

The Impact of Thyme, Rosemary, and Basil Extracts on the Chemical, Sensory and Microbiological Quality of Mackerel Balls Stored at -18°C

Kekik, Biberiye ve Fesleğen Ekstraktlarının -18°C'de Depolanan Uskumru Köftelerinin Kimyasal, Duyusal ve Mikrobiyolojik Kalitesine Etkisi

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Abstract: The effect of adding 0.05% natural herb extracts obtained from rosemary (*Rosmarinus officinalis*), thyme (*Thymbra spicata*), and basil (*Ocimum basilicum* L.) on mackerel balls during 10 months of frozen storage (-18°C) were investigated concerning sensory (raw and cooked), biochemical (PV- peroxide value, FFA- free fatty acids, TVB-N -total volatile basic nitrogen, TBA- thiobarbituric acid and pH) and microbiological analyses (TVC- total viable count). The results indicated that the total phenolic compounds of plant extracts were determined as 38.13 mg GAE/g, 81.85 mg GAE/g, and 21.08 mg GAE/g for thyme, rosemary, and basil, respectively. The shelf life of raw fish balls was found as eight months for the control and basil groups and ten months for rosemary and thyme groups. 0.05% basil extract gave the fishball an intense taste and odor and a bitter taste. TVB-N, TBA, FFA, PV, and pH values remained lower than the acceptability limits during the storage period in all groups. The plant extracts, especially rosemary and thyme, were effective for inhibiting bacterial growth and the values of biochemical parameters. Therefore, it was concluded that natural extracts could be added to fish products as an antioxidant to prolong the shelf life of fish.

Keywords

- Lipid oxidation
- Thyme
- Rosemary
- Basil
- Fishball

Özet: Uskumru köftelerine %0,05 biberiye (*Rosmarinus officinalis*), kekik (*Thymbra spicata*) ve fesleğen (*Ocimum basilicum* L.)'den elde edilen doğal bitki ekstraktlarının ilave edilmesinin duyusal (çiğ ve pişmiş), biyokimyasal (PV-peroksit değeri, FFA-serbest yağ asitleri, TVB-N-toplam uçucu bazik nitrojen, TB-tiobarbitürik asit ve pH) ve mikrobiyolojik kalitesi (TVC-toplam canlı sayısı) üzerine etkileri köftelerin dondurularak 10 ay depolanması (-18°C) süresince araştırılmıştır. Sonuçlar bitki ekstraktlarının toplam fenolik bileşiklerinin kekik, biberiye ve fesleğen için sırasıyla 38,13 mg GAE/g, 81,85 mg GAE/g ve 21,08 mg GAE/g olduğunu göstermiştir. Çiğ balık köftelerinin raf ömrü kontrol ve fesleğen grupları için 8 ay, biberiye ve kekik grupları için 10 ay olarak bulunmuştur. %0,05 fesleğen ekstraktı, balık köftesine yoğun bir koku ve acı bir tat vermiştir. Depolama süresince tüm gruplarda TVB-N, TBA, FFA, PV ve pH değerleri kabul edilebilirlik limitlerinin altında kalmıştır. Bitki ekstreleri, özellikle biberiye ve kekik, bakteri üremesini ve biyokimyasal parametrelerin değerlerini engellemede etkili olmuştur. Bu nedenle, balıkların raf ömrünü uzatmak için antioksidan olarak balık ürünlerine doğal ekstraktlar eklenebileceği sonucuna varılmıştır.

Anahtar kelimeler

- Lipid oksidasyonu
- Kekik
- Biberiye
- Fesleğen
- Balık köftesi



1. INTRODUCTION

Fish balls are commonly ball-shaped products obtained from minced fish with/without the inclusion of vegetables, starches, and condiments (Tee & Siow, 2014; Yuxiang Zhang et al., 2019). They are steamed, boiled, or deep-fried (Feng et al., 2017; Huda et al., 2010). In several countries such as China, Singapore, Japan, the United States, Germany, Australia, and the countries in Southeast Asia, it is commonly consumed food and available in supermarkets worldwide (Feng et al., 2017; Yuxiang Zhang et al., 2019). Besides its high nutritional value, the consumption of fish balls prevents the problem of drowning due to the fish bones since the bones are completely taken during the procedure (Feng et al., 2017). However, the fishball is a particularly perishable product as it is made of fish meat (Kok & Park, 2007) containing a high level of long-chain PUFAs, especially DHA and EPA (Ali et al., 2019; Petricorena, 2015). In addition, it is more sensitive to oxidative deterioration than other meat products. Lipid oxidation can cause degradation of taste, odor, color, texture, and also the production of toxic composites (Kanner, 1994). During frozen storage, lipid hydrolysis, oxidation, and protein denaturation in seafood have been reported to have a major effect on product acceptability (Tironi et al., 2010). To ensure freshness and longer the shelf life, fish balls are mostly sold as frozen (Hilgarth et al., 2018; Russell, 1990).

Growing demand for ready-to-cook fish products with high quality and prolonged shelf life has led to the improvement of various novel techniques to preserve quality characteristics and obtain innocuous products (Maftoonazad & Badii, 2012). Synthetic preservative agents have been employed to prolong the storage life of foods, but they are tightly controlled due to toxicological and health issues (Wilson & Bahna, 2005). Therefore, food products preserved with natural extracts have become popular due to the perception of consumers against chemical preservatives (Holley & Patel, 2005). It is challenging to find influential and non-toxic procedures to delay spoilage and prolong the shelf life of fish (Gao et al., 2014). The usage of herbal essential oils and their extracts has been used in many food products (Aliakbarlu & Khalili Sadaghiani, 2015; Hashemi Gahruie et al., 2015). Current demand in food manufacturing is the decrease or replacement of chemically synthesized additives. The usage of plant extracts or plant essential oils as natural food preservatives is a method to avoid the growth of microorganisms or to preserve food from oxidation (Bubonja-Sonje et al., 2011).

Rosemary (*Rosmarinus officinalis*) has been employed as an antioxidant or food additive (Seabra et al., 2011). Its antioxidant capacity is mainly due to phenolic diterpenes, rosmarinic acid, carnosol, and carnosic acid (Rocío Teruel et al., 2015). In addition, carvacrol and thymol extracted from thyme improved the permeability of ATP, resulting in fatal damage to bacterial cells (Burt, 2004). Basil has also demonstrated antimicrobial and antioxidant activities. Rosmarinic acid is one of the most significant phenolics in basil (Sharafati-Chaleshtori et al., 2015). It has been reported that rosmarinic acid and its derivatives have antioxidant, antibacterial, and medicinal properties (Petersen & Simmonds, 2003). Thus, the current study aimed to research the antioxidant and antimicrobial effects of three plant extracts (basil, thyme, and rosemary) on the quality of fishball concerning sensory, chemical, and microbiological assessments during frozen storage.

2. MATERIALS and METHODS

2.1. Plant extract

Rosemary (*R. officinalis* L.), thyme (*Thymbra spicata*), and basil (*Ocimum basilicum* L.) were extracted by the method of (Chen et al. (1992).

2.2. Preparation of samples

Atlantic mackerel (*S. scombrus*) were bought from a local fish processing plant (Pakyürek, Adana, Turkey). After frozen fish were thawed in a fridge, they were gutted and washed. After that, the fish was cooked in boiling water for 5 min. The flesh of fish was separated from the bones and skins manually. The minced fish was divided into four groups (the control with no extract, 0.05% rosemary, 0.05% thyme, and 0.05% basil extract). Fish balls were made by the method of López-Caballero et al. (2005) with minor modifications. Ingredients consisted of 0.5% salt, 5% water, 2% egg white (Lick A.Ş., Turkey), and 10% wheat starch (Kent Gıda A.Ş., Turkey) with/without plant extract was added to minced fillets, mixed thoroughly by a mixer (Easy Max Compact, France) and then shaped into a fishball (3cm, 15±1.2 g) by hands. Fish balls were placed into each polyethylene bag (24x38cm, high-density polyethylene (HDPE)), whose density is typically between 0.94-0.97 g/cm³ and higher melting point (135°C, typically stable at 110°C). Every package contained ten fish balls and was kept at -18 °C for ten months. They were analyzed monthly in terms of sensory, chemical, and microbiology analyses. Data were obtained from three packages of fish balls.

2.3. Sensory analysis

Sensory analyses of raw fish balls (appearance, color, odor, and general acceptability) were assessed by the method of de Koning and Mol (1991). Each assessment was carried out on each test day by a minimum of six trained panelists. Fish balls were evaluated at 24 °C under normal daylight circumstances. On each analysis day, three packs of fish balls were randomly selected for each group to assess their sensory attributes, both raw and cooked. Sensory evaluation of cooked mackerel meatballs was assessed by Paulus et al (1979). Fish balls were presented to panelists after they were fried in sunflower oil at 185 °C for 2-3 minutes.

2.4. Chemical analyses

The kjeldahl procedure was used for the determination of protein content (AOAC, 1998) using a Buchi Digestion System (BÜCHI Labortechnik, Flawil, Switzerland) and a Kjeltex Distillation Unit B-324 (BÜCHI Labortechnik). Protein level was determined as a percentage of N×6.25. Lipid was measured by Bligh and Dyer's (1959) method. Ash level was found as described by AOAC Method 920.153 (2002), and moisture content was found by AOAC Official method 950.46 (2002). Peroxide value (PV) was carried out by AOCS Method Ja 8-87 (1994) and expressed as milliequivalents of peroxide oxygen per kilogram of fat. The total phenolic levels of plant extracts were performed by Singleton et al. (1999). The TVB-N content of samples was measured by Antonocopoulos (1973) and expressed as milligrams TVB-N per kilogram of muscle. Free fatty acid analysis (FFA) was found by AOCS Official Method (Ca 5a-40, 1997) and expressed as a percentage of oleic acid. Thiobarbituric acid (TBA) values of fish balls were measured by the method of Tarladgis et al. (1960) and expressed as TBA values in milligrams of malondialdehyde (MA) per kilogram of flesh.

2.5. Microbiological analyses

Plate count agar (standard methods agar) was used enumeration of mesophilic bacteria. Ten grams of fish meat from each fish ball group were weighed, and then these samples were homogenized for 2 minutes in the stomacher device by adding 90 mL of Ringer's solution. After, decimal dilutions were made up to 10⁻⁸, and then 0.1 mL of each dilution was pipetted onto the surface of plate count agar plates in triplicate. Then, all plates were incubated at 30°C for two days. Afterward, all plate count agar plates were incubated at 30 °C for two days for mesophilic bacteria growth.

2.6. Statistical analysis

Statistical analyzes were done using the SPSS version 22 software (SPSS, Chicago, Illinois, USA). The data of control, thyme, rosemary, and basil groups were subjected to analysis of variance and Duncan's multiple range tests.

3. RESULTS and DISCUSSION

3.1. Sensory analyses

The sensory analyses of raw fish balls are given in Table 1. Sensory scores of fish balls in all groups regarding appearance, odor, color, and general acceptability reduced towards the end of the storage period. However, the scores for each sensory trait of samples with rosemary and thyme were significantly higher ($p < 0.05$) than those of the control samples at any time during frozen storage. There were significant differences ($p < 0.05$) in fish ball groups after the 6th month of storage in terms of general acceptance (Table 1). As a result of sensory analyses, the control and basil groups were rejected at nine months by panelists and considered unacceptable, while rosemary and thyme groups were still acceptable at the end of the storage (10 months) period. It was found that thyme and rosemary extract was more effective on sensory evaluation and prolonged the shelf life by two months compared to the control and basil groups.

Table 1. Changes in sensory parameters of the raw fish ball during frozen storage (-18°C)

Sensory parameters	Storage time (month)	Groups			
		Control % 0.05	Thymus % 0.05	Rosemary % 0.05	Basil % 0.05
Appearance	0	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	1	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	2	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	3	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	4	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	5	4.9±0.25 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	4.9±0.25 ^{ax}
	6	4.4±0.48 ^{bxy}	4.9±0.25 ^{ax}	4.9±0.25 ^{ax}	4.3±0.29 ^{by}
	7	4.0±0.00 ^{cx}	4.3±0.29 ^{bx}	4.4±0.25 ^{bx}	4.1±0.25 ^{bx}
	8	3.1±0.25 ^{dy}	4.0±0.00 ^{cx}	4.1±0.25 ^{bx}	3.3±0.29 ^{cy}
	9	2.6±0.25 ^{ey}	3.4±0.25 ^{dx}	3.6±0.48 ^{cx}	2.5±0.00 ^{dy}
	10	2.4±0.25 ^{ey}	3.1±0.25 ^{ex}	3.0±0.00 ^{dx}	2.3±0.29 ^{dy}
Colour	0	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	1	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	2	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	3	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	4	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	5	4.6±0.48 ^{bx}	4.8±0.50 ^{ax}	5.0±0.00 ^{ax}	4.6±0.48 ^{ax}
	6	4.1±0.25 ^{cx}	4.4±0.48 ^{ax}	4.5±0.58 ^{ax}	4.3±0.29 ^{bx}
	7	3.9±0.25 ^{cx}	4.1±0.25 ^{bx}	4.3±0.29 ^{bx}	4.1±0.25 ^{bx}
	8	3.1±0.25 ^{dy}	4.0±0.00 ^{bx}	4.1±0.25 ^{bx}	3.1±0.25 ^{cy}
	9	2.5±0.00 ^{ey}	3.4±0.25 ^{cx}	3.5±0.41 ^{cx}	2.3±0.29 ^{dy}
	10	2.3±0.29 ^{ey}	3.0±0.25 ^{dx}	3.1±0.25 ^{dx}	2.1±0.25 ^{dy}
Odour	0	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	1	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	2	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	3	4.9±0.25 ^{abx}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	4.9±0.25 ^{abx}
	4	4.6±0.48 ^{abx}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	4.8±0.50 ^{abx}
	5	4.5±0.58 ^{bcx}	4.8±0.50 ^{abx}	4.8±0.50 ^{ax}	4.5±0.58 ^{bcx}
	6	4.1±0.25 ^{cdy}	4.5±0.58 ^{bcx}	4.9±0.25 ^{ax}	4.3±0.30 ^{cdy}
	7	4.0±0.00 ^{dy}	4.3±0.25 ^{cy}	4.8±0.29 ^{ax}	4.0±0.00 ^{dy}
	8	3.3±0.29 ^{ey}	4.1±0.25 ^{cx}	4.3±0.29 ^{bx}	3.3±0.29 ^{ey}
	9	2.1±0.25 ^{fy}	3.3±0.29 ^{dx}	3.5±0.58 ^{cx}	2.3±0.29 ^{fy}
	10	2.0±0.41 ^{fy}	3.0±0.00 ^{dx}	3.0±0.00 ^{dx}	2.0±0.00 ^{fy}
General acceptance	0	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	1	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	2	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	3	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}
	4	4.9±0.25 ^{ax}	5.0±0.00 ^{ax}	5.0±0.00 ^{ax}	4.9±0.25 ^{abx}
	5	4.8±0.29 ^{ax}	4.9±0.25 ^{ax}	4.9±0.25 ^{ax}	4.63±0.48 ^{bcx}
	6	4.3±0.29 ^{bxz}	4.8±0.29 ^{axy}	4.9±0.07 ^{ax}	4.4±0.25 ^{cdyz}
	7	4.0±0.00 ^{by}	4.1±0.25 ^{by}	4.5±0.00 ^{bx}	4.1±0.25 ^{dy}
	8	3.3±0.29 ^{cy}	4.0±0.00 ^{bx}	4.3±0.29 ^{bx}	3.3±0.29 ^{ey}
	9	2.6±0.25 ^{dy}	3.4±0.25 ^{cx}	3.5±0.41 ^{cx}	2.3±0.29 ^{fy}
	10	2.3±0.29 ^{ey}	3.0±0.00 ^{dx}	3.0±0.00 ^{dx}	2.0±0.00 ^{fy}

n:3, values represent mean ±SD. Values in the same column followed by different letters (a, b, c, d, e, f) indicate significant differences ($p < 0.05$) during storage periods. Values in the same row followed by different letters (x, y, z) indicate significant differences between groups ($p < 0.05$). A score of 5 represents 'like very much, a score of 4 'like', a score of 3 'neither like or dislike' while a score of 2-1 was regarded as 'dislike'.

Sensory assessments of cooked fish balls (color, odor, taste, general acceptability, and texture) are given in Table 2. Sensory scores in all groups decreased during storage of 10 months. The addition of 0.05% basil extract gave the samples a bitter taste and odor compared to the extracts of thyme and rosemary used at the same concentration. Therefore, the thyme and rosemary groups were the most

preferred by the sensory panel compared to the basil group. Many researchers have reported that the decrease in taste and aroma scores is caused by composites such as ketone and aldehyde, which are released because of lipid oxidation during storage and negatively affect the taste of products (Flores et al., 2004; Montel et al., 1998).

Table 2. Changes in sensory parameters of the cooked fish ball during frozen storage (-18°C).

Storage time (month)	Groups	Color	Odor	Taste	Texture	General acceptance
0	Control	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}
	Tymus	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}
	Rosemary	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}
	Basil	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}
1	Control	9.00±0.00 ^{ax}	8.83±0.41 ^{ax}	8.58±0.49 ^{aby}	9.00±0.00 ^{ax}	8.83±0.41 ^{ax}
	Tymus	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}
	Rosemary	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}
	Basil	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}
2	Control	9.00±0.00 ^{ax}	8.83±0.41 ^{ax}	8.42±0.49 ^{abx}	9.00±0.00 ^{ax}	8.67±0.49 ^{ax}
	Tymus	9.00±0.00 ^{ax}	8.83±0.41 ^{abx}	8.83±0.41 ^{ax}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}
	Rosemary	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}	8.92±0.20 ^{abx}	9.00±0.00 ^{ax}	9.00±0.00 ^{ax}
	Basil	8.92±0.20 ^{ax}	8.58±0.49 ^{abx}	8.75±0.42 ^{ax}	9.00±0.00 ^{ax}	8.83±0.41 ^{abx}
3	Control	8.83±0.41 ^{ax}	8.50±0.55 ^{ax}	8.33±0.52 ^{bx}	8.67±0.52 ^{abx}	8.63±0.52 ^{ax}
	Tymus	8.75±0.27 ^{ax}	8.75±0.42 ^{abx}	8.83±0.26 ^{ax}	8.92±0.20 ^{ax}	8.83±0.41 ^{abx}
	Rosemary	8.92±0.20 ^{ax}	8.75±0.27 ^{abx}	8.83±0.26 ^{abx}	8.83±0.26 ^{ax}	8.92±0.20 ^{abx}
	Basil	8.75±0.42 ^{abx}	8.50±0.55 ^{abx}	8.50±0.45 ^{ax}	8.67±0.52 ^{ax}	8.67±0.52 ^{abx}
4	Control	8.75±0.42 ^{ax}	8.42±0.49 ^{ax}	8.17±0.26 ^{bx}	8.58±0.49 ^{abx}	8.08±0.20 ^{bx}
	Tymus	8.58±0.38 ^{ax}	8.50±0.55 ^{abx}	8.33±0.26 ^{bx}	8.67±0.52 ^{ax}	8.50±0.55 ^{bxc}
	Rosemary	8.67±0.52 ^{ax}	8.50±0.55 ^{bxc}	8.50±0.55 ^{bxc}	8.67±0.52 ^{ax}	8.58±0.49 ^{bxc}
	Basil	8.50±0.55 ^{bxc}	8.33±0.26 ^{bxc}	8.00±0.55 ^{bx}	8.17±0.61 ^{bx}	8.42±0.49 ^{bx}
5	Control	8.25±0.27 ^{bx}	7.75±0.61 ^{bx}	7.50±0.55 ^{cy}	8.17±0.26 ^{by}	7.92±0.20 ^{bz}
	Tymus	8.50±0.45 ^{ax}	8.33±0.26 ^{cx}	8.25±0.27 ^{bx}	8.58±0.49 ^{ax}	8.42±0.20 ^{cx}
	Rosemary	8.58±0.38 ^{ax}	8.25±0.27 ^{cdx}	8.33±0.26 ^{cx}	8.58±0.20 ^{ax}	8.33±0.26 ^{cdx}
	Basil	8.33±0.42 ^{cx}	8.00±0.55 ^{cdx}	7.92±0.49 ^{bxy}	7.83±0.26 ^{by}	7.92±0.20 ^{cy}
6	Control	7.67±0.52 ^{bxc}	7.50±0.55 ^{bx}	7.17±0.41 ^{cdy}	7.50±0.55 ^{cy}	7.33±0.52 ^{cy}
	Tymus	7.75±0.61 ^{bx}	7.58±0.45 ^{cx}	7.67±0.52 ^{cxy}	7.75±0.42 ^{bx}	7.83±0.41 ^{dx}
	Rosemary	7.75±0.42 ^{bx}	8.00±0.00 ^{dex}	7.83±0.26 ^{dx}	8.08±0.20 ^{bx}	8.00±0.00 ^{dex}
	Basil	7.50±0.55 ^{dx}	7.50±0.55 ^{dx}	7.33±0.52 ^{cxy}	7.00±0.00 ^{cz}	7.17±0.26 ^{dy}
7	Control	7.58±0.58 ^{bxc}	7.33±0.52 ^{bxy}	6.83±0.41 ^{dey}	7.33±0.52 ^{cxy}	7.33±0.52 ^{cxy}
	Tymus	7.50±0.55 ^{bx}	7.50±0.49 ^{cx}	7.50±0.55 ^{cx}	7.58±0.66 ^{bx}	7.50±0.55 ^{dx}
	Rosemary	7.67±0.52 ^{bx}	7.83±0.41 ^{ex}	7.67±0.52 ^{dx}	7.83±0.41 ^{bx}	7.67±0.52 ^{efx}
	Basil	7.42±0.49 ^{dex}	6.83±0.52 ^{ey}	7.33±0.52 ^{cxy}	6.75±0.42 ^{cy}	6.83±0.41 ^{dey}
8	Control	7.33±0.52 ^{cx}	6.50±0.55 ^{cy}	6.33±0.52 ^{ey}	6.67±0.52 ^{dy}	6.50±0.55 ^{cy}
	Tymus	7.33±0.52 ^{bx}	7.00±0.00 ^{dy}	6.50±0.55 ^{dy}	7.08±0.20 ^{cxy}	7.00±0.00 ^{exy}
	Rosemary	7.50±0.55 ^{bx}	7.67±0.52 ^{ex}	7.50±0.55 ^{dx}	7.33±0.52 ^{cx}	7.50±0.55 ^{fx}
	Basil	7.33±0.41 ^{dex}	6.67±0.52 ^{ey}	6.17±0.26 ^{dy}	6.67±0.52 ^{cy}	6.67±0.52 ^{ey}
9	Control	6.75±0.42 ^{dx}	5.92±0.49 ^{dxy}	5.67±0.52 ^{fy}	6.50±0.55 ^{dx}	6.00±0.00 ^{cy}
	Tymus	6.83±0.41 ^{ax}	6.25±0.42 ^{ey}	6.33±0.52 ^{dex}	6.50±0.55 ^{dx}	6.17±0.41 ^{fy}
	Rosemary	7.00±0.00 ^{cx}	7.00±0.00 ^{fx}	6.50±0.55 ^{ex}	6.67±0.52 ^{dx}	7.00±0.00 ^{gx}
	Basil	7.00±0.00 ^{ex}	5.67±0.52 ^{fz}	5.50±0.45 ^{ey}	6.50±0.55 ^{cx}	6.00±0.00 ^{fy}
10	Control	6.33±0.52 ^{dx}	4.92±0.20 ^{ey}	5.00±0.32 ^{gy}	6.25±0.42 ^{dx}	5.42±0.49 ^{dy}
	Tymus	6.50±0.55 ^{ax}	5.83±0.41 ^{fx}	6.00±0.00 ^{ex}	6.33±0.52 ^{dx}	6.00±0.00 ^{fx}
	Rosemary	6.50±0.55 ^{dx}	6.00±0.00 ^{gx}	6.25±0.27 ^{ex}	6.50±0.55 ^{dx}	6.33±0.41 ^{hx}
	Basil	6.33±0.52 ^{fx}	4.50±0.55 ^{gy}	4.33±0.41 ^{fz}	6.33±0.52 ^{cx}	5.00±0.55 ^{gy}

n:3, values represent mean ±SD. Different letters (a,b,c,d,e,f) in the same column show significant differences (P<0.05). Different letters (x,y,z) in the same row show significant differences (P<0.05). In the sensory evaluation based on a scale of 1 to 9, a score of 9 indicates "very good quality", a score of 7-8 indicates "good quality", a score of 5-6 indicates the "acceptable limit", while a score of 1-4 indicates "bad or unacceptable".

The general acceptability value of cooked samples decreased during storage, in this study. The decline in general acceptability was attributed to the loss of taste and texture caused by microbial infestation, degradation of protein, and lipid oxidation (Lorenzo et al., 2013). For the raw samples, the shelf life of fishball was 8 months for the basil and the control groups whereas the rosemary and thyme groups had shelf lives of 10 months. However, for the cooked samples, all groups can be consumed until the end of 10-month storage period. This result proposed that cooking might eliminate some unwanted volatile compounds in fish meat. Chytiri et al. (2004) also reported that raw trout meat was rejected on the 14th day of storage, while cooked trout were rejected on the 19th day of storage. Thyme and rosemary groups in the cooked fish balls were the most effective compared to the control and basil groups as found in raw fish balls. Thyme and rosemary extracts enhanced the flavor of fish balls by giving a pleasant taste and aroma. Similar results were found in the other studies (Schilling et al., 2018; Zhao et al., 2019).

Uçak et al., (2011) tested two different concentrations of rosemary extracts (0.4% and 0.8%) on fish burgers made from Atlantic mackerel. The addition of 0.4% rosemary extracts was the most effective on the organoleptic quality of the fish burgers. Pezeshk et al. (2011) researched the effects of turmeric and shallot extracts on rainbow trout (*Oncorhynchus mykiss*) stored at 4°C for 20 days. They reported that the use of natural extracts maintained the good quality of the products. They also noticed an improvement in the sensory characteristics especially when turmeric and shallot extracts were combined. These findings suggest that adding natural plant extracts could help to enhance the sensory characteristics of fish and fish products.

3.2. Changes in chemical quality

3.2.1. Proximate composition

Lipid, protein, ash, and moisture contents of raw fish balls were determined as 12.42%, 17.02%, 1.53%, and 59.51% respectively. Similar values were observed for chub mackerel burgers with natural (green tea, sage, oregano, and laurel) extracts by Ozogul and Uçar, (2013) and also for Atlantic mackerel fish burgers with rosemary extract by Uçak et al. (2011).

3.2.2. The total phenolic compounds (TPC)

Herbs as preservative agents have been used to prolong the shelf-life of food, especially lipid-rich products (Kivilompolo et al., 2007) due to their antioxidant properties related to their phenolic compounds (Vallverdú-Queralt et al., 2015). In the current study, the highest value of phenolic level was reported for rosemary (81.85 mg GAE/g) with the most effective on fish balls, followed by thyme (38.13 mg GAE/g) whereas basil (21.08 mg GAE/g) extract had the lowest content of phenolic compounds. Similarly, Stanciu et al. (2017) observed that rosemary had the highest TPC while basil had the lowest TPC among the examined seven plant spices (basil, thyme, sage, coriander, mint, tarragon, rosemary). Melissa and rosemary, oregano, and thyme have been reported to have high contents of phenolic compounds (Ulewicz-Magulska & Wesolowski, 2019).

Skendi et al. (2017) reported a similar total phenolic compound (38.2 mg GAE/g) for thyme with the methanolic extract. However, Gedikoğlu et al. (2019) showed that the TPC in the range of 13.13 - 15.13 mg GAE/g for the dry weight (DW) for *Thymbra spicata* and *Thymus vulgaris* ethanol and methanol extracts. Roby et al. (2013) also observed much lower (from 4.75 to 8.10 mg GAE/g DW) total phenolic compounds for thyme for four solvents (hexane, ethanol, methanol, and diethyl ether) than that found in this study. The mean total phenolic levels for 15 basil cultivars were in the range of 3.47 mg - 17.58 mg GAE/g DW (Kwee & Niemeyer, 2011). Złotek et al. (2017) indicated that basil leaves contained 16.21 ± 0.51 mg GAE/g DW of total phenolic compounds. The earlier studies revealed that the TFC of rosemary

was 40.2 mg GAE/g (Skendi et al., (2017) and 31.73 mg GAE/g (Yeşilsu & Özyurt, 2019) which were lower than that of rosemary in this study. The differences in total phenols content of herbs can be explained by the geographical properties, growing conditions, chemical composition of the species, harvesting time, plant's part used in analyses (Rahimmalek et al., 2009; Stanciu et al., 2017), and also storage conditions and the method of analysis (Skendi et al., 2017).

3.2.3. Peroxide value (PV)

PV was measured to evaluate the creation of prime lipid oxidation products that define the degree of lipid oxidation. During frozen storage, the changes in PV of all samples are given in Table 3. PV values were measured as 0.28 meq O₂/kg in all groups at the beginning of the storage period. A rise in the PV values of all groups was noticed during the storage period (Table 3). There were significant differences (P<0.05) in PV between the control and the other groups except for the basil group. At the end of frozen storage, the highest values were obtained for the control and the basil groups (12.98 and 12.77 meq O₂/kg respectively) while the lowest PV was obtained from rosemary (8.18 meq O₂/kg) and thyme (11.22 meq O₂/kg) groups. The acceptability limit is 20 meq O₂/kg oil for PV of fish oil according to Connell (1995). In this study, PV values of all fish ball samples did not reach this limit during frozen storage.

Table 3. Changes in TBA (mg MA/kg), FFA (% oleic acid) and PV (meq O₂/kg) content of fishball during frozen storage (-18°C).

Storage months	Control	Thymus	Rosemary	Basil
TBA (mg MA/kg)				
0	0.49±0.00 ^{fx}	0.49±0.00 ^{ex}	0.49±0.00 ^{fx}	0.49±0.00 ^{fx}
1	0.59±0.03 ^{fx}	0.54±0.01 ^{ey}	0.53±0.02 ^{fy}	0.58±0.01 ^{fx}
2	1.77±0.08 ^{ex}	1.40±0.04 ^{fy}	1.33±0.06 ^{ey}	1.83±0.07 ^{ex}
3	1.64±0.07 ^{ex}	1.50±0.24 ^{fx}	1.36±0.09 ^{ex}	1.60±0.35 ^{ex}
4	1.83±0.32 ^{ex}	1.65±0.17 ^{fx}	1.49±0.24 ^{ex}	1.75±0.42 ^{ex}
5	2.82±0.55 ^{dx}	2.37±0.44 ^{ey}	2.05±0.18 ^{dy}	2.87±0.05 ^{dx}
6	2.69±0.81 ^{dx}	2.54±0.39 ^{ex}	2.45±0.23 ^{cdx}	2.67±0.61 ^{dx}
7	3.02±0.18 ^{dx}	2.89±0.32 ^{dx}	2.67±0.37 ^{cx}	2.92±0.22 ^{dx}
8	3.80±0.17 ^{cx}	3.55±0.33 ^{cx}	3.10±0.21 ^{by}	3.75±0.44 ^{cx}
9	5.81±0.64 ^{bx}	4.03±0.41 ^{by}	3.34±0.90 ^{by}	5.43±0.45 ^{bx}
10	6.87±0.57 ^{ax}	5.96±0.19 ^{ay}	4.39±0.39 ^{az}	6.43±0.58 ^{axy}
FFA (% Oleic acid)				
0	2.02±0.12 ^{dx}	2.02±0.12 ^{ex}	2.02±0.12 ^{cx}	2.02±0.12 ^{hx}
1	2.34±0.07 ^{cdx}	2.16±0.09 ^{dex}	2.12±0.26 ^{cx}	2.13±0.11 ^{ghx}
2	2.42±0.08 ^{cx}	2.20±0.09 ^{dey}	2.20±0.10 ^{cy}	2.23±0.08 ^{fghy}
3	2.41±0.08 ^{cx}	2.33±0.28 ^{cdx}	2.31±0.13 ^{bcx}	2.34±0.20 ^{efgx}
4	2.46±0.14 ^{cx}	2.39±0.09 ^{bcdx}	2.25±0.05 ^{cx}	2.45±0.23 ^{defx}
5	2.88±0.21 ^{bx}	2.53±0.21 ^{bcy}	2.30±0.02 ^{bcy}	2.57±0.17 ^{dexy}
6	2.83±0.20 ^{bx}	2.57±0.12 ^{bcy}	2.34±0.09 ^{bcy}	2.65±0.17 ^{cdx}
7	2.88±0.51 ^{bx}	2.67±0.12 ^{abx}	2.62±0.43 ^{abx}	2.70±0.14 ^{bcdx}
8	3.18±0.08 ^{abx}	2.88±0.15 ^{ay}	2.69±0.08 ^{ay}	2.86±0.06 ^{abcy}
9	3.30±0.20 ^{ax}	2.92±0.14 ^{ay}	2.75±0.18 ^{ay}	2.97±0.23 ^{abxy}
10	3.35±0.15 ^{ax}	2.89±0.18 ^{ay}	2.88±0.07 ^{ay}	2.99±0.05 ^{ay}
PV (meq O₂/kg)				
0	0.28±0.09 ^{gx}	0.28±0.09 ^{gx}	0.28±0.09 ^{hx}	0.28±0.09 ^{ix}
1	0.29±0.17 ^{gx}	0.25±0.04 ^{gx}	0.24±0.05 ^{hx}	0.25±0.02 ^{ix}
2	1.85±0.44 ^{fx}	1.54±0.14 ^{fy}	0.21±0.02 ^{hz}	1.23±0.21 ^{hy}
3	4.12±0.68 ^{dx}	2.29±0.40 ^{ey}	1.29±0.25 ^{gy}	1.85±0.75 ^{ghy}
4	3.10±0.54 ^{ex}	2.53±0.04 ^{exy}	2.01±0.10 ^{fy}	2.29±0.35 ^{ey}
5	4.23±0.86 ^{dx}	3.28±0.81 ^{dx}	3.00±0.08 ^{ex}	4.21±0.33 ^{fx}
6	7.62±0.84 ^{cx}	6.35±0.06 ^{cy}	5.86±0.37 ^{dy}	5.46±0.62 ^{ey}
7	8.10±0.33 ^{cx}	7.44±0.38 ^{bxy}	6.67±0.65 ^{bcy}	7.79±0.53 ^{dx}
8	9.87±0.13 ^{bx}	7.98±0.11 ^{bz}	6.26±0.45 ^{cdt}	8.63±0.36 ^{cy}
9	10.51±0.19 ^{by}	8.07±0.38 ^{bz}	6.90±0.33 ^{bt}	11.45±0.64 ^{bx}
10	12.98±0.46 ^{ax}	11.22±0.82 ^{ay}	8.18±0.31 ^{az}	12.77±0.39 ^{ax}

n:3, values represent mean ±SD. Different letters (a,b,c,d,e,f) in the same column show significant differences (P<0.05). Different letters (x,y,z) in the same row show significant differences (P<0.05).

It was found that the application of plant extract groups especially rosemary and thyme had significant effects on postponing lipid oxidation in frozen fish balls. This could be explained by that rosemary and thyme groups showed a more pro-oxidant effect than the basil group on fish balls. The antioxidant efficiency of rosemary, thyme, and basil extracts is because of a high content of phenolic compounds (Ying Zhang et al. 2010; Rizea et al. 2012; Rocío Teruel et al. 2015; Skendi et al. 2017). Shi et al. (2019) and Özyurt et al. (2011) also observed the anti-oxidative effect of rosemary extract on mud shrimp (*Solenocera melanthero*) and sea bream (*Sparus aurata*) during frozen storage respectively. Similarly,

Ozogul and Uçar, (2013) indicated that additions of laurel, green tea, and oregano extract reduced lipid oxidation in fish burgers during frozen (-18 °C) storage.

3.2.4. Free Fatty Acid (FFA)

FFA is found to determine the degree of lipid hydrolysis (De Abreu et al., 2011; Shi et al., 2019). Variations in FFA of frozen fish balls during the storage period are presented in Table 3. The initial FFA level was measured as 2.02%. This value rose in all groups during the storage and the FFA value was found at 3.35% in the control group, while the FFA value of thyme, rosemary, and basil was found at 2.89%, 2.88%, and 2.99% respectively at the end of the storage. During the storage, the FFA values of the control were higher than those of the plant extract groups ($p < 0.05$). No significant differences were found among the FFA values of the groups with plant extract (except the 6th month) ($p > 0.05$). The data exhibited that the addition of plant extract was significant ($p < 0.05$) effect on the prevention of FFA development during the frozen storage of fish balls. Serdaroğlu and Felekoğlu (2005) also indicated that the FFA levels of sardines treated with rosemary extract were lower than the control group during storage at -20°C. Moreover, Ozogul and Uçar, (2013) reported that thyme extract added to the fish burger significantly reduced FFA compared to the control group.

3.2.5. Thiobarbituric Acid Reactive Substances (TBARs)

The TBARs value was one of the significant indices for measuring lipid oxidation (Kuley et al., 2012). TBA value is widely used to evaluate rancidity levels and is mostly correlated to the improvement of secondary products for oxidation (Schormüller 1968). At the beginning of the storage, the TBA value was 0.49 mg MA/kg and increased significantly during the storage period (Table 3). This increase was higher in the control group than in the plant extract groups ($p < 0.05$). It was determined that the TBA values of the control and plant extract groups did not reach the acceptability limit value of 7-8 mg MA/kg specified by Schormüller (1969) at the end of storage. The lowest TBA values were observed in rosemary-treated groups during storage. The decrease in TBARs could be associated with the phenolic compounds. As a result of their potential antioxidant capability, phenol-enriched plant extracts were commonly used in food processing and storage (Sun et al., 2017). The use of various plant extracts has been found to affect a decrease TBARS levels in fish and fish products (Alghazeer et al., 2008; Gahruie et al., 2017; Li et al., 2013; Martínez et al., 2019; Shi et al., 2019; Yi et al., 2011).

Similar results were found for the sage, oregano and grape seed (SOG) extracts which were applied to delay lipid oxidation in hairtail fish balls (Guan et al., 2019). They reported that TBARs values were in the range of 1.17 - 2.07 mg MDA/kg for the control, whereas TBARs values were reduced to 0.27–0.56 mg MDA/kg in the SOG-treated sample Cadun et al. (2008) also reported that TBA value in marinated shrimp applied with rosemary extract was 2.7 times lower than that of the control group at the end of storage. The impacts of adding rosemary extract on the oxidation of cooked sea bream during frozen storage were evaluated by Özyurt et al. (2011). They observed that samples treated with rosemary extract mostly gave lower TBA values than those of the untreated samples. Based on the data found in the present study, treatment with rosemary and thyme extract was more effective on the TBA value.

3.2.6. Total Volatile Basic Nitrogen (TVB-N)

TVB-N is one of the extensively used fish quality indexes to assess the degree of spoilage by endogenous enzymes and bacteria. TVB-N in fish consists mainly of ammonia, dimethylamine, and trimethylamine. (Pezeshk et al., 2011; Sallam et al., 2007). The rise in TVB-N value is due to the action of spoilage bacteria and endogenous enzymes (Ruiz-Capillas & Moral, 2005). However, during the storage period, the TVB-N level of some species showed fluctuations (Özyurt et al. 2007; Papadopoulos et al.

2003). TVB-N values of fishball samples during frozen storage are presented in Table 4. In this study, during the storage period, fluctuations, and significant differences ($p < 0.05$) for TVB-N values were observed between the control and treated with the plant extracts groups. The initial TVB-N value of all the groups was determined as 11.15 mg /100g and increased at the end of storage. The TVB-N values for the control group were the highest (19.43 mg /100g) ($p < 0.05$), followed by basil (18.62 mg/100g), thyme (18.22 mg/100g), and rosemary (18.13mg/100g) at the end of storage period. TVB-N values in all groups didn't reach the maximum values, which is the refusal limits of TVB-N 35-40 mg /100g of fish and fishery products (Huss, 1995).

Table 4. Changes in TBV-N, pH and TCV contents of fish ball during frozen storage (-18°C)

Storage months	Control	Thymus	Rosemary	Basil
TVB-N (mg /100g)				
0	11.15±0.04 ^{dx}	11.15±0.04 ^{dx}	11.15±0.04 ^{dx}	11.15±0.04 ^{hx}
1	15.29±0.03 ^{cx}	13.59±0.35 ^{cy}	12.92±0.35 ^{cz}	12.57±0.02 ^{gz}
2	18.59±0.81 ^{ax}	17.89±0.37 ^{ax}	15.78±0.01 ^{by}	17.67±0.79 ^{bex}
3	17.30±0.35 ^{bx}	15.94±0.36 ^{cy}	16.97±0.02 ^{cdy}	16.98±0.01 ^{cdx}
4	15.34±0.04 ^{cx}	15.35±0.03 ^{bx}	15.31±0.10 ^{bx}	15.16±0.44 ^{fx}
5	16.71±0.64 ^{bx}	15.56±0.42 ^{by}	15.35±0.02 ^{by}	15.79±0.80 ^{efxy}
6	17.19±0.83 ^{bx}	15.34±0.01 ^{byz}	14.87±0.82 ^{bz}	16.25±0.78 ^{dexy}
7	18.70±0.03 ^{ax}	18.17±0.68 ^{ax}	14.89±0.44 ^{by}	18.52±0.34 ^{abx}
8	18.88±0.40 ^{ax}	18.11±0.08 ^{axy}	17.69±0.83 ^{ay}	18.83±0.72 ^{axy}
9	19.27±0.83 ^{ax}	16.04±0.74 ^{by}	15.33±0.01 ^{by}	18.81±0.67 ^{ax}
10	19.43±0.03 ^{ax}	18.22±0.42 ^{ay}	18.13±0.04 ^{ay}	18.62±0.82 ^{abxy}
pH				
0	6.14±0.07 ^{gx}	6.14±0.07 ^{fx}	6.14±0.07 ^{fx}	6.14±0.07 ^{gx}
1	6.39±0.01 ^{cdx}	6.24±0.02 ^{ez}	6.19±0.04 ^{et}	6.29±0.01 ^{efy}
2	6.45±0.04 ^{abx}	6.26±0.03 ^{ez}	6.25±0.02 ^{dz}	6.32±0.02 ^{dexy}
3	6.37±0.04 ^{dex}	6.26±0.04 ^{dexy}	6.20±0.04 ^{ey}	6.34±0.05 ^{cdx}
4	6.43±0.04 ^{abcx}	6.35±0.03 ^{abyz}	6.34±0.01 ^{cz}	6.41±0.09 ^{abxy}
5	6.48±0.02 ^{ax}	6.39±0.04 ^{ay}	6.38±0.02 ^{by}	6.44±0.01 ^{ax}
6	6.45±0.20 ^{abx}	6.32±0.04 ^{bcy}	6.34±0.03 ^{cy}	6.42±0.02 ^{ax}
7	6.23±0.05 ^{fx}	6.30±0.00 ^{cdy}	6.36±0.01 ^{bex}	6.29±0.01 ^{efy}
8	6.41±0.05 ^{bcdy}	6.37±0.01 ^{ay}	6.48±0.01 ^{ax}	6.38±0.00 ^{bcy}
9	6.34±0.01 ^{ey}	6.28±0.01 ^{dez}	6.37±0.03 ^{bx}	6.26±0.02 ^{fx}
10	6.18±0.04 ^{gx}	6.09±0.04 ^{gy}	5.88±0.03 ^{gz}	6.17±0.02 ^{gx}
TVC (log cfu/g)				
0	3.85±0.12 ^{ax}	3.85±0.12 ^{ax}	3.85±0.12 ^{ax}	3.85±0.12 ^{ax}
1	4.01±0.11 ^{ax}	3.58±0.12 ^{ay}	3.54±0.22 ^{ay}	3.56±0.07 ^{by}
2	4.00±0.05 ^{ax}	3.56±0.02 ^{ay}	3.46±0.26 ^{aby}	3.53±0.02 ^{by}
3	4.00±0.11 ^{ax}	3.54±0.09 ^{ay}	3.39±0.17 ^{aby}	3.50±0.17 ^{by}
4	3.69±0.09 ^{abx}	3.49±0.43 ^{abxy}	3.30±0.00 ^{aby}	3.50±0.17 ^{bxy}
5	3.60±0.00 ^{abx}	3.24±0.34 ^{aby}	3.23±0.30 ^{by}	3.39±0.00 ^{by}
6	3.47±0.00 ^{abx}	3.00±0.00 ^{bz}	3.00±0.00 ^{bz}	3.30±0.00 ^{bcy}
7	3.30±0.30 ^{bx}	3.00±0.00 ^{bz}	3.00±0.00 ^{bz}	3.21±0.17 ^{bcy}
8	3.30±0.00 ^{bx}	3.00±0.00 ^{bz}	3.00±0.00 ^{bz}	3.20±0.00 ^{bcy}
9	3.15±0.21 ^{bx}	3.00±0.00 ^{bx}	2.97±0.35 ^{bx}	3.15±0.39 ^{bex}
10	3.12±0.00 ^{bx}	3.00±0.00 ^{by}	3.00±0.00 ^{by}	3.06±0.01 ^{cy}

n:3, values represent mean ±SD. Different letters (a,b,c,d,e,f) in the same column show significant differences ($P < 0.05$). Different letters (x,y,z,t) in the same row show significant differences ($P < 0.05$).

As reported in previous studies, lower TVB-N values were found compared to the groups treated with plant extracts during storage (Kenar et al. 2010; Özyurt et al. 2012; Ozogul and Uçar 2013; Martínez et al. 2019; Shi et al. 2019). The rise in TVB-N values was significantly ($P < 0.05$) reduced in plant extract groups. This is due to the role of extracts used as an antimicrobial agent for bacterial growth (Sacchetti et al., 2005). Especially rosemary and thyme groups were observed to maintain low TVB-N values during the storage period. The results suggested that the reduction of TVB-N in the samples with plant extracts can be associated with an inhibition impact of herb extracts on bacteria.

3.2.7. pH

The pH value was determined as 6.14 in all groups at the beginning of storage (Table 4). During the storage period, pH values fluctuated. There were significant differences ($p < 0.05$) between the control and plant extract groups. The highest pH values were observed at the 5th month of storage for the control (6.48), thyme (6.39), and basil (6.44) groups. At the end of the storage period, the pH values were found 6.18 for control, 6.09 for thyme, 5.88 for rosemary, and 6.17 for basil group. The results indicated that the pH value of the rosemary group was significantly lower than those of the other groups ($p < 0.05$). Similarly, Ibrahim and El-Sherif (2008) studied the effects of three extracts (black cumin, thyme, and rosemary) and their combination on frozen tilapia fillets at -18°C . They found that plant extracts retarded pH values compared to the control, although pH values increased in all groups during the storage period. It was reported that the rise in pH value might result from a raise in volatile bases including trimethylamine and ammonia formed by microbial enzymes or either endogenous (Chaijan et al., 2005) while the reduction in pH value could be caused by the acidic compounds formed during bacterial metabolism (Yi et al., 2011).

3.3. Microbiological Analyses

The initial TVC value was determined as 3.85 log cfu/g for all fish ball groups (Table 4). Plant extracts groups showed a significant difference ($p < 0.05$) in TVC among groups. The microbiological limit suggested by ICMSF, (1986) as 7 log cfu/g was not exceeded in any group during storage. With an increase in storage time, TVC showed a decrease (Table 4). The same decrease was found by Ozogul and Uçar (2013) for mackerel hamburgers with laurel, oregano, sage, and green tea extracts during frozen (-18°C) storage. Lakshmisha et al. (2008) also found that the initial TVC value in the mackerel fillet was 4.46 log cfu / g and the TVC value decreased due to the inhibition of bacterial activity during frozen storage at -18°C .

Our findings were also in accordance with the research on Atlantic mackerel fish burgers conducted by Uçak et al. (2011). In this research, it was found that especially rosemary and thyme extracts were more effective in controlling the growth of microorganisms. It could be due to the total phenolic content of plants. Bubonja-Sonje et al. (2011) measured the antioxidant and antibacterial efficacy of polyphenols derived from rosemary, cocoa, and olive oil extract. They found that the most effective antimicrobial and the antioxidant agent was rosemary extract used as a food additive. The antioxidant and antimicrobial activity of the extract of rosemary is primarily due to carnosic acid and carnosol (Moreno et al. 2006). In addition, Burt (2004) found more than 30 antimicrobial composites in oregano extracts, especially significant quantities of carvacrol.

4. CONCLUSIONS

The extracts of rosemary and thyme proved their efficacy by improving the shelf life of the frozen fish ball samples; 10 months for the rosemary and thyme and 8 months for basil and the control groups. The

plant extracts with antioxidant properties maintained the sensory scores (appearance, color, and odor) of the fish ball, and the TBV-N, PV, FFA, and TBA values were lower than those of the control. In addition, the natural extracts reduced the microbial propagation since TVC was lower than that of the untreated fish ball. Therefore, plant extracts especially thyme and rosemary could be applied in the seafood industry to extend the storage period, improve the sensory attributes, control the biochemical changes and delay the deterioration of seafood since plant extracts are an easily accessible source of natural antioxidants and antimicrobials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Editing: EB, YÖ; Methodology: EB, YÖ; Performing the experiment: EB, MD, YU, SDT; Data analysis: EB; Article writing: EB; Supervision: EB, YÖ. All authors approved the final draft.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

DATA AVAILABILITY STATEMENT

Data used in this study are available from the corresponding author upon reasonable request.

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