.

Tilapia in aquaculture is an important and common species in Egypt. It is generally consumed as fresh, or minimally processed, but usage of marinated Tilapia is not common in Egypt. In this study, the usage of Tilapia in marinade production was tried using different acetic acid concentrations with different methods. The aim of the study was to produce Tilapia marinade, to determine its microbiological and best sensory quality during storage at 4°C.

Materials and Methods

Raw material

Fresh cultured tilapia fish (*Oreochromis niloticus*) was purchased in November 2014 from local market, Suez, Egypt. Total 10 kg fish were obtained and transferred to the laboratory in an iced box with crushed ice. Fish were headed, filleted, skinned, washed and marinated. Samples (10 g) were taken from skinned fish fillet before and during the marinating process for microbiological analysis.

Marinating process

Productions of cold and warm marinated tilapia fillets were carried

as shown in (Fig.1). Marinating was done by immersing the fish fillets into solutions containing acetic acid and NaCl. These solutions were selected for the present study.
Solution A: 2% acetic acid, 3% NaCl, 3% gelatin.
Solution B: 3% acetic acid, 3% NaCl, 3% gelatin.
Solution C: 5% acetic acid, 6% NaCl.
Solution D: 2.5% acetic acid, 3% NaCl.
Solution A, and B were used in warm marinating, fish fillets were kept in these solutions during all the process. Solution C used as initial solution during maturation in cold marinating process then changed to solution D as a packaging solution. Warm marinated fillets were kept in plastic jar containers with addition of gelatin. (Karim, and Bhat, 2009). (Fig. 1) (AOAC, 1995).
The ratio of fish to solution was 1:1. Immersing process was performed at ambient temperature (25 °C). Packed fish were stored at 4 °C and analyzed at different time intervals to determine the microbiological and sensory quality of the product (Goko€glu *et al*., 2004).

Analysis

Before every analysis, two fish fillets from the jar were randomly taken and all the fish were pooled. Then the fish flesh were homogenized and analyzed for the following parameters:

pH measurement

Fish samples were homogenized with distilled water at ratio of 1:9 (W/V) in a blender. The pH was measured by dipping the pH electrode into a mixture of homogenized sample according to AOAC (1995).

Sensory analyses:

In Sensory evaluation studies, marinated fish were assessed on the basis of appearance, odour, taste and texture characteristics using a 5-point scale. A score of 5.0, 4.0, 3.0, 2.0 and 1.0 indicated excellent, very good, good, fair and poor quality, respectively. Sensory evaluations were conducted using five experienced panelists (Meilgoard *et al*., 2007).

Bacteriological sampling and analysis:

Sampling was analyzed for microbiological investigations for 54 days of warm marinated fish with solution A, and B. Before starting marinating, fishes were processed into fillets and microbiological loads were tested and represented by sample zero. Fillets were steamed (steamed sample), marinated and stored at refrigeration temperature (4°C). Samples were taken at day 1, 2, 3, 7, and 54 day intervals. Whereas sampling extended for up to 42 days with cold marinated fish with solutions C and D.

For all microbiological counts, **10 g** samples were homogenized in **90** ml 0.1% peptone water (LAB M).

From this 10^{-1} dilution, other decimal dilutions were prepared. Total viable count was determined by the spread plate method, using Plate Count Agar (PCA) (Difco, 0479-17) as the medium. Total coliform was enumerated by spread-plating on to Brilliant Green agar (BG), *Escherichia coli* on Sorbitol MacKoncy (SMAC), and *Salmonella* sp. on XLD. Plates were incubated at 37^0C for 24 hours and colony forming units (CFU) were counted (Santo *et al*, 2008). Readings obtained with 30 to 300 colonies on a plate were used to calculate bacterial population numbers, recorded as CFU per gram of sample (Al-Harbi and Uddin, 2005). Three to five representatives of each colony type were then streaked on additional Tryptcase Soy Agar slants (TSA). The isolates were then subjected to biochemical tests (citrate, Methyl red, Voges–Proskauer, indole, H_2S production, and triple sugar iron) for identification.

Statistical Analysis

Data were treated by analysis of variance using SPSS (2006). Significance was established at $p < 0.05$. Means and standard deviations were estimated.

Results and Discussion

The microbiological determinations were performed to assess the procedures related to the processing of the tilapia marinated fillets. Results of the quantitative estimation of aerobic bacteria in warm marinated fish using 2% acetic acid (solution A) and 3% acetic acid (solution B) are shown in Table 1. Steam temperature decreased slightly the initial bacterial counts in zero sample for both acetic acid concentrations from 6 log CFU/g to 5.5 log CFU/g. The salting and marinating processes reduced the total bacterial viable count to 4.6 log CFU/g in two days with an agreement with ***Furutani et al (2013).*** Results fluctuated to 5.9, 4.6, and 5.5 log CFU/g for samples 1, 2, and 3, respectively in the first 3 days. Results agreed also with ***Kin et al (2011)*** indicate that the use of acetate salts minimizing bacterial counts of fish fillets during the first 7 days from application ($P < 0.05$).

The initial quality of fish used in this study was good as indicated by a low initial number of suspected bacteria. *Salmonella, coliform,* and *E. coli* were not detected during the first days of storage. No defined result was found on selective plates of SMAC, XLD, and BG with both methods for up to 3 days (estimated less than 2 log CFU/g). It was reported that sorbic, benzoic, gluconic, acitric acids, and acetic acid in this case, inhibited the growth of bacteria ***(Poligne and Collignan, 2000; Cadun et al, 2005; Bjo¨rkroth, 2005).*** Total bacterial count increased after day 7, and by the end of storage period (54 days) increased more to reach 8.9, 8, 8.8, and 8.6 Log CFU/g on SMAC, PCA, XLD, and BG in

marinated fish using solution A. In marinated fish using Solution B, coliform count (8.1 log CFU/g) on BG was less than that (8.6 log CFU/g) in solution A, E.coli, and Salmonella count were the same, and a slight increase was detected in total bacterial count on PCA (8.1 log CFU/g). ***O¨zogul et al (2009)*** had reported similar results with reduction of total bacterial count after application of warm marinating with 4% acetic acid solution to be decreased to 3.2 logs CFU/g. After that, total bacterial count started to increase to 4.3 log CFU/g at the end of storage period. Increased bacterial count after 7 days may also be the result of lactic acid bacteria growth that added to the total count in low acid marinated fish ***(Silva and White 1994).***

Cold marinating was another process used to marinate tilapia without any steaming. The results of the microbiological tests of the cold marinated tilapia fillets using solutions C, and D conserved under refrigeration (4°C) are shown in Table 2. Bacterial load started with high count reached up to 7 log/g CFU and a little higher on SMAC, PCA, XLD, and BG (Table.2). Complete reduction after 2 days of application of 5% acetic acid solution, as bacterial count reduced and estimated to be less than 2 log CFU/g on SMAC, XLD, and BG. As can be seen, all the results obtained for *E.coli*, total coliform, and Salmonella sp. were within the acceptance limits for consumption after 2 days from marinating. On the other hand, tiny anaerobic bacteria grew on PCA from the 3rd day until the end of storage period. It was reported that the marinating process reduced the number of Enterobacteriacae and H_2S-producing bacteria within 3 days, while the total viable count and the lactic acid bacteria slightly increased during the storage due to lower pH that favor the growth of lactic acid bacteria ***(Giuffrida et al, 2007).***

Suspected colonies were selected from SMAC, XLD, and BG in warm and cold marinating processes were tested biochemically on Citrate, Methyl red, Vogus Proskour, and triple sugar iron slants. Results were positive for *E-coli* and negative for Salmonella sp.

The pH in fresh fish flesh is almost neutral. In the post-mortem period, decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh ***(Shenderyuk & Bykowski, 1989).*** The increase in pH indicates the loss of quality. Marinades have a low pH due to acetic acid content. During the storage of marinades, lactic acid bacteria can grow and cause the amino acids to degrade. Thus, the formation of carbon dioxide and other decarboxylation products is observed. These products bind

acetic acid and the pH of marinade rises ***(Shenderyuk & Bykowski, 1989).*** pH levels of samples marinated with 5% acetic acid solution started from 4.9± 1 for the first 9 days then rose after 38 and 42 days to be 5.5, and 6.2. This increase might be explained because of changing the marinating solution to use packaging solution with 2.5% acetic acid (solution D). pH value in raw fish flesh was found 6.5. ***Kristinsson et al (2005)*** found a pH value of 6.5 in Tilapia too. The pH value in tilapia found by ***Santo et al. (2008) and Abelti (2013)*** were 6.4, 6.1 respectively. There was a significant difference ($p < 0:05$) in pH between samples marinated in 2 and 3%, acetic acid solutions and these did not change significantly ($p > 0:05$) during storage (Fig. 3). Other researchers reported insignificant changes in pH levels of marinated fish. ***Poligne and Collignan (2000)*** found that the pH levels of anchovies pickled with acetic acid increased from 3.90 to 4.21 after 20 d of storage and then remained constant until the end of the storage. ***Goko€glu et al. (2004)*** found that pH of marinated sardine in 2 and 4% acetic acid solutions did not change significantly ($p > 0:05$) during storage for up to 150 days.

Sensory scores of marinated Tilapia significantly decreased ($p < 0:05$) throughout the storage especially except marinated fish using 2% acetic acid solution. Sensory scores of Tilapia marinated with acetic acid solution of 3% were significantly higher ($p < 0:05$) than those found in Tilapia marinated with acetic acid of 2% and 5% (Fig.2). The samples had ‘‘very good’’ and its best quality for up to 54 days for 2% acetic acid, and first 3 days for 3% and 5% acetic acid solutions and that was agreed with ***(Goko€glu et al, 2004).***

Conclusion

It was concluded that, the results showed that warm marinated tilapia could be consumed safely throughout the first week from storage period while the cold marinated tilapia (*Oreochromis niloticus*) fillet could be stored safely for up to 42 days at 4℃.

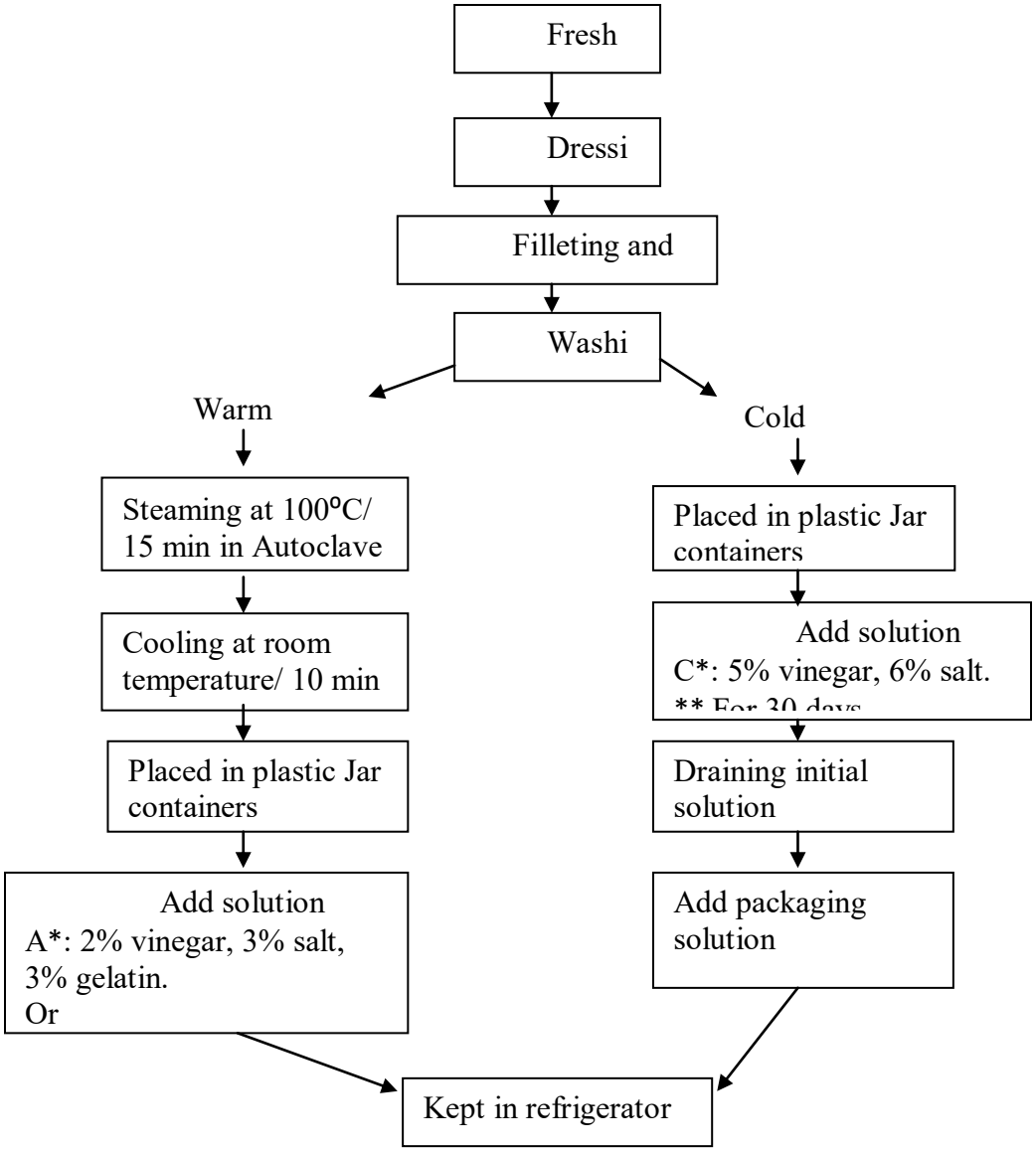

Fig 1: |*Outlines of cold and warm marinated Tilapia (Oreochromis niloticus) fillets.*

** Fish to solution ratio, 1:1 ** Until maturation*

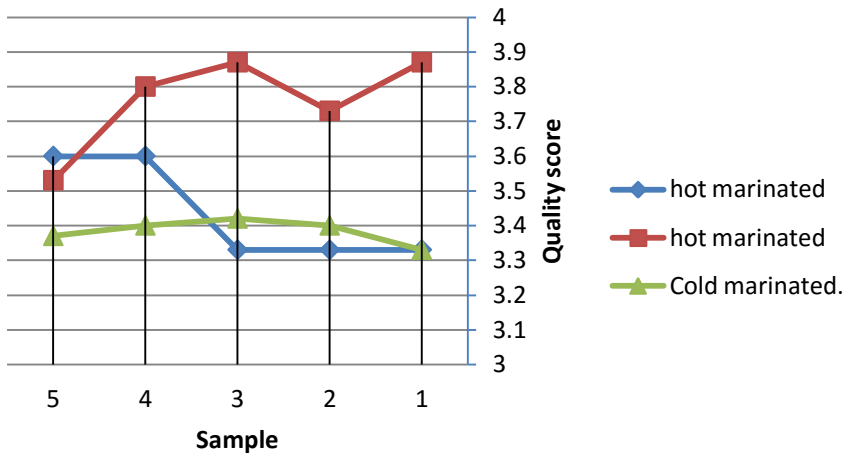

Fig 2: *Sensory scores of marinated Tilapia (Oreochromis niloticus) during storage at 4 ℃.*

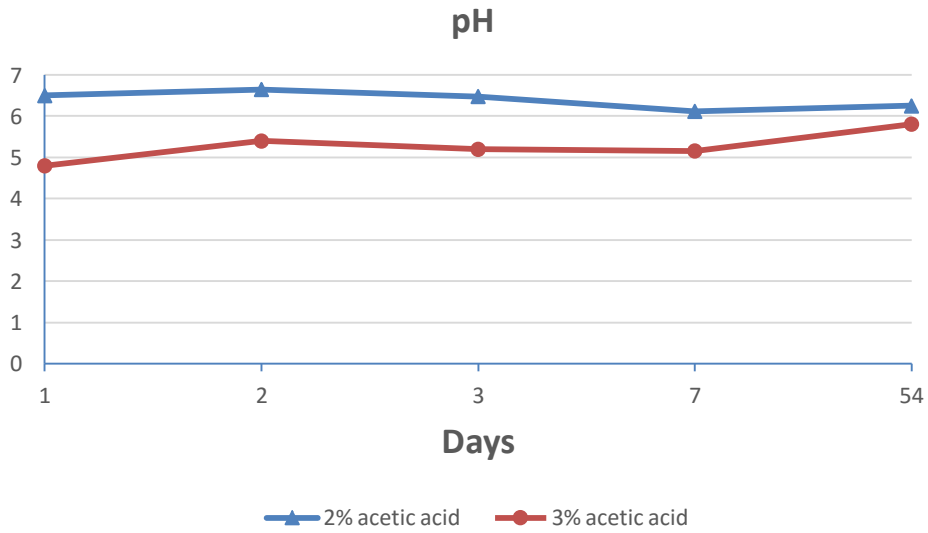

Fig 3: *pH values of marinated Tilapia (Oreochromis niloticus) during storage at 4 C.*

Table 1: Bacterial load (CFU/g) of marinated fish using 2% acetic acid (solution A), 3% acetic acid (solution B).

	WARM MARINATING USING 2% ACETIC ACID					WARM MARINATING USING 3% ACETIC ACID			
DAYS	sample	Log (CFU/ g)				Log (CFU/ g)			
		SMAC	**PCA**	**XLD**	**BG**	**SMAC**	**PCA**	**XLD**	**BG**
1	zero	<2 EST	6	<2 EST	<2 EST	<2 EST	6	<2 EST	<2 EST
1	steamed	<2 EST	5.5	<2 EST	<2 EST	<2 EST	5.5	<2 EST	<2 EST
1	1	<2 EST	5.9	<2 EST	<2 EST	<2 EST	5.9	<2 EST	<2 EST
2	2	<2 EST	4.6	<2 EST	<2 EST	<2 EST	4.6	<2 EST	<2 EST
3	3	<2 EST	5.5	<2 EST	<2 EST	<2 EST	5.5	<2 EST	<2 EST
7	4	8.6	6.8	7.9	8.0	8.1	6.4	8.1	7.1
54	5	8.9	7.5	8.8	8.6	8.9	8.4	8.8	8.1

Table 2: Bacterial load (CFU/g) of cold marinated fish using 5% acetic acid (solution C) and 2.5% acetic acid (solution C) .

DAYS	SAMPLE	LOG (CFU/ SAMPLE UNIT)			
		SMAC	**PCA**	**XLD**	**BG**
1	zero	7.2	7.4	7.3	7.0
1	1	7.4	8.5	8.5	7.5
2	2	8.5	8.7	8.8	8.3
4	3	<2 EST	7.5 anaerobic	<2 EST	<2 EST
7	4	<2 EST	7.7 anaerobic	<2 EST	<2 EST
9	5	<2 EST	8.4 anaerobic	<2 EST	<2 EST
38	6	<2 EST	8.9 anaerobic	<2 EST	<2 EST
42	7 (solution D)	<2 EST	8.3 anaerobic	<2 EST	<2 EST

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تغيرات الجودة و الحمل الميكروبى الخاص بأسماك البلطى المخللة و المخزنة فى درجة التبريد

البلطى من أهم الأنواع المستزرعة تجاريا في مصر وهي الأكثر شيوعا للتسويق كما الأسماك الطازجة. ومع ذلك، فإن استخدام البلطى فى انتاج اسماك مخللة غير شائع فى الثقافة المصرية. والهدف من هذا البحث هو إنتاج البلطي المخلل باستخدام تركيزات مختلفة حامض الخليك مع تحديد حمولتها البكتيرية وأفضل جودة حسية للمنتج أثناء التخزين في درجة ٤ مئوية.و قد تم تجهيز الاسماك عن طريق قطع الرأس و تنظيف الاحشاء و عمل شرائح ثم تخليل الاسماك و حفظها فى الثلاجة حتى انتهاء فترة التخزين. تم تقييم المنتج عن طريق قياس درجة الحموضة، وتحليل الحسية، والتحاليل الميكروبيولوجية. و عملية التخليل الساخن تمت باستخدام محاليل تحتوي على حامض ٢، ٣٪ الخليك و ٣٪ كلوريد الصوديوم ثم وضعه فى برطمان بلاستك مع التغطية باستخدام ٣٪ من الجيلاتين. استخدمت لعملية التخليل الباردة تركيزات أعلى من حمض الخليك (٥٪) وكلوريد الصوديوم (٦٪) ثم نقل الى محلول التعبئة والتغليف من حمض الخليك ٢,٥٪ و وكلوريد الصوديوم ٣% . وقد زاد الحمل البكتيري بعد ٧ أيام فى شرائح المخلله بالطريقة الساخنة مع أفضل الدرجات الحسية في ٣ أيام الأولى باستخدام ٣٪ محلول حامض الخليك. أفضل نتائج حسية لشرائح المخللة بالطريقة الباردة أقل من تلك التي تنتمي للتخليل الساخن. كما ان لا يوجد أي تغيير معنوي في قيم الرقم الهيدروجيني أثناء التخزين.(p> 00:05)