SİDAS MEDYA
Akademik Gıda® / Academic Food Journal
ISSN Print: 1304-7582, Online: 2146-9377
http://www.academicfoodjournal.com

Akademik Gıda 10(4) (2012) 19-23

Research Paper / Araştırma Makalesi

Shelf Life of Anchovy (*Engraulis engrasicholus,* L.1758) Patties Stored at 4°C

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Received (Geliş Tarihi): 20.11.2012, Accepted (Kabul Tarihi): 20.12.2012
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ABSTRACT

In this study, shelf life of anchovy patties stored at 4°C was determined. Sensory properties, pH, total volatile base nitrogen (TVB-N), thiobarbituric acid (TBA), trimethyl-amine (TMA) and total mesophilic aerobic bacteria (TMAB), total yeast-mold (TYM) and total coliform bacteria (TCB) counts of patties were determined daily. According to sensory and microbiological quality criteria, anchovy patties became inconsumable at the 8th day of storage. Effect of storage time on the sensory score, value of pH, TVB-N, TBA, TMA, TMAB, TYM and TCB of patties was found significant ($p<0.05$).

Key Words: Anchovy, Pate, Shelf life, Sensory, Microbiological

4°C'de Depolanan Hamsi Balığı (*Engraulis engrasicholus*, L.1758) Burgerlerin Raf Ömrü

ÖZET

Bu çalışmada hamsi balığından elde edilen balık patesi 4°C'de muhafaza edilmiş ve raf ömrünün tespit edilmesi amaçlanmıştır. Balık patesi örneklerinde günde 1 kez olmak üzere, duyusal analizler, pH, toplam uçucu bazik azot (TVB-N), trimetilamin azot (TMA), tiyobarbütirik asit sayısı (TBA), toplam mezofil bakteri (TMB) sayısı, toplam maya ve küf sayısı ve toplam koliform bakteri sayısı analizleri yapılmıştır. Duyusal ve mikrobiyolojik kalite kriterleri göz önüne alındığında, hamsi balığından elde edilen balık patesi 8. günde tüketilemez kalite özelliği göstermiştir. Depolama süresi boyunca, duyusal puanlar, pH değerleri, TVB-N miktarı, TBA miktarı, TMA miktarı, TMB miktarı, TMK miktarı ve TKB miktarı üzerine depolama süresinin etkisi önemli bulunmuştur ($p<0.05$).

Anahtar Kelimeler: Hamsi, Burger, Raf ömrü, Duyusal, Mikrobiyolojik

INTRODUCTION

It was stated that quantity of caught anchovy, that is one of the most important fish types among sea fishes in Turkey, is 228.491 tons. According to 2011 data, anchovy comprises approximately 53% of total production among caught sea fishes [1]. Techniques of drying, cooling, freezing, canning, smoking, marination and imitate are most commonly used ones. Some of these technologies that are applied traditionally from past to present are improved by modifying nowadays. Besides durability, different flavors, oppurtunity of transportation to far distances gains for product, easiness and cost of practise is also important in acceptability of technology [2]. Consumption of processed seafood is very required and beneficial in terms of preservation and storage of product, further utilization from products and enhancing the employment oppurtinities, reducing enviromental pollution, recovery of waste and providing easiness for consumer [3]. Lee [4] stated that, minced meat based seafood, maximize values of available seafood sources due to innovator formulation strategies in seafood industry. Also stated that, fish is good source that can be converted to ready food material in consumed food materials consumed by a large segment of the society. Increasing of production of seafood based food is important in containing healthy food features [5]. Anchovies are very suitable for patties

because of their flavor and odor [6]. Generally, they are consumed as fresh. Fish patties are among ready-to-eat food and producted from various types of fishes. Consumption of them are not very common in Turkey [7]. In this study, anchovy patties were obtained by adding various ingredients after boiling process of anchovies, and it was aimed to determine shelf life of anchovy patties in refrigeration conditions.

MATERIALS and METHODS

Pattie Preparation

Fresh anchovy fishes (*Engraulis engrasicholus*, L.1758) with an average lenght of 8±1 cm were used. In total 10 kg of fresh anchovies were provided from a fisher in Sinop located in Black Sea Region, Turkey. After anchovies had been eviscerated, they were washed with tap water. Then, fish patties were prepared from the fresh anchovies. Modified formulation of anchovy patties were prepared from Yerlikaya et al. [7]. Headed and gutted anchovies were boiled in boiling water for 5 min. Bones of the fishes were picked up and then fillets minced with a blender. Ingredients were added to minced fish according to the following formulation: 3% boiled potatoes, 0.8% semolina, 0.6% crumb, 1.3% egg, 1.7% onion, 1.1% olive oil, 0.25% salt, 0.1% black pepper, 0.07% red pepper, 0.07% cumin, 0.07% thyme, 0.07% white pepper. Minced fish and ingredients were kneaded and shaped by hand. Ten anchovy patties were placed into each polystyrene box. The polystyrene boxes were wrapped in strech films and stored in a refrigerator at 4°C. Quality control analyses were made once a day for anchovy patties during storage until their spoilage in terms of sensory.

Chemical Analyses

Every analysis was made as three parallels. About 10 g of samples were taken, made smaller and 20 mL distilled water was added onto sample. The mixture was homogenized in a homogenizer (IKA Yellow Line DI 25 Basic) and the pH values were measured using an apparatus (Werkstatten 82362 Weilheim, Germany) [8]. Total volatile base nitrogen amount (TVB-N) was determined according to method of Lucke and Geidel modified by Antonacopoulas and expressed as mg TVB-N per 100 g fish flesh [9, 10]. Thiobarbituric acid (TBA) value was determined according to Tarladgis et al. [11]. Trimethyl-amine nitrogen (TMA-N) amount was determined according to Dyer developed by Bysted et al. [2].

Microbiological Analyses

A sample of 10 g was taken for microbiological analysis and transferred into 90 mL sterile physiological saline solution (0.85% NaCl). After homogenized, proper dilutions were prepared by taking samples from this homogenate [13]. Total mesophilic aerobic bacteria, yeast-mold and coliform bacteria counts was determined by using the pour plate method. Plate Count Agar (PCA, Merck, Germany) was used as a medium for total mesophilic aerobic bacteria count and petri dishes were incubated for 3 days at 28°C. For yeast-mold count, Potato Dextrose Agar (PDA, Merck, Germany) was used as a medium and again petri dishes were incubated at for 3 days at 28°C. To count coliform bacteria, Violet Red Bile Agar (VRBA, Merck, Germany) was used as medium and petri dishes incubated for 24 h at 35°C. Results were given as log cfu/g [9, 14].

Sensory Assessment

Taste panels were composed of six members who had been experienced in sensory evaluation. The anchovy patties were fried in a deep fryer pan (MOULINEX Minuto) for 4 min. After frying, they were cooled to 50°C and samples were served to panelists for evaluation of the sensory attributes (appearance, odor, flavor, texture) by using modified form of Schormuller [15]. The evaluation was made by giving scores between 1-5 and indicated as: 5→Very good, 4→Good, 3→Acceptable, 2→Bad, 1→Very bad [16,17].

Statistical Analysis

The Minitab 15 (Minitab Inc. USA) program was used to search for significant differences between mean values of different results. Differences between means were analyzed by one-way analysis of variance (ANOVA). The results are presented as mean±SE.

RESULTS and DISCUSSION

pH value of fresh anchovy was determined as 6.10. The pH levels in anchovy patties increased from 6.37 to 6.80 at the end of the storage period of 8 days. pH value of fresh fish flesh was almost neutral. Decomposition of nitrogenous components in post mortem period causes to increase in pH in fish flesh [18]. Turhan et al. [19] reported that pH values of anchovy patties increased from 6.33 to 6.56 after 10 days of storage. In addition Kilinc [6] determined that pH value of anchovy patties was 6.14 at first day of storage and then increased to 6.36 at the end of the storage period of 5 days. These results were very similar to our findings. Besides, pH values of sardine patties studied by Kilinc et al. [20] found similar to our study.

The TVB-N value of fresh anchovy was determined as 5.60 mg/100 g. In the present study, TVB-N values increased significiantly ($p<0.05$) during storage of anchovy patties. The initial TVB-N content of anchovy patties was 7.00 mg/100 g. This value increase to 26.50 mg/100 g at the end of the storage period of 8 days. There is some differences in quality classification according to TVB-N value for seafood in terms of different researchers. According to Varlik et al. [9], seafood was evaluated as ‘very good’, if TVB-N value is lower than 25 mg/100 g; ‘good’, if TVB-N value is between 25-30 mg/100 g, ‘marketable’, if TVB-N value is between 30-35 mg/100 g and ‘spoiled’, if TVB-N value is 35 mg/100 g or higher than this value. So, the anchovy patties stayed in ‘‘good’’ quality limits for TVB-N values at the end of the storage. Kilinc et al. [20] reported that TVB-N value of sardine patties was 13.66 mg/100 g at the first day of storage period, when it was 29.55

mg/100 g at the end of the storage period (at day 6). The values in our study were found lower than Kilinc et al. [20]. TVB-N values of control group that is made from trout with similar method and ingredients to our study [21].

The TBA value of fresh anchovy was determined as 1.25 mg MA/kg. At the beginning of the storage period, the TBA value of anchovy patties was determined as 1.37 mg MA/kg. At the end of the storage period of 8 days, TBA values of anchovy patties were found to be 6.78 mg MA/kg. According to the results of TBA analysis, statistically differences were determined ($p<0.05$). Schormuller [15] stated that TBA amount that is used to determine oxidation level is less than 3 mg MA/kg in very good material, must not be higher than 5 mg MA/kg in good material and acceptability limit value is 7-8 mg MA/kg. Anchovy patties that were stayed in acceptability limit values in the present study, sardine patties of Kilinc et al. [20] stayed in acceptability limit values, similarly. Yerlikaya et al. [7] stated that, it was investigated quality changes of anchovy patties at 4°C. Values of TVB-N and TBA of anchovy patties increased, acidity and sensory scores decreased for storage period and they kept acceptable quality properity for 6 days. Despite, TVB-N value did not exceed acceptability limit value after 6th day of storage, the product was spoiled in terms of sensory properties, similarly in our study. Oksuztepe et al. [21] investigated effect of addition sodium lactate at the rate of 0.5%, 1% and 2% on meat balls that were obtained from fresh rainbow trout and stored at 4°C. They reported that TVB-N and TBA values increased during storage period and did not exceed acceptability limit values in all samples. TBA values of fish burgers made from fresh and frozen-thawed trout fillets by Taskaya et al. [22] were found quite lower than our values. It may be said that this difference arising from fish species besides ingredients.

The TMA value of fresh anchovy was determined as 2.25 mg/100 g. The TMA values of anchovy patties were founded to be 2.51 mg/100 g and 5.86 mg/100 g at day 1 and at the end of the storage period of 8 days, respectively (Table 1). TMA is produced by the decomposition of trimethylamine N-oxide caused by bacterial spoilage and enzymatic activity [23]. Acceptable TMA value for fish is stated as 5-10 mg/100 g [24]. In our study, TMA values did not exceed limit values during storage and inter-day difference was found significant statistically ($p<0.05$). Boran and Kose [25] reported that TMA values of whiting meat balls obtained from plain mince and surimi exceeded acceptability limit value at day 10 of storage period. TMA values of the sardine patties were determined as 1.20-5.01 at the first day of storage period and at the end of the storage period, respectively [20] and these results are very similar to our findings.

Table 1. Chemical analysis results of anchovy patties stored at 4°C

Analyses	Storage time (days)								
	Fresh	1	2	3	4	5	6	7	8
TVB-N (mg/100 g)	5.60±0.00^{a}	7.00±0.00^{b}	11.2±0.02^{c}	12.60±0.05^{d}	14.00±0.00^{e}	18.20±0.09^{f}	21.00±0.00^{g}	23.40±0.1^{h}	26.50±0.2^{i}
TBA (mg/MA/kg)	1.25±0.00^{a}	1.37±0.02^{b}	2.41±0.1^{c}	2.82±0.01^{d}	3.07±0.07^{e}	3.46±0.03^{f}	4.79±0.04^{g}	5.48±0.05^{h}	6.78±0.02^{i}
pH	6.10±0.00^{a}	6.37±0.00^{ab}	6.30±0.02^{c}	6.32±0.03^{bc}	6.13±0.01^{a}	6.28±0.02^{c}	6.40±0.04^{d}	6.70±0.00^{e}	6.80±0.02^{f}
TMA (mg/100 g)	2.25±0.00^{a}	2.51±0.03^{b}	2.69±0.01^{c}	3.00±0.00^{d}	3.06±0.06^{e}	3.66±0.09^{f}	4.72±0.04^{g}	5.27±0.03^{h}	5.86±0.02^{i}

Means in rows with different superscripts are significantly different ($p<0.05$). n=3, values are shown as mean ± standard error of triplicates. TVB-N: Total volatile base nitrogen; TBA: Thiobarbituric acid; TMA: Trimethyl-amine nitrogen

Table 2. Microbiological analysis results of anchovy patties stored at 4°C (log cfu/g)

Analyses	Storage time (days)								
	Fresh	1	2	3	4	5	6	7	8
Total mesophilic aerobic bacteria	4.48±0.00^{a}	4.78±0.01^{b}	4.98±0.00^{c}	5.34±0.02^{d}	5.66±0.04^{e}	5.71±0.02^{f}	5.80±0.02^{g}	5.94±0.00^{h}	6.65±0.01^{i}
Total yeast-mold	4.18±0.00^{a}	4.51±0.00^{b}	4.93±0.02^{c}	5.04±0.04^{d}	5.53±0.01^{e}	5.62±0.02^{f}	5.76±0.02^{g}	5.88±0.01^{h}	6.56±0.01^{i}
Total coliform bacteria	2.48±0.00^{a}	2.54±0.00^{b}	2.45±0.03^{a}	2.32±0.02^{c}	2.18±0.01^{d}	2.00±0.00^{e}	1.93±0.03^{f}	1.79±0.03^{g}	1.70±0.00^{h}

Means in rows with different superscripts are significantly different ($p<0.05$). n=3, values are shown as mean ± standard error of triplicates. cfu: colony forming units.

Total mesophilic aerobic bacteria, total yeast-mold and total coliform bacteria counts of fresh anchovy were determined as 4.48 log cfu/g, 4.18 log cfu/g and 2.48 log cfu/g, respectively. The initial counts of total mesophilic aerobic bacteria, total yeast-mold and total coliform bacteria were 4.78 log cfu/g, 4.51 log cfu/g and 2.54 log cfu/g, respectively. These values were determined to 6.65 log cfu/g, 6.56 log cfu/g, 1.70 log cfu/g at day 8 of storage, respectively (Table 2). The initial microbiological quality is very important for the shelf life of fish products. Total viable count is an important criterion for quality evaluation. The maximum of 10^7 cfu/g is the acceptability of fresh and frozen fish, but not for fish patties, as recommended by the International Commission of Microbiological Standards for Foods [26]. There is also no standard for fish patties in Turkey, but according to patties standard, maximum total viable count was given as 10^6 cfu/g [27]. When total mesophilic aerobic bacteria and yeast-moldcounts exceeded acceptability limit values at day 8 of storage ($p<0.05$), total coliform bacteria counts decreased during storage. It is thought that decrease of coliform bacterias during storage is related with used ingredients in the patties and storage of the patties in refrigeration conditions (4±1°C) between 1.8°C and 4.4°C that are the minimum growth temperatures for this group bacterias [28]. In another study related with anchovy patties stated that total bacteria count was $1.6x10^6$ cfu/g at the end of the storage period (at 5 day). In the same study, determined that yeast-mold counts were <10 cfu/g during storage [6].

Changes in the sensory quality of fish patties are shown in Table 3. When evaluated in terms of sensory quality criterias, anchovy patties obtained from anchovy and stored in refrigeration condition at 4°C, exceeded acceptability limit value with 1.20±0.18 at day 8 of storage. The most important criteria for the quality of product for the storage of food is sensory analysis results. A product cannot be marketed unless sensory analysis results are favorable [29]. When the fish pate made from anchovy and stored in refrigeration conditions at 4°C was evaluated in terms of sensory quality criterias, it exceeded acceptability limit value with 1.50±0.00 at day 8 of storage. In the study that was investigated the microbiological, sensory and color changes in anchovy patties, the total bacteria count increased when sensory values decreased. It is determined that, the anchovy patties were in 'acceptable' quality property until storage of 4 days and exceeded acceptability limit values at day 5 of storage in terms of microbiological and sensory quality criterias [6]. In another study spoilage of sardine patties according to sensory analysis results at day 5 of storage was stated [20]. Several factors such as the used ingredients, hygienic conditions during process and initial case of the fish may be listed for reason of longer shelf life in our study. Boran and Kose [25] reported that plain mince based meat balls have the shortest shelf life with 9 days when precooked mince have the longest shelf life with 11 days in terms of sensory quality values. In another study Bilgin et al. [3], recovered fillet residuals as fish paste after hot smoking of pikeperch and tench fishes. The shelf life of the product at 4±1°C was stated as 9 days. These results are similar to our study.

Table 3. Sensory assessment results of anchovy patties stored at 4°C

Anchovy patties	Storage time (days)							
	1	2	3	4	5	6	7	8
Appearance	5±0.00^{a}	5±0.00^{a}	4.3±0.21^{b}	4±0.00^{b}	3.3±0.21^{c}	3.1±0.16^{c}	2.6±0.21^{d}	1.3±0.21^{e}
Texture	5±0.00^{a}	4.6±0.21^{ab}	4.5±0.22^{bc}	4.1±0.16^{c}	4.1±0.16^{c}	3.5±0.22^{d}	2.6±0.21^{e}	1.1±0.16^{f}
Odor	5±0.00^{a}	5±0.00^{a}	4.3±0.21^{b}	4±0.25^{b}	3.5±0.22^{c}	3.1±0.16^{c}	2.6±0.21^{d}	1.1±0.16^{e}
Flavor	5±0.00^{a}	5±0.00^{a}	4±0.25^{b}	4±0.00^{b}	3.6±0.21^{bc}	3.3±0.21^{c}	2.8±0.16^{d}	1.5±0.22^{e}
Average	5±0.00	4.9±0.05	4.2±0.22	4.0±0.10	3.6±0.20	3.2±0.18	2.6±0.19	1.2±0.18
Quality	Very good	Very good	Very good	Good	Acceptable	Acceptable	Acceptable	Spoiled

Means in rows with different superscripts are significantly different ($p<0.05$). n=3, values are shown as mean ± standard error of triplicates.

CONCLUSION

Consequently, shelf life of anchovy patties in refrigeration conditions was determined as 8 days. In addition, boiling process applied to anchovies while making anchovy patties was seen as appropriate and necessary application. In addition, when compared with another fish meat ball studies, it was seen that initial freshness criterias, microbiological load of the product, hygienic condition of environment and product formulation affected shelf life. The shelf life of anchovy patties may be extended by various antimicrobials, antioxidants ve chemical substances (e.g. potassium sorbate, herbal oil, herbal extract) and using effective packaging methods.

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