

Effect of rosemary (*Rosmarinus officinalis*) extract on the protection of the fishballs from knife fish (*Chitala chitala*) and striped catfish by-product (*Pangasianodon hypophthalmus*) against spoilage during frozen storage

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Abstract

The study aimed to investigate the effect of rosemary (*Rosmarinus officinalis*) extract on the quality changes of fishballs from knife fish (*Chitala chitala*) and striped catfish by-product (*Pangasianodon hypophthalmus*) during frozen storage. Fishballs were supplemented with rosemary extracts at two concentrations of 13 mg/kg and 156 mg/kg. The control treatment was the sample without extract. The products were steamed, vacuum packed in PA bags and stored at a temperature from -18°C to -20°C. Sampling was done at 0, 30, 60, 90, 120, 150 and 180 days of storage period for analysis of pH, Total volatile base nitrogen (TVB-N), total viable bacteria count (TVC), pathogenic bacteria, sensory properties, peroxide value (PV), Thiobarbituric acid reactive substances (TBARs). Results showed that *Escherichia coli* and *Salmonella* spp. were absent in the fishballs, reflecting the safety of products for consumers. Adding rosemary extract at 156 mg/kg in fishballs displayed more effectiveness in inhibiting the formation of lipid oxidation and microbial growth when compared to the control sample during the frozen storage. Based on the results of physicochemical and sensory quality, the supplementation of rosemary extracts showed a significant promise for improving the quality of fishballs. Fishballs from knife fish and striped catfish by-products could be maintained their sensory properties and prolonged their shelf-life up to 180 days of frozen storage.

1. Introduction

Fish and aquatic products are the major protein sources in the human diet (Min *et al.*, 1988). Fishball is considered one of the most popular foods in Southeast Asian countries. However, fishball is highly perishable due to the high contents of protein and moisture (Kok and Park, 2007). Furthermore, seafood products are characterized by their naturally high content of essential n-3 polyunsaturated fatty acids (PUFAs), which makes fish more susceptible to oxidation (Olcott, 1962). Thus, the lipid oxidation in these products should be controlled in order to maintain the nutritional value as well as the organoleptic quality of fishery products (Lin *et al.*, 2009). In Vietnam, knife fish (*Chitala chitala*) is a favourite fish of consumers thanks to the properties of fish flesh such as toughness, unique flavour and high nutrition (Tran *et al.*, 2015). Knife fish are frequently used in fishball production on a small scale although the product cost is relatively high. In the processing of frozen striped catfish (*Pangasianodon hypophthalmus*) fillets, the trimming red meats and fats from the fillets, known as the by-products, accounted for a relatively

high proportion and mostly utilized in animal feed processing in the Mekong Delta. Therefore, the use of these by-products needs to be concerned to increase the profits of the processing industry (Nguyen, 2011). In addition, the lipid level of catfish by-products is comparatively high (7.8%), leading to the consideration of eliminating the spare fat and controlling the lipid oxidation when reprocessing this by-product.

Since ancient times, spices, herbs and their extracts have been supplemented in food not only as flavouring agents but also as traditional medication as well as food preservatives (Nakatani, 1994; Singh *et al.*, 2005; Corbo *et al.*, 2009; Ozogul and Ucar, 2012). Rosemary (*Rosmarinus officinalis* L.), a popular herbal belonging to the Lamiaceae family, is acknowledged for its high antioxidant activity. Rosemary extracts have been documented to indicate a high antioxidant activity and are extensively applied in the food industry. The antioxidative capacity of rosemary is primarily associated with its phenolic compounds, which can scavenge free radicals by donating a hydrogen atom

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(Basaga et al., 1997). Several studies exhibited that the common trend of adding herbal extracts to fish products could improve their quality during storage (Banerjee, 2006; Sarkardei and Howell, 2008; Rohlík et al., 2010). A recent study by Aref et al. (2018) revealed the effective improvement of rosemary extract on some quality characteristics of ready-to-eat fish fingers made from catfish (*Clarias gariepinus*) during frozen storage. The allowable dose of rosemary extract in the food and beverages reviewed by EFSA, and in the European Union was up to 400 mg/kg (Nieto et al. 2018). In the study on fishball production from knife fish and striped catfish by-product, Nguyen et al. (2020) reported that the addition of 156 mg/kg of rosemary extract could improve sensory quality, TVC as well as inhibit lipid oxidation of fishballs during refrigerated storage ($4\pm 1^\circ\text{C}$).

These findings above may also be useful in the food industry since the herbal extracts could be used as natural preservatives to defend food from spoilage and foodborne pathogens contamination. In this study, the rosemary extract was used for supplementation of fishballs from knife fish and striped catfish by-product to study their influence on the microbiological, chemical and organoleptic quality of the fishballs during frozen storage.

2. Materials and methods

2.1 Preparation of rosemary extract

The rosemary plant was obtained from Da Lat city (Vietnam) and transported to Can Tho city (Vietnam). The stem and leaves of the plant were collected for preparing rosemary extract. All collected parts of plants were then washed to remove mud and dust; the rotten and damaged parts were also discarded. Samples were air-dried in shade for some days and then put in an oven at about 50°C until well-dried. After that, they were ground into fine powder with a blender and stored in sealed containers in a dry and cool place. The extraction in hot water (100°C) was done as follows the description by Huynh et al. (2013). The antioxidant activity of rosemary extract, which showed its capacity in scavenging 50% of the DPPH (2,2'-diphenyl-1-picrylhydrazyl) solution (IC_{50}), was determined at 13 mg/L (Thiangthum et al., 2012). The minimum inhibitory concentration of rosemary extract against *Aeromonas hydrophila* was determined at a concentration of 156 mg/L (Sarker et al., 2007). These two concentrations of rosemary extract were used for supplementing fishballs in this study.

2.2 Experimental design

Twenty knife fish was bought at a local market in Can Tho city (Vietnam). Fish weight ranged from 300 to

350 g/fish while the fish length was from 55 to 60 cm. The knife fish was washed with clean water and scraped to collect the fish meat. Striped catfish by-product (4.5 kg) was obtained at a seafood processing company in Can Tho city. The fishballs from knife fish and striped catfish by-product were prepared as following the processed study of Nguyen et al. (2020). The striped catfish by-product removed was fat, skin and red meat. After that, the fish by-product was firstly washed three times with iced water at the ratio of 1:1 (w:v) in 10 mins and subsequently cleansed with 0.5% NaCl solution (5 mins). The final washing step of the striped catfish by-product was cleansing with water and draining off water. In the next step, knife fish meat and striped catfish by-product were mixed at the ratio (%) of 70:30 and roughly ground in 1 min. Next, the fish paste was blended with 5% cornstarch and seasoned with 1.5% NaCl, 3% sugar and 1% pepper. Fish paste was supplemented with rosemary extract at different concentrations of 0 mg/kg, 13 mg/kg and 156 mg/kg. Then, the steps of grinding (10 mins), shaping fishballs (20 g/piece) and steaming (10 mins) were conducted as well. After that, fishball samples were cooled, dipped in liquid nitrogen, packed and vacuumed in PA bags. The product is stored at -18°C to -20°C in the freezer. In each treatment, 161 fishballs were divided into 7 PA bags. In total, 21 vacuum packages of fishballs were prepared for three treatments.

Samples were collected on day 0, 30, 60, 90, 120, 150 and 180. At each sampling time, each PA bag containing 23 fishballs was taken in each treatment for determining TVC (3 balls), *E. coli* (3 balls) and *Salmonella* spp. (3 balls). The other 3 balls were taken for measuring the texture, then grinding to analyze pH values and TVB-N, 7 balls for sensory evaluation, 4 balls for PV and TBARs analysis.

2.3 Determination of the proximate composition of raw materials

Before the storage experiment, the flesh of knife fish and striped catfish by-products were minced and determined the proximate composition comprised moisture, ash, protein and lipid contents (AOAC, 2016). The data collected will be the basis for experimental design and establishing the processed steps in the fishballs processing.

2.4 Total viable bacteria count

Samples were taken aseptically in a vertical laminar and 10 g was transferred to a sterile tube and homogenized with 90 mL of sterile normal saline water for 60 s. From this first 10^{-1} dilution, other decimal dilutions were prepared. A portion (1 mL) of these dilutions was pipetted into sterile Petri dishes and 15 mL

PCA medium at 45°C was added. TVC was determined by counting the number of colony-forming units after incubation at 30°C for 48 hrs. Petri dishes containing from 25 to 250 colonies were selected for counting according to Nordic Committee on Food Analyses (Nordic Committee on Food Analysis, 2006).

2.5 *Escherichia coli* and *Salmonella* spp. determination

The determination of *E. coli* and *Salmonella* spp. was conducted on 3 fishballs for each species at each sampling day of storage at the microbiological laboratory of Intertek Vietnam Limited Company-Total Quality Assurance provider. The tests were performed according to the testing methods of ISO 16649-2:2001 and ISO 6579-1:2017, respectively.

2.6 pH

The pH was determined in duplicate in a 1:1 (w:v) mixture of minced fishballs and 0.15 M KCl by a digital pH meter (C1020, Consort, Germany) equipped with a combined glass-electrode, according to the method described in Hultmann *et al.* (2012).

2.7 Total volatile base nitrogen

Total volatile basic nitrogen (TVB-N) was measured following the method described by Velho (2001). Five grams of sample were loaded into a Kjeldahl tube, followed by 2 g MgO and 50 mL distilled water. Each tube was then agitated and placed in the Kjeldahl distillation system. The distillation was performed for 5 mins, and then the distillate was collected in a flask containing 25 mL boric acid 1% (a mixed indicator of methyl red/methylene blue 2:1). Afterwards, the boric acid solution was titrated with a 0.1 N sulfuric acid solution.

2.8 Texture analysis

The texture of fishballs was determined using a texture analyzer (Model TA.XTplus Texture Analyzer, Stable Micro Systems, Godalming, UK). The conditions of the texture analyzer were as follows: pretest speed, 1.0 mm/s, posttest speed, 10.0 mm/s, distance, 5.0 mm, trigger type, auto, and trigger force, 5 g. Gel strength values (peak force of the compression cycle) of fishballs were measured by using a P/5S probe (5 mm spherical stainless, Stable Micro Systems).

2.9 Sensory property

Sensory analyses were carried out at each sampling time (day 0, 30, 60, 90, 120, 150) during the storage period. The sensory quality of fishball samples was evaluated by seven trained members according to the method of Amerine *et al.* (1965). At each sampling time,

fishball products were thawed in a microwave and warmed in a hot pan (90 –100°C) for 3–4 mins. The general acceptability of fishballs was scored for sensory characteristics by panellists. A hedonic scale from 1 to 9 was used to evaluate fishball, ranking 9 represented for very good quality, 7–8 for good quality, 5–6 for acceptable and a score of 1–4 for bad or unacceptable.

2.10 Peroxide value

Peroxide values were determined through the spectrophotometric ferric thiocyanate method of International IDF Standards (1991). Samples (7.5 g) were extracted by 30 mL of chloroform: methanol mixture (2:1) (v:v) for 3 hrs. After centrifuging at 700×g at 25°C for 5 mins, the lower phase was collected for determination of fat content and considered as the sample extract for the latter analysis. The sample extract (1 mL) was mixed with 3.9 mL chloroform: methanol (2:1). Then, 50 µL of Fe²⁺ solution (0.018 M) was added and later with 50 µL NH₄SCN 30%. The solution was stirred on a vortex mixer for 15 s. The absorbance of the sample was measured at 480 nm against a blank that contained all the reagents, except the sample. Peroxide values, expressed as milliequivalents (meq) peroxide/kg fat, were calculated based on the concentration of Fe³⁺ determined from the regression line ($y = ax + b$) and the fat content of the fish samples.

2.11 Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances were determined according to the spectrophotometric method of Raharjo *et al.* (1992). Samples were homogenized and extracted duplicated in TCA 5%. After centrifugation at 1050×g for 15 mins at 4°C, collected and filled up the supernatant to 50.0 mL in a volumetric flask. In the test tubes, 2.0 mL of each extracted sample and TEP standard solution was added, following an addition of 2.0 mL of TBA reagent 80 mM. The solution was stirred on a vortex mixer for 15 s and placed in a water bath at 94°C for 5 mins. Samples were cooled in a cold-water bath and the absorbance with the spectrophotometer at 530 nm was measured.

2.12 Statistical analysis

All data were expressed as mean ± standard deviation, calculated using Microsoft Excel software. The data of all parameters analyzed at each sampling time were subjected to analysis of variances (one-way ANOVA) using SPSS 16.0 (SPSS Inc, Chicago, II, USA).

3. Results and discussion

3.1 Proximate composition

Proximate analysis was determined in the raw materials before any treatment with *Rosmarinus officinalis* extract. The proximate composition of knife fish flesh and striped catfish by-product were presented in Table 1. The chemical composition of knife fish was characterized by high moisture (79.1%) and protein (18.6%) and low lipid (1.19%) contents. This was similar to the study of Le *et al.* (2018) which reported knife fish muscle containing amount of moisture 78.9%, protein 18.8% and lipid 1.20%.

Table 1. Proximate composition of raw materials

Proximate composition (%)	Knife fish	Striped catfish by-product
Moisture	79.1±1.12	75.9±0.62
Protein	18.6±0.75	12.4±0.31
Lipid	1.19±0.16	6.64±0.50
Ash	1.03±0.19	3.44±0.25

Values are presented as mean±SD in wet basis (n = 3).

Regarding striped catfish trimming by-product, the moisture content of the protein and lipid contents were determined as 75.9, 12.4 and 6.64%, respectively. The fat content was relatively higher than that of Vietnamese striped catfish fillets reported by Orban *et al.* (2008) and Karl *et al.* (2010) due to the presence of trimmed fat comprised in the by-products during filleting. Protein was recorded in a slightly higher content when compared to that of commercial fillet products (13.6-15.7%) (Orban *et al.*, 2008; Karl *et al.*, 2010). It was in accordance with the lower moisture level of the striped catfish by-products during the processing and handling stages. From the above results, it can be seen that the nutritional values of striped catfish by-products were comparable to the chemical composition of commercial fillets, except for lipid content. Therefore, when using this by-product for fishball production, not only the trimming stage of removing subcutaneous fat but also the lipid oxidation of the product during storage should be controlled.

3.2 Microbiological quality

Preservation at freezing temperatures is known as an effective method in the prevention of microbiological as well as pathogenic loads of various food products due to its capability to depress metabolic activities (Chaisowwong *et al.*, 2011; Noor *et al.*, 2014; Noor *et al.*, 2015; Ro *et al.*, 2015). The presence of *E. coli* and *Salmonella* spp. in fishballs of three treatments was tested during frozen storage. The results showed that both bacteria were not detected in 25 g of fishball samples collected throughout the study period (data not shown). *Escherichia coli* and *Salmonella* spp. are well-

known pathogenic microorganisms for etiological dominance in causing enteric diseases in humans (Mody *et al.*, 2014; Waturangi *et al.*, 2015). In our study, the absence of *E. coli* and *Salmonella* spp. in both the control and the fishballs treated with rosemary extract shows the consumer safety of such stored frozen fishballs.

In Vietnam, TVC is one of the standardized parameters which is required for the evaluation of the quality of fishballs (QCVN 8-3: 2012/BYT). Changes in TVC values of fishballs during the 180-day frozen storage are indicated in Figure 1. For all treatments, a marginal decline in TVC was perceived over the freezing storage period. Freezing could decrease the total viable counts, as also presented in other studies on fish and fish products (Tokur *et al.*, 2016).

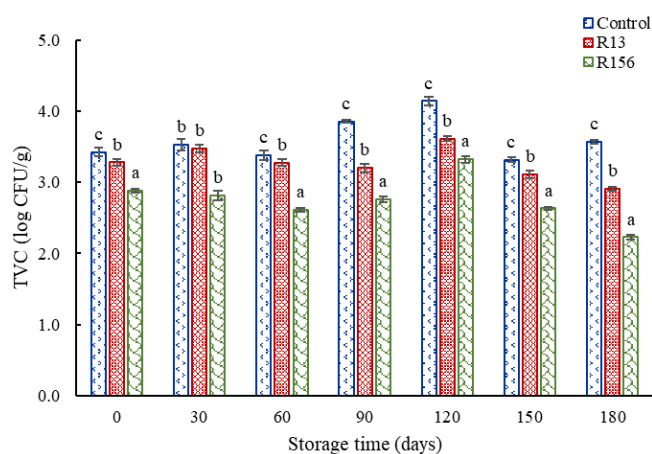


Figure 1. TVC values of fishballs during frozen storage. (mean±SD, n = 3). Bars with different notations within the same day are significantly different between treatments ($p > 0.05$). R13: fishballs treated with rosemary extract at the concentration of 13 mg/kg, R156: fishballs treated with rosemary extract at the concentration of 156 mg/kg

In this study, it was obvious to discern that the treatments containing rosemary extract (R13 and R156) were able to significantly lower the TVC (and thus inhibit microbial growth) compared to the control at all sampling times. Especially, the higher concentration of rosemary extract (R156) showed the lowest TVC values, demonstrating the strongest antibacterial effectiveness. These are in good agreement with the result of the antimicrobial activity testing, where ethanolic rosemary extract showed high activity against *Aeromonas hydrophila* (Al Laham and Al Fadel, 2014). Furthermore, Hamidpour *et al.* (2017) revealed that rosemary extract was responsible for its preservative and medicinal properties. The presence of phenolic compounds, rosmarinic acid, carnosic acid, rosmaridiphenol, carnosol and rosmanol derived from rosemary extract could provide an association with the bacterial cell membrane leading to the modified action on the permeability and integrity of bacterial cells.

The TVC values of fishballs in our study were within the allowable limit of 5 log CFU/g according to the national technical regulation of microbiological contaminants in food of the Vietnam Ministry of Public Health (2012) and much lower than the maximum limits (7 log CFU/g) for fresh and frozen fish established by the International Commission on Microbiological Specifications for Foods (Suvanich *et al.*, 2000). These findings indicated that the shelf life of fishballs stored in the freezer would be up to 180 days.

3.3 pH

Changes in pH values of fishballs during frozen storage observed in this study were given in Table 2. The pH values of all samples during frozen storage were ranging from 6.60 to 6.79. The increase of pH values can be caused by the growth of fish spoilage bacteria leading to the production of volatile basic components, such as ammonia and trimethylamine (Ruiz-Capillas and Moral, 2001). It is proposed that the acceptable limit of pH values in fish ranged from 6.8 to 7.0 (Köse *et al.*, 2006; Özyurt *et al.*, 2007). In this study, the pH values remained under 7, corresponding with a slightly increasing tendency with insignificant differences, indicating that the fishballs were not affected by the spoilage. A similar increasing trend of pH was presented in the publication of Tokur *et al.* (2006) in carp fish burgers, Duman and Özpölat (2012) in fishballs from *Capoeta trutta*. In addition, the low pH values obtained are in correspondence with the study of Kılınççeker (2012) in using thyme and green tea extracts for the characteristic improvement of frozen fishballs from sabut fish (*Tor grypus*).

In this study, at each sampling day, the treatment R156 showed the significantly lowest values ($p < 0.05$) among other treatments, apart from the first day and the 150-day of storage. This may be due to the antimicrobial capacity of rosemary extract, which was evidenced in the protection against many microorganisms (Fernandez-Lopez *et al.*, 2005). Aref *et al.* (2018) found that the use of rosemary contributed to the reduced pH values in ready-to-eat fish fingers made from catfish (*Clarias gariepinus*) during 5 months of frozen storage (-18°C). Unexpectedly, differences were not observed between

the R13 treatment and the control (Table 2). In the present study, the treatment R156 was seen to be able to reduce pH values (compared to control) better than the R13 treatment.

3.4 TVB-N

The term Total volatile basic nitrogen (TVB-N) includes the measurement of trimethylamine (TMA), dimethylamine (DMA), ammonia and other volatile basic nitrogenous compounds which are produced by protein degradation due to bacterial and enzymatic actions in the progress of seafood spoilage (Huss, 1995). TVB-N is one of the most extensive indicators used for the quality and freshness assessment of aquatic products (Yi *et al.*, 2011). Changes in the mean TVB-N values of fishball samples over the freezing period are depicted in Figure 2. In our study, TVB-N values of all samples had a slightly growing trend from the beginning to the end of process storage. This increase was related to ammonia and other volatile amines in the fishballs by enzymatic action (Mazorra-Manzano *et al.*, 2000). A similar trend was presented in the work of Aref *et al.* (2018) and Zhao *et al.* (2019). In addition, there was an observation of the lower significant differences in TVB-N values of R156 treatment in comparison with the control from day 30 to the end of the storage period, which may be attributed to the antioxidant and antimicrobial activities of

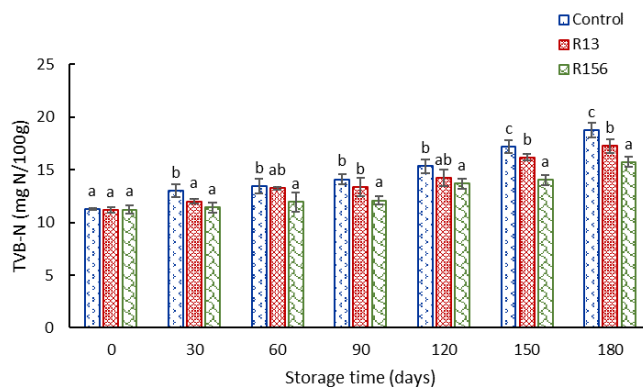


Figure 2. TVB-N values of fish balls during frozen storage (mean±SD, n = 3). Bars with different notations within the same day are significantly different between treatments ($p > 0.05$). R13: fish balls treated with rosemary extract at the concentration of 13 mg/kg, R156: fish balls treated with rosemary extract at the concentration of 156 mg/kg

Table 2. The changes of pH values of fishballs during frozen storage

Treatment	Storage time (days)						
	0	30	60	90	120	150	180
Control	6.71±0.04 ^a	6.77±0.02 ^b	6.70±0.04 ^b	6.71±0.02 ^b	6.78±0.06 ^b	6.70±0.02 ^b	6.79±0.04 ^b
R13	6.67±0.05 ^a	6.75±0.01 ^b	6.66±0.01 ^{ab}	6.69±0.03 ^b	6.74±0.03 ^{ab}	6.67±0.05 ^b	6.74±0.04 ^{ab}
R156	6.67±0.02 ^a	6.71±0.02 ^a	6.63±0.02 ^a	6.65±0.02 ^a	6.67±0.06 ^a	6.60±0.02 ^a	6.69±0.03 ^a

Values are mean ± SD (n = 3). Values with different superscript within the same column are significantly different ($p > 0.05$). R13: fishballs treated with rosemary extract at the concentration of 13 mg/kg, R156: fishballs treated with rosemary extract at the concentration of 156 mg/kg

Rosmarinus officinalis extract (Moreno et al., 2006). A similar finding was stated in the study of Yi et al. (2011) that tea polyphenols can effectively preclude protein degradation and decline the TVB-N content of *Collichthys* fishball. Lower TVB-N values in samples treated with rosemary (*Rosmarinus officinalis*) extract was recorded as reported by Aref et al. (2018) and Zhao et al. (2019). These results of TVB-N in all treatments, from the initial to the end of frozen storage, were lower than the maximum acceptable limit value that was emphasized as 25 mg N/100 g for a very good quality (Varlık et al., 1993).

3.5 Sensory quality

3.5.1 Texture of fishballs

Gel strength, a representative of textural characteristics, is the main determinant of fish-paste product quality. In the present study, the texture of fishballs was determined by measuring gel strength. Changes in the gel strength values of fishball samples over the frozen storage period are illustrated in Figure 3. During the preservation experiment, no significant difference in texture was observed between the control and treated fishballs, meaning that the rosemary had no effect on the texture of the fishballs in this study. Gel strength values of all treatments tend to be stable within 90 days of storage. Afterwards, a gradually decreasing trend was observed over the frozen storage. After more than 90 days, the texture decreased due to the activity of autolytic enzymes (such as collagenase and ATPase) which can decompose proteins from the connective tissue and the spoilage by bacteria (Lakshmanan et al. 2003). Our results obtained are in agreement with what they reported that frozen storage reduced the gel-forming ability of muscle protein from striped catfish (*Pangasianodon hypophthalmus*) (Akter et al., 2013).

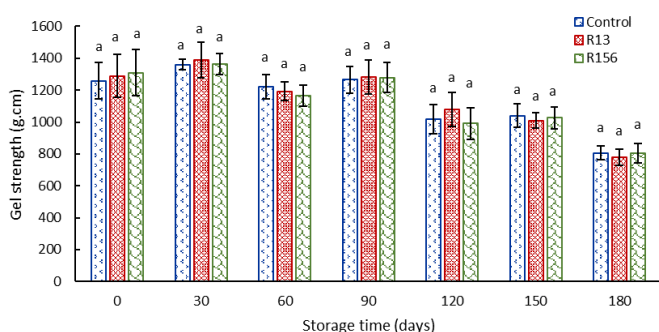


Figure 3. Gel strength of fishballs during frozen storage (mean±SD, n = 3). Bars with different notations within the same day are significantly different between treatments ($p > 0.05$). R13: fishballs treated with rosemary extract at the concentration of 13 mg/kg, R156: fishballs treated with rosemary extract at the concentration of 156 mg/kg

3.5.2 Sensory properties

The general acceptability scores were attained by

pooling the scores of sensory attributes, i.e., colour, odour, flavour and texture which were indicated in Figure 4. The fishballs were considered to be acceptable for human consumption until the general acceptability score reached 5 (Amerine et al., 1965). The acceptability of fish and aquatic products during frozen storage is influenced by the variation of their sensory attributes. Overall, the sensory scores displayed a similar pattern of increasing unacceptability of all experimental fishballs. However, these groups maintained their fresh quality after 180 days of storage period. Additionally, no significant differences were found between the sensory scores of the control fishballs and treatment R13, whereas sensory scores were found to be significantly higher in the R156 treatment. The results of the gradual decreasing tendency in sensory scores obtained in this study are corresponding to those given by Yerlikaya and Gökoğlu (2010) and Hassanin (2013), who explained that lipid oxidation and oxidation of ferrous to ferric iron contributed to a darker colour of the fishball during frozen storage. Also, the lipid oxidation led to the variation of texture and resulted in taste losses and odour changes.

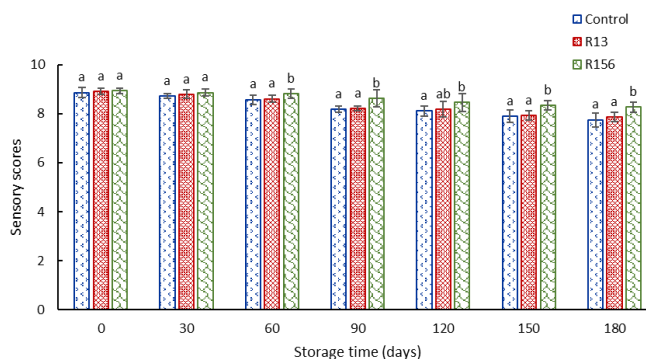


Figure 4. Sensory scores of fishballs during frozen storage (mean±SD, n = 7). Bars with different notations within the same day are significantly different between treatments ($p > 0.05$). R13: fishballs treated with rosemary extract at the concentration of 13 mg/kg, R156: fishballs treated with rosemary extract at the concentration of 156 mg/kg

The remarkable finding of organoleptic quality in this study showed that the fishballs with a higher concentration of rosemary extract (156 mg/kg) were preferred by the panellists from day 60 onwards. According to Aref et al. (2018), the combination of chitosan and rosemary showed effectiveness in improving the sensory properties of fish fingers during 5 months stored at -18°C . Rosemary is well known as an ordinary aromatic spice widely applied in the food industry (González-Trujano et al., 2007). The supplementation of 0.4% rosemary extract could improve the sensory characteristic of Atlantic mackerel fish burgers (Uçak et al., 2011).

From the general acceptability point of view, the

treatment of R156 could be the best option for fishballs throughout the storage period.

3.6 Lipid oxidation

The peroxide value (PV) is the most ordinary measure of lipid hydroperoxides, which are called primary lipid oxidation products (Ólafsdóttir *et al.*, 1997), while thiobarbituric reactive species (TBARs) are extensively utilized to measure secondary oxidation products which peroxides are oxidized to aldehydes and ketones (Hamilton, 1997). Both parameters were used for evaluating the oxidative stability of fishballs during frozen storage. The effect of rosemary extract on the variations in peroxide value (PV) and thiobarbituric acid reactive substances (TBARs) index during the freezing storage period are given in Figures 5 and 6.

All fishballs treated with rosemary extract dramatically decreased the PV and TBARs values throughout the storage period as compared to the control (except for days 90 and 120). The lowest PV values (from 1.49 to 3.24 meq peroxide/kg fat) were obtained in the treatment of R156, while the control was recorded the highest results, ranging from 1.95 to 4.31 over 180 days of storage. Besides, TBARs values of all treated fishballs were considerably lower ($P \leq 0.05$) than that of the control (Figure 6), apart from treatment R13 at day 90 and 120 of storage. The lowest TBARs value (0.26 mg MDA/kg) was recorded in the fishballs treated with rosemary extract at the concentration of 156 mg/kg ($P \leq 0.05$). The impressive effect of rosemary extract on the retardation of lipid oxidation is due to the strong radical scavenging activity and considerably high phenolic content of the rosemary extract (Moreno *et al.*, 2006). Noteworthy, the oxidative measurement by PV and TBARs index of all fishballs were lower than the acceptable limit of 5 meq peroxide/kg (Guran *et al.*, 2015) and of 5-8 mg MDA/kg (Schormuller, 1969 cited by Duman and Peksezer, 2016), respectively. Aref *et al.* (2018) documented the antioxidative responsibility of rosemary extract on ready-to-eat fish fingers made from catfish (*Clarias gariepinus*) during frozen storage for 5 months. The antioxidant activity of rosemary extract is attributed to the presence of phenolic diterpenes which can postpone or inhibit lipid oxidation (Erdmann *et al.*, 2017) by donating a hydrogen atom for breaking the free radical chain reactions (Aruoma *et al.*, 1992; Basaga *et al.*, 1997). These compounds were pointed out that they act as metal ion chelators, thus reducing the formation of the reactive species of oxygen (Fang and Wada, 1993). Furthermore, oil-soluble carnosic acid was found as one of the principal antioxidant compounds of rosemary extract that can stabilize unsaturated fatty acids and delay their deterioration (Houlihan *et al.*, 1985; Estévez

et al., 2005). Chen *et al.* (1992) confirmed that carnosic acid indicated higher effectiveness on peroxidation of membrane lipids than artificial antioxidants (BHA, BHT and propyl gallate). In this study, rosemary extract at the concentration of 156 mg/kg was seen to be more active than that of 13 mg/kg in protecting lipids of fishballs from oxidation.

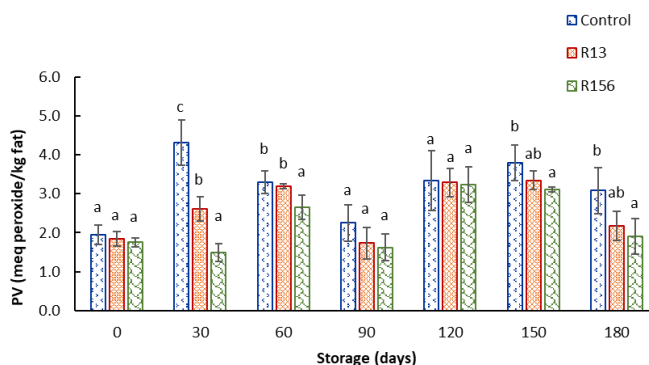


Figure 5. PV values of fishballs during frozen storage (mean±SD, n = 4). Bars with different notations within the same day are significantly different between treatments ($p > 0.05$). R13: fishballs treated with rosemary extract at the concentration of 13 mg/kg, R156: fishballs treated with rosemary extract at the concentration of 156 mg/kg

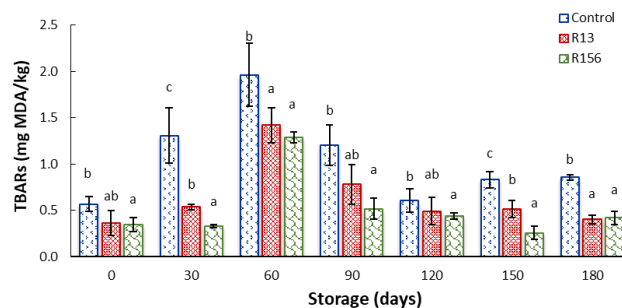


Figure 6. TBARs values of fishballs during frozen storage (mean±SD, n = 4). Bars with different notations within the same day are significantly different between treatments ($p > 0.05$). R13: fishballs treated with rosemary extract at the concentration of 13 mg/kg, R156: fishballs treated with rosemary extract at the concentration of 156 mg/kg

4. Conclusion

The treatment of 156 mg/kg rosemary extract (R156) could reduce the pH values during the frozen storage period, whereas the concentration of 13 mg/kg (R13) did not indicate this property. Both concentrations of rosemary extract did not impact the texture of fishballs compared to the control group. *E. coli* and *Salmonella* spp. were not detected in the fishballs product, reflecting the safe quality of products for the consumer. Based on the TVC value and sensory quality, it was shown that the control and the two treatments of rosemary extract allowed the shelf life of fishballs to be extended for up to 180 days. From the data, it can be concluded that using the addition of 156 mg/kg of rosemary extract in fishballs from knife fish and striped catfish by-products

could maintain their good quality characteristics in terms of sensory assessment. These conclusions were supported by the findings of chemical quality analyses.

Conflict of interest

The authors declare no conflict of interest.

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