

## Shelf-Life of Refrigerated Raw Anchovy (*Engraulis encrasicolus*) Patties

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**Özet:** Soğukta Muhafaza Edilen Hamsi (*Engraulis encrasicolus*) Köftelerinin Raf Ömrü. Bu çalışmada soğukta muhafaza edilen hamsi köftelerinin raf ömrünün belirlenmesi amaçlanmıştır. Hamsi köfteleri 10 günlük muhafaza süresince 2'şer gün arayla duyuşal, kimyasal ve mikrobiyolojik yönden analiz edilmiştir. Total volatile baz azotu (TVB-N) ve trimetil amin (TMA) miktarları muhafaza süresince artmış ( $P<0.05$ ) ve 10. günde sırasıyla, 52.6 mg/100 g ve 10.6 mg/100 g' a ulaşmıştır. Köftelerin pH değerlerindeki deęişme az, fakat önemli bulunmuştur ( $P<0.05$ ). Toplam ve koliform bakteri, proteolitik mikroorganizma ve maya-küf sayıları muhafaza süresince önemli düzeyde artmıştır ( $P<0.05$ ). Ürünlerin kabul edilebilirliği muhafaza süresinin artışıyla azalmıştır ( $P<0.05$ ). Hamsi köftelerinin  $4\pm 2$  °C' de maksimum raf ömrü 6 gün olarak belirlenmiştir.

**Anahtar kelimeler:** hamsi köftesi, raf ömrü, soğutma.

**Abstract:** The objective of this study was to determine the maximum shelf-life of raw anchovy patties preserved under refrigeration conditions. Organoleptic, chemical, and microbiological analysis of anchovy patties were performed every other day throughout 10 days. Total volatile basic nitrogen and trimethylamine values increased throughout storage ( $P<0.05$ ) and reached 52.6 and 10.6 mg/100 g at the 10<sup>th</sup> day, respectively. There was slight but significant change in pH values of the patties ( $P<0.05$ ). All microbial counts, total and coliform bacteria, proteolytic microorganism, and yeast-mold count, significantly increased throughout storage ( $P<0.05$ ). Acceptability of the products significantly decreased as storage time increased ( $P<0.05$ ). Maximum shelf-life of anchovy patties was found 6 days at  $4\pm 2$ °C.

**Key Words:** anchovy patty, shelf-life, refrigeration.

### Introduction

Anchovy (*Engraulis encrasicolus*) is a small salt water fish of approximately 12 cm in length. Anchovy is mostly hunted inshore of the Black Sea, but also in the waters of the Marmara, Mediterranean, and East Atlantic (Ozdamar 1991). Turkey ranks first (241 000 tons) in anchovy production among 25 countries in the world (Anonymous 1997). Anchovy in Turkey is processed mostly into fish flour and fish oil while the rest is consumed as human food such as anchovy patties. Anchovy patties are

produced by washing; removal of head, viscera, and backbone; grinding; mixing with several spices, and shaping the final patty. Anchovy patties are generally produced in small scale as a cottage industry, and therefore there is no established standard production method.

Several developments concerning anchovy processing and preservation have been published. Anil (1985) studied the effect of freezing ( $-18\pm 2$ °C) on chemical and microbiological properties of ground anchovies containing 700 g/kg moisture, 180 g/kg protein, 100 g/kg fat, and 15 g/kg ash. He found that ground

anchovies can be preserved in the freezer ( $-18\pm 2^{\circ}\text{C}$ ) for up to 6 months without any significant chemical and microbiological changes. Varlik (1988) found that anchovy frozen at  $-4^{\circ}\text{C}$  and stored at  $-18^{\circ}\text{C}$  did not show any quality deterioration within 3 months. He concluded that products preserved at  $-18^{\circ}\text{C}$  can be consumed safely up to 9 months. Damarli et al. (1992) also found that fish patties preserved in a freezer ( $-18^{\circ}\text{C}$ ) can maintain the initial quality up to 9 months and fish patties between 9 to 12 months may be consumed without any health concern. Varlik and Heperkan (1990) studied the organoleptic, chemical, and microbiological properties of anchovy preserved with ice cooling (about  $11^{\circ}\text{C}$ ). It was found that anchovy patties can be preserved only 2 days before the onset of quality deterioration.

Perrez-Villarreal and Pozo (1992) studied the sensory, biochemical, and microbiological aspects of the ripening of salted anchovy. They found that water content and pH remained constant during ripening (6-8 months), protein nitrogen decreased and non-protein nitrogen increased during ripening. Hydroperoxide and thiobarbituric (TBA) values varied significantly throughout ripening and total bacteria count reached a maximum value at 4-5 months. Egiazagryan et al. (1983) studied the preservation of Black Sea anchovies in aromatized and Georgian oil. They found that anchovies can be stored for 6 months at  $-5^{\circ}\text{C}$  and the changes in chemical and sensory properties of both preserves were increase in only titratable acidity and volatile basic nitrogen.

The overall goal of this study was to monitor quality of anchovy patties under refrigeration conditions and to determine the maximum shelf-life of the fish patties.

#### **Materials and Methods**

Fish was purchased from sea food shops in Samsun, Turkey. Corn starch, salt, onion, and spices were obtained from local grocery stores.

#### **Sample preparation**

Anchovies were first gutted and the heads and the backbones of fishes were removed. The fishes were then washed and drained free of water. The cleaned fish were ground with a meat grinder. A mixture of 100 g corn starch, 20 g salt, 50 g onion, 5 g black pepper, 3 g cumin, and 2 g allspice was added to 820 g ground fish and the mixture was homogenized in a blender. The anchovy patties (10-12 g) were wrapped with polyethylene film, placed in cardboard boxes and stored in a refrigerator at  $4\pm 2^{\circ}\text{C}$ .

#### **Analytical techniques**

Anchovy patties were analyzed for total solids, protein, fat, ash, and salt (Lees 1975), for starch (Uluoz 1965), for total volatile basic nitrogen, TVB-N, (Lees 1975), and trimethylamine, TMA, (Gokalp et al. 1993). Fish patties were enumerated for counts of total bacteria, coliform, and yeast and mold (Collins and Lyne 1984) and for proteolytic microorganism (Lee and Kraft 1992).

#### **Sensory analysis**

Anchovy patties were evaluated for appearance, texture, odor, and flavor. Samples were prepared for sensory tests as follows: Anchovy patties were removed from the refrigerator and cooked in a conventional oven at  $320^{\circ}\text{C}$  for 12 min, 7 min for one side and 5 min for the other side. The cooked samples were cooled to room temperature and served to panelists. Flavor evaluation was med in only cooked samples while

other tests were done in uncooked samples. Anchovy patties were evaluated based on a 10 point Hedonic scale, where 1 represented dislike extremely and 10 represented like extremely. The panel consisted of 8-12 untrained members from the Department of Food Engineering at Ondokuz Mayıs University in Turkey.

All experiments were done in duplicate and the results were presented as average.

### Statistical analysis

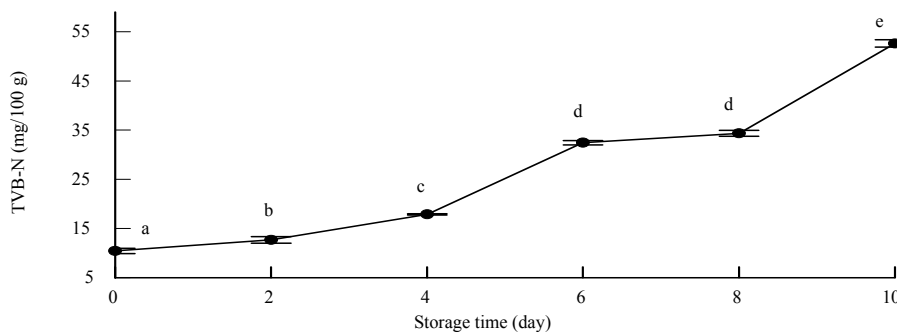
The effect of storage time on biochemical, microbiological, and sensory properties of anchovy patties was analyzed by one way ANOVA using Minitab statistical software (Anonymous 1996). Mean comparisons were performed using Fisher's Least Significant Difference test at  $p < 0.05$ .

## Results and Discussion

### Chemical and biochemical properties

Average composition of anchovy patties contained  $343.3 \pm 2.0$  g/kg total solids,  $137.3 \pm 1.5$  g/kg protein,  $81.6 \pm 1.4$  g/kg fat,  $27.6 \pm 0.7$  g/kg ash,  $17.3 \pm 0.3$  g/kg salt, and  $94.7 \pm 3.5$  g/kg starch. This composition is slightly higher in total solids and ash, and lower in fat and protein compared to composition of fresh anchovy reported by Anil (1985). This difference is due to formulation of anchovy with starch and spicy which increased total solids and decreased fat and protein content.

TVB-N, TMA, and pH of the samples are shown in Figure 1, 2, and 3, respectively.



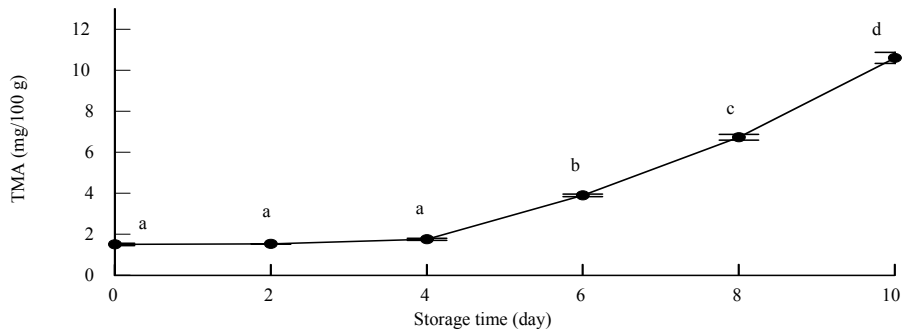
**Figure 1.** Total volatile basic nitrogen content of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P < 0.05$ ).

TVB-N values continuously increased throughout storage ( $P < 0.05$ ). The increase in TMA values of fish patties was not significant up to sixth day of storage ( $P > 0.05$ ). Spoilage of fish generally occurs at 30-35 mg/100 g TVB-N and 6-8 mg/100 g TMA (Ludorf and Meyer 1973, Regenstein and Rege

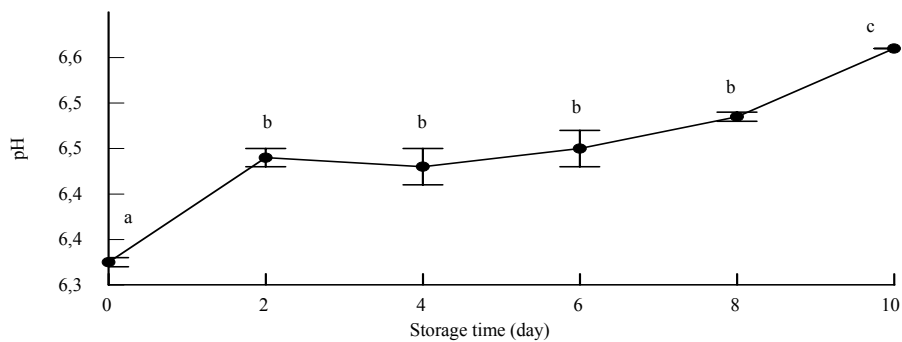
1991). The anchovy patties in this study reached  $32.44 \pm 0.61$  mg/100 g TVB-N value at the sixth day,  $6.73 \pm 0.20$  mg/100 g TMA value at the eighth day and exceeded the maximum limits for both TVB-N and TMA at the tenth day. Trimethylamine-N-oxide (TMAO) is

oxidized into TMA by trimethylamine oxidase enzyme. Fresh fish contains very little or no TMA. It has been established that TMA and basic nitrogen compounds increase as fish is spoiled (Jhaveri et al. 1982, Gokalp et al. 1993, Varlik et al. 1993). Values for pH of fish patties changed significantly over 10 days period

( $P < 0.05$ ), but pH range was relatively small, 6.33 to 6.56 which is lower than the critical limit of 6.8-7.0. The increase in pH was expected, because microorganisms and enzymes release free oxygen and hydrogen, increasing hydroxyl ion concentration and thus causing a rise in pH (Inal 1992).



**Figure 2.** Trimethylamine content of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P < 0.05$ ).

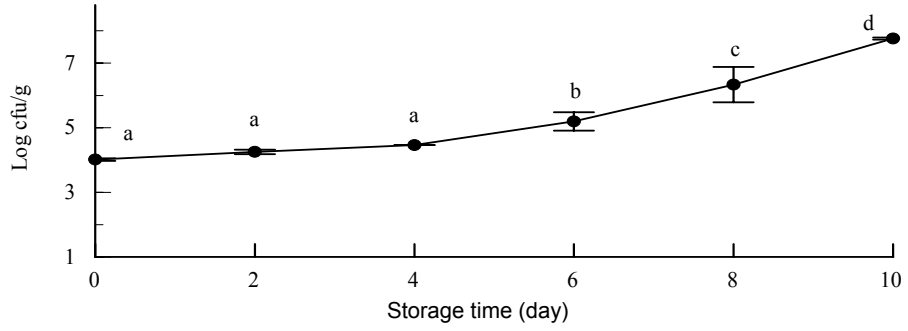


**Figure 3.** pH values of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P < 0.05$ ).

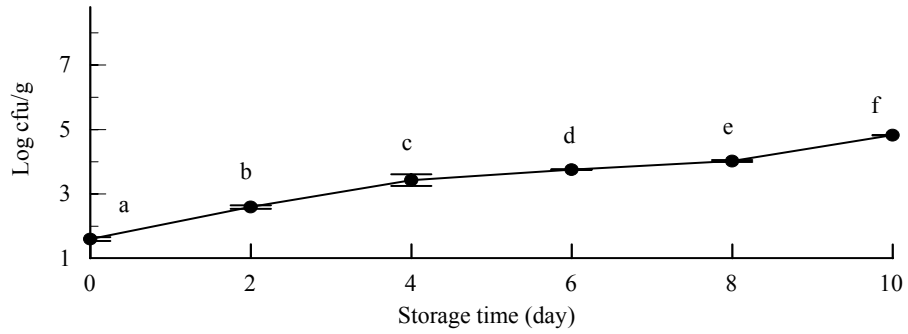
### Microbial properties

All microbiological counts increased over time (Figure 4, 5, 6, and 7). The fastest growth rate was observed in the total

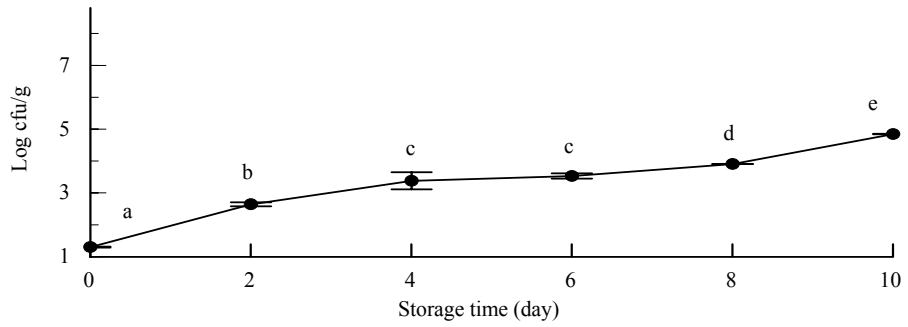
bacteria (Figure 4), coliform (Figure 5) and proteolytic count (Figure 6) in contrast to the slowest growth rate in the yeast and mold count (Figure 7).



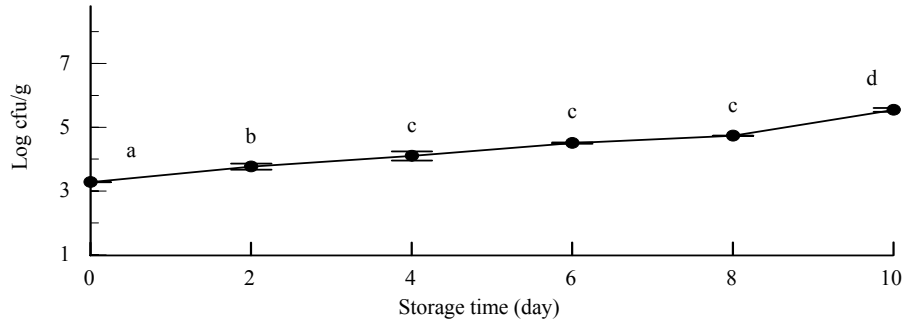
**Figure 4.** Total bacteria counts of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P<0.05$ ).



**Figure 5.** Coliform bacteria counts of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P<0.05$ ).



**Figure 6.** Proteolytic bacteria counts of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P<0.05$ ).



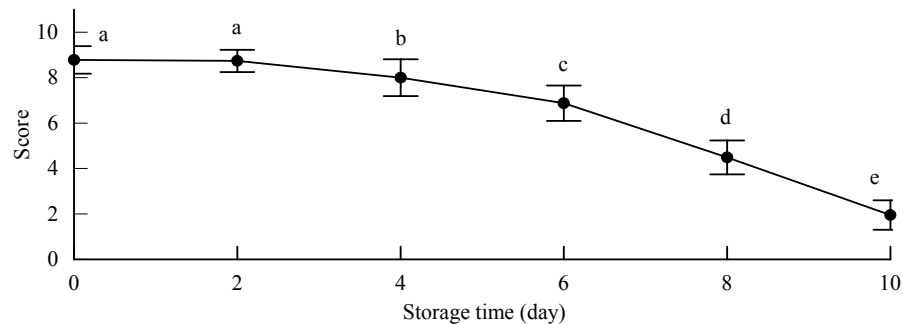
**Figure 7.** Yeast and mold counts of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P < 0.05$ ).

Total bacteria counts of the anchovy patties increased from  $1.0 \times 10^4$  cfu/g (log 4) at the first day to  $5.8 \times 10^7$  cfu/g (log 7.76) at the tenth day ( $P < 0.05$ ). Total bacteria count exceeded the critical limit of  $10^6$  /g on the eighth day (Varlik et al. 1993). Coliform bacteria, an important food safety indicator, increased significantly throughout storage ( $P < 0.05$ ), ranging from  $2.0 \times 10^1$  (log 1.3) to  $6.9 \times 10^4$  cfu/g (log 4.84). Proteolytic bacteria break down nitrogen compounds in fish patties and cause heavy off-flavor in the product. Proteolytic bacteria grew significantly in the anchovy patties throughout storage ( $P < 0.05$ ), ranging

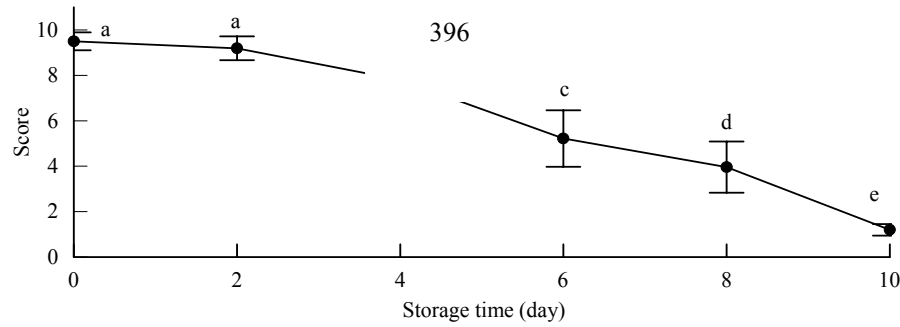
from  $4.0 \times 10^1$  (log 1.6) to  $6.7 \times 10^4$  cfu/g (log 4.82). Since most proteolytic bacteria in fish are psychrotrophs these microorganisms were able to grow under refrigeration conditions and contributed the spoilage of the product (Lee and Kraft 1992).

#### Sensory properties

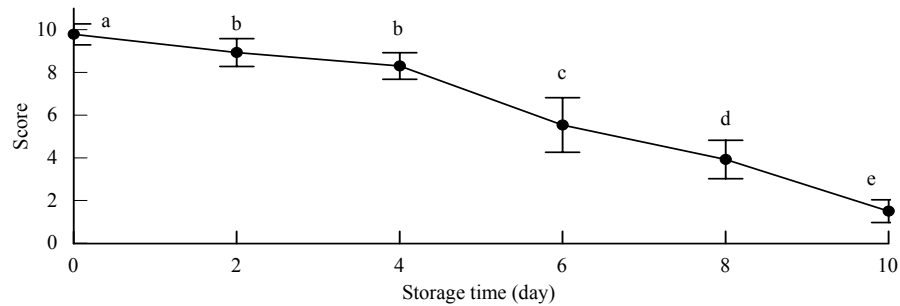
Sensory attributes, appearance, texture, odor, and flavor, significantly decreased ( $P < 0.05$ ) as the storage time increased (Figure 8, 9, 10, and 11).



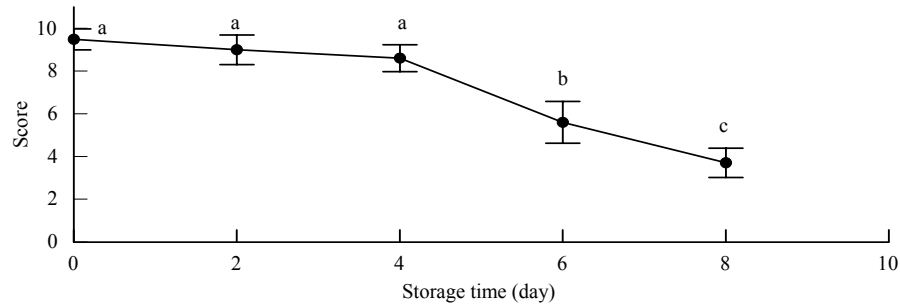
**Figure 8.** Appearance scores of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P<0.05$ ).



**Figure 9.** Texture scores of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P<0.05$ ).



**Figure 10.** Odor scores of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P<0.05$ ).



**Figure 11.** Flavor scores of anchovy patties. Means of days within each experiment having the same letter are not significantly different ( $P<0.05$ ).

Appearance and texture of fresh and two days old fish patties were rated the best choice ( $P>0.05$ ) while these attributes were significantly decreased for the rest of the storage ( $P<0.05$ ). The

samples stored for 10 days were strongly criticized for an off-color and soft texture.

After six days of storage, anchovy patties were criticized for a strong odor ( $P<0.05$ ). Flavor results showed that fish

patties kept initial quality up to sixth day of storage ( $P>0.05$ ), started deterioration after sixth day, and completely spoiled at the tenth day of storage. Samples stored 10 days could not be evaluated for flavor due to an off-color, soft texture, and a strong odor. Thus, sensory characteristics of anchovy patties showed a positive relationship with biochemical and microbiological aspects.

### Conclusions

Fish patties preserved at  $4\pm 2^{\circ}\text{C}$  kept the initial freshness up to sixth day, started spoilage after 8th day and exceeded the consumable limit at the tenth day. This study raises concern for the consumer safety of raw anchovy products preserved in a refrigerator more than 6 days and the need for product development research to extend shelf-life and to improve processing and preservation methods of refrigerated raw anchovy patties.

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