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Original article

GAMMA-IRRADIATED ROSEMARY (*ROSMARINUS OFFICINALIS*) DIPS TREATMENT EFFECT ON QUALITY OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FILLETS DURING REFRIGERATED STORAGE

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Summary

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The effect of the gamma-irradiated extract of rosemary and butylated hydroxyanisole (BHA) antioxidant on sensory, chemical and microbiological changes of rainbow trout fillets stored at 4 ± 1°C was investigated for 16 days. The fillets were divided into: control (C), 750 and 1500 rosemary extract (RE) (immersed in 750 and 1500 ppm rosemary extract); 250 and 500 gamma-irradiated rosemary (GIR), (immersed in 250 and 500 ppm gamma-irradiated rosemary) and 250 and 500 BHA (immersed in 250 and 500 ppm BHA). The shelf life of fillets was reported to be 8 days for C, and 12 days for 500 GIR group according sensory, microbiological and chemical parameters. The pH, peroxide value (PV), total volatile basic nitrogen (TVB-N), free fatty acid (FFA), thiobarbituric acid (TBA), psychrotrophic counts (PTC), and total viable aerobic bacterial counts (TVC) values were 7.62 ± 0.21, 17.17 ± 1.6, 51.07 ± 1.1, 6.05 ± 0.13, 3.42 ± 0.18, 12.03 ± 0.19, and 13.16 ± 0.16 respectively for C group and also, 7.42 ± 0.16, 15.2 ± 1.7, 42.5 ± 1.5, 4.48 ± 0.39, 2.59 ± 0.15, 9.98 ± 0.15, and 10.5 ± 0.15 respectively for 500 GIR group. Finally, the following trend in effectiveness was reported: gamma-irradiated rosemary PBS extract > BHA > rosemary PBS extract.

Key words: dip treatment, gamma-irradiated rosemary, rainbow trout fillet, quality parameters

INTRODUCTION

Undoubtedly, fresh aquatic products are the most sensitive animal food stuffs to deterioration due to their high contents of moisture and nutrients and high pH value. Consequently, using methods to extend the shelf life of different aquatic food products is crucial (Song *et al.,* 2012). Synthetic antioxidants have been broadly used to prevent oxidation of fish fillet. Antioxidants enable uniform incorporation into the membrane phospholipids and as a result, they can effectively inhibit oxidation processes (Lauridsen *et al.,* 1997). However, applying synthetic antioxidants such as BHA is under question due to their potential action in carcinogenesis which can bring the rejection of these supplementations by consumer priority taste (Pezeshk *et al.,* 2015).

In the last decades, plant ingredients have been applied increasingly and in different forms, these plants have begun to replace the synthetic preservatives (Pezeshk *et al.,* 2015). An example is rosemary which has been considered as a strong antioxidant in fish processing studies in different forms such as oil and nanoemulsion (Özogul *et al.,* 2016). Also, some authors used it in fish diet to improve fish muscle quality and resistance of fish muscle against oxidation (Hernández *et al.,* 2014; Rezanejad *et al.,* 2019a). Moreover, use of nanotechnology products has become increasingly popular due to improving some factors such as food texture, taste, sensory factors, colour modification and stability during storage (McClements *et al.,* 2009). Accordingly, it is reported gamma irradiation process can boost antioxidant activity in extracts of rosemary (Rezanejad *et al.,* 2019b).

Unquestionably, aquaculture in Iran has been focused on rainbow trout. Based on Iran Fishery Organization announcement in 2012, rainbow trout production was 131 thousand tonnes (Esmaeili *et al.,* 2017) and it is estimated to reach 200 thousand tonns in the near future. Due to the fact this fish is primarily consumed fresh, whole and ungutted, many works have been done toward expanding the shelf lives of these foods (Özogul *et al.,* 2017).

With this respect, to best of our information, there has been no study to evaluate the effects of applying different forms of rosemary (oil and gamma-irradiated) in comparison with synthetic antioxidant (BHA) in fillet. For these reasons the goal of our research was to estimate physical, microbial and sensory factors in refrigerated rainbow trout fillet treated with gamma-irradiated extract of rosemary.

MATERIALS AND METHODS

Gamma irradiated rosemary PBS extract

Gamma irradiated rosemary was prepared as described by Rezanejad *et al.* (2019c). Rosemary leaves were obtained from the Institute of Medicinal Plants herbarium (1394/O/037 for *R. officinalis L.*), Karaj, Iran. Rosemary leaves were washed and dried at room temperature in dark and ground to powder (Rezanejad *et al.*, 2019a). The rosemary PBS extract was prepared and irradiated at dose 30 kGy (gamma dose rate 0.22 Gy/s) as nanoparticles. After the irradiation process the samples were stored at 4 °C for further experiments and analysis.

Sample preparation and storage conditions

The purchased fish, from a fish farm in Mahmoud Abad (Lorestan province, Iran), were dipped in ice-cold water at the laboratory. The dead fish were immediately scaled, gutted and washed in chilled potable water (0 °C). Fish immersion (1:2.5, fish: solution) was carried out during 1 hour and then fillets were packed in nylon/polyethylene pouches. Seven treatments were applied as followed: group C (control), group 750 RE and group 1500 RE (immersed in 750 and 1500 ppm rosemary PBS extract), group 250 GIR and group 500 GIR (immersed in 250 and 500 ppm gamma-irradiated rosemary PBS extract); group 250 BHA and group 500 BHA (immersed in 250 and 500 ppm Butylated hydroxyanisole) Following this, samples with weight of 150 g were then stored in a chilling cabinet at 4 °C up to 16 days. At selected time intervals (1, 4, 8, 12, and 16 days) four packages were removed from each treatment for chemical, microbial and sensory analysis.

Analytical determinations

Complete methods of chemical factors assessment were reported in Rezanejad *et al.* (2019b). Briefly, total volatile basic nitrogen (TVB-N) value was assayed by the micro-diffusion method (Goulas & Kontominas, 2007). The TVB-N value (N mg /100 g of fish) was determined according to the consumption of sulphuric acid.

Assessment of peroxide value (PV) was done per AOAC (1999). In the first step, the titration was run against standard solution of sodium thiosulfate (25 g/L). Finally, PV was calculated and expressed as mill equivalent peroxide /kg of sample: PV (meq/kg) = 1000×(S×N)/W where *S:* volume of titration (mL), *N:* normality of sodium thiosulfate solution (*N* =0.01), and *W:* sample weight (kg) (Sallam, 2007).

For assessment of thiobarbituric acid value by colorimetric method, after dissolving the sample with an aliquot (1 mL) of 1-butanol, 5.0 mL of the mixture was pipetted into a dry stopper test tube with 5 mL TBA reagent (prepared by dissolving 200 mg 2-TBA in 100 mL 1-butanol, filtered, stored at 4 °C for use freshly not more than 7 days). TBA value (mg of malonaldehyde equivalents/kg of tissue) was calculated by the formula TBA=50× (As - Ab)/200 (Ojagh *et al.,* 2010).

pH was measured according to Sallam & Samejima (2004) method. pH value of the filtered sample was assessed with a digital pH meter (model GLP 22; Crison Industrial Company, Barcelona, Spain) standardised at pH 4 and 7.

For free fatty acid (FFA) measurement, AOAC (1998) method was used. After titration with sodium hydroxide (0.1N) acid value was calculated as: Acid value=(56.1×N×V)/W; where; N: normality; V: volume of consumed sodium hydroxide, and W: fish sample weight. Finally, FFA was presented as FFA (%) = Acid value × ½

Bacteriological analyses

Bacteriological counts (total viable aerobic bacterial counts (TVC) and psychrotrophic counts (PTC) were investigated by the method of Ojagh *et al.,* (2010). Plate count agar (PCA, Merk, Darmstadt, Germany) were used for determination in the pour plate method. The inoculated plates were incubated at 37 °C for 2 days for TVC and at 10 °C for 7 days for psychrophilic counts. All counts were expressed as $\log_{10}$ CFU/g and performed in duplicate (Sallam, 2007).

Sensory assessment

Seven panelists (trained for 2 months in 4 hours each week) took four samples at regular intervals. Ten categories were ranked: from 0 to 10, with score below 6 being unacceptable. Before to start sample evaluation, the panelists were familiarised with the flavour (off-odour), texture and colour attributes of fish muscle. Following this, 140 g fillet sample were presented individually to the panelists (20 g to each of them) in plastic cups covered with a lid randomly. Also, light, temperature and humidity in individual booths were under control (Goulas & Kontominas, 2007).

Statistical analyses

A randomised design was performed according to the SPSS (SPSS 21, Richmond, USA) statistical analysis package. Normality and homogeneity of data variances were conducted by the Kolmogorov-Smirnov test and Bartlett's test. Finally, for comparison of pH, PV, TVB-N, TBA, FFA, PTC, TVC, texture, colour, odour, and overall score in seven treatments in each day and also comparison of each factor at days 1, 4, 8, 12 and 16, the Duncan test at the 95% confidence level were used.

RESULTS

In the present study, pH values over the period of storage are reported in Table 1. On day 1 average pH value was 6.67±0.27 and increased steadily to 7.67±0.17 in 750 RE group by the 16th day. Although pH on day 16 had no significant difference among treatments, rosemary had positive effects on decreasing pH so that in 500 GIR group it reached 7.42. It should be noticed that this value in control group on 12th day of storage reached 7.4.

PV changes in the present study are reported in Table 1. This factor on day 16 in 500 BHA (15.4±1.5) and 500 GIR (15.2±1.7) groups was significantly lower than that in controls (17.17±1.6) ($P<0.05$).

Again, considering the results of Table 1, it can be said that TVB-N experienced a gradual increase from initial value 11.05±0.26 to 51.07±1.1 in control group. At the end of 16th day, the lowest TVB-N level was observed in 500 GIR group (42.5±1.5 mg/100 g).

Table 2 indicates the changes of FFA in rainbow trout fillet during the 16-day storage. A gradual increase in FFA content was observed in all treatments as a result of the frozen storage. Significant differences were reported between the control and the rosemary groups (with different concentrations and forms) ($P<0.05$). Lipid hydrolysis developed at a slower rate in the treated groups, especially in the rosemary group.

In the present study, by the 12th day, lower FFA concentration (4.48±0.39) was observed in 500 GIR group, whereas other groups had no significant difference.

Samples treated with rosemary demonstrated low levels of TBA (Table 2). The initial value in the present research was 0.11±0.02. TBA in fish sample treated with 500 GIR was significantly lower vs the other treatments.

Changes of PTC and TVC on fillets throughout storage are shown in Table 3. PTC and TVC on the 1st day was on the average 2.45 and 2.94 $\log_{10}$ CFU/g, respectively. There were no significant differences among the various samples within 4 days of storage ($P<0.05$). From the 8th day of storage, untreated fillets showed rapidly growing counts, attaining 12.3 and 13.16, respectively by the 16th day, which was significantly higher than other groups except 750 RE for PTC and

Table 1. pH, muscle peroxide value (PV, meq/kg), and total volatile base nitrogen (TVB-N, meq/kg) during 16-day storage (4 °C) of rainbow trout fillet immersed in different forms and concentrations of rosemary. Values are means ± SEM of four samples

		Control	750 RE	1500 RE	250 GIR	500 GIR	250 BHA	500 BHA
pH	Day 1	6.62±0.27 C	6.67±0.2 C	6.73±0.17 D	6.67±0.16 B	6.8±0.22 C	6.65±0.27 C	6.77±0.21 C
	Day 4	6.75±0.13 BC	6.77±0.17 C	6.85±0.25 CD	6.7±0.26 B	6.87±0.17 C	6.77±0.47 BC	6.9±0.29 BC
	Day 8	6.97±0.17 bB	7.22±0.18 aB	7.05±0.12 abBC	6.92±0.09 bB	7±0.14 abBC	7.07±0.12 abAB	7.15±0.17 abAB
	Day 12	7.4±0.18 A	7.37±0.2 B	7.28±0.09 AB	7.3±0.18 A	7.25±0.17 AB	7.2±0.16 A	7.35±0.13 A
	Day 16	7.62±0.21 A	7.67±0.17 A	7.45±0.13 A	7.52±0.09 A	7.42±0.16 A	7.45±0.13 A	7.43±0.15 A
PV	Day 1	2.27±0.26 E	2.3±0.18 E	2.38±0.24 E	2.05±0.23 E	2.12±0.16 E	2±0.29 E	2.07±0.35 E
	Day 4	5.18±0.26 aD	4.65±0.4 bcD	4.85±0.14 D	4.47±0.21 cD	4.65±0.2 bcD	4.28±0.17 cD	4.3±0.27 cD
	Day 8	8.03±0.29 aC	7.95±0.12 aC	7.85±0.23 aC	7.82±0.17 aC	7.25±0.21 bC	7.67±0.3 aC	7.22±0.4 bC
	Day 12	11.73±1.1 aB	11.85±1.67 aB	11.94±1 aB	11.68±1.25 aB	10.7±0.9 bB	11.32±0.95 abB	11.2±0.68 abB
	Day 16	17.17±1.6 aA	17.25±1.2 aA	17.16±1.5aA	16.98±1.3 aA	15.2±1.7 cA	16.06±0.8 bA	15.4±1.5 cA
TVB-N	Day 1	11.05±0.26 E	10.97±0.69 E	11.07±0.22 E	11.17±0.27 E	10.95±0.34 E	11.08±0.49 E	11.1±0.6 E
	Day 4	19.97±0.45 Da	18.95±0.28 Db	18.47±0.53 Dbc	18.38±0.22 Dbc	17.96±0.42 Dc	18.97±0.51 Db	18.42±0.57 Dbc
	Day 8	26.72±1.2 Ca	25.02±1.04 Cb	23.75±1.1 Cc	23.9±1.04 Cc	22.6±0.98 Cd	24.12±0.97 Cc	24±1.13 Cc
	Day 12	39.8±0.09 Ba	37.12±1.24 Bb	35±1.11 Bc	35.22±1.18 Bc	31.5±1.22 Bd	35.9±0.95 Bc	35.4±1.1 Bc
	Day 16	51.07±1.1Aa	48.77±1.01 Ab	45.52±1.6 Ac	46.03±1.3 Ac	42.5±1.5 Ad	46.72±1.3 Ac	45.27±0.9 Ac

Dietary treatments: 750 RE and 1500 RE (immersed in 750 and 1500 ppm rosemary extract), 250 GIR and 500 GIR (immersed in 250 and 500 ppm gamma irradiated rosemary); 250 BHA and 500 BHA (immersed in 250 and 500 BHA). Means with common letter labels are not significantly different ($P>0.05$). Different capital letters (A–E) within the same column (different storage day) indicate significant differences ($P<0.05$) and different lowercase letters (a–e) within the same row (different concentrations and forms) indicate significant differences ($P<0.05$).

Table 2. Free fatty acids (FFA, oleic acid %) and thiobarbituric acid (TBA, mg of malonaldehyde equivalents/kg of tissue) during 16-day storage (4 °C) of rainbow trout fillet immersed in different forms and concentrations of rosemary. Values are means ± SEM of four samples

		Control	750 RE	1500 RE	250 GIR	500 GIR	250 BHA	500 BHA
FFA	Day 1	0.34±0.17 D	0.33±0.18 D	0.35±0.16 D	0.34±0.14 D	0.36±0.14 D	0.34±0.13 D	0.33±0.15 D
	Day 4	0.62±0.09 D	0.59±0.31 D	0.51±0.1 D	0.53±0.18 D	0.49±0.12 D	0.58±0.09 D	0.56±0.11 D
	Day 8	1.92±0.17 Ca	1.87±0.26 Cab	1.73±0.3 Cab	1.77±0.17 Cab	1.53±0.25 Cb	1.85±0.12 Cab	1.9±0.14 Ca
	Day 12	3.22±0.46 Ba	3.01±0.27 Ba	2.79±0.22 Bab	3.03±0.26 Ba	2.45±0.26 Bb	3.11±0.24 Ba	2.97±0.16 Ba
	Day 16	6.05±0.13 Aa	5.35±0.24 Abc	4.93±0.34 Ac	5.4±0.25 Abc	4.48±.0.39 Ac	5.77±0.22 Aab	5.57±0.54 Aab
TBA	Day 1	0.11±0.02 E	0.1±0.02 E	0.11±0.02 E	0.12±0.03 E	0.1±0.03 E	0.11±0.02 E	0.1±0.02 E
	Day 4	0.81±0.06 D	0.8±0.13 D	0.82±0.02 D	0.79±0.11 D	0.71±0.12 D	0.82±0.11 D	0.84±0.07 D
	Day 8	1.57±0.13 Cab	1.61±0.1 Cab	1.59±0.11 Cab	1.62±0.12 Ca	1.42±0.11 Cb	1.58±0.1 Cab	1.6±0.13 Cab
	Day 12	2.33±0.16 Ba	2.31±0.09 Ba	2.25±0.09 Ba	2.19±0.1 Ba	2.01±0.09 Bb	2.31±0.08 Ba	2.28±0.1 Ba
	Day 16	3.42±0.18 Aa	3.17±0.08 Abc	2.99±0.14 Acd	3.04±0.09 Acd	2.59±0.15 Ad	3.29±0.12 Aab	3.12±0.11 Abc

Dietary treatments: 750 RE and 1500 RE (immersed in 750 and 1500 ppm rosemary extract), 250 GIR and 500 GIR (immersed in 250 and 500 ppm gamma irradiated rosemary); 250 BHA and 500 BHA (immersed in 250 and 500 BHA). Means with common letter labels are not significantly different ($P>0.05$). Different capital letters (A–E) within the same column (different storage day) indicate significant differences ($P<0.05$) and different lowercase letters (a–e) within the same row (different concentrations and forms) indicate significant differences ($P<0.05$).

Table 3. Total viable aerobic bacterial counts (TVC, log10 CFU/g) and psychotropic counts (PTC, log10 CFU/g) during 16-day storage (4 °C) of rainbow trout fillet immersed in different forms and concentrations of rosemary. Values are means ± SEM of four samples

		Control	750 RE	1500 RE	250 GIR	500 GIR	250 BHA	500 BHA
PTC	Day 1	2.48±0.07 E	2.47±0.12 E	2.55±0.19 E	2.40±0.15 E	2.48±0.15 E	2.38±0.16 E	2.47±0.14 E
	Day 4	4.48±0.23 D	4.54±0.26 D	4.19±0.3 D	4.23±0.22 D	4.31±0.18 D	4.38±0.16 D	4.41±0.25 D
	Day 8	7.49±0.28 Ca	7.46±0.14 Ca	7.24±0.31 Cab	7.14±0.23 Cab	7.02±0.15 Cb	7.48±0.22 Ca	7.25±0.22 Cab
	Day 12	10.39±0.21 Ba	10.26±0.26 Ba	9.56±0.22 Bb	9.23±0.18B bc	9.02±0.25 Bc	10.16±0.26 Ba	10.05±0.19 Ba
	Day 16	12.03±0.19 Aa	11.98±0.11 Aa	11.01±0.26 Ac	10.44±0.18 Ad	9.98±0.15 Ae	11.47±0.3 Ab	11.17±0.29 Abc
TVC	Day 1	2.9±0.11 E	2.94±0.18 E	2.96±0.09 E	2.98±0.19 E	2.95±0.24 E	2.89±0.0.12 E	2.99±0.14 E
	Day 4	5.25±0.13 D	5.27±0.11 D	5.29±0.23 D	5.11±0.11 D	5.18±0.15 D	5.23±0.12 D	5.29±0.17 D
	Day 8	8.18±0.19 Ca	8.09±0.16 Cab	8.13±0.16 Cab	7.89±0.18 Cb	7.49±0.15 Cc	8.29±0.14 Ca	8.2±0.10 Ca
	Day 12	11.21±0.21 Ba	10.99±0.13 Ba	10.46±0.37 Bb	10.55±0.22 Bb	9.48±0.23 Bc	11.1±0.18 Ba	10.89±0.14 Ba
	Day 16	13.16±0.16 Aa	12.67±0.17 Abc	11.51±0.34 Ad	11.79±0.11 Ad	10.5±0.15Ae	12.9±0.13 Aab	12.57±0.17 Ac

Dietary treatments: 750 RE and 1500 RE (immersed in 750 and 1500 ppm rosemary extract), 250 GIR and 500 GIR (immersed in 250 and 500 ppm gamma irradiated rosemary); 250 BHA and 500 BHA (immersed in 250 and 500 BHA). Means with common letter labels are not significantly different ($P>0.05$). Different capital letters (A–E) within the same column (different storage day) indicate significant differences ($P<0.05$) and different lowercase letters (a–e) within the same row (different concentrations and forms) indicate significant differences ($P<0.05$).

250 BHA for TVC (P<0.05). On day 12, 500 GIR for PTC (9.02 ± 0.25) and TVC (9.48±0.23) were significantly lower compared to the other groups (P<0.05).

Changes in the texture, colour, odour and overall score of rainbow trout fillets in dip rosemary treatments during storage at 4°C are shown in Table 4. No significant differences were observed between control and rosemary groups on day 1 for texture, odour, overall acceptance and for colour index on the 1st and 4th day of storage. During storage of rainbow trout fillets, a gradual decline for all sensory factors was reported from day 8 onward (P<0.05). In this sense, texture, colour, odour and overall score reached unacceptable scores (5.6±0.24, 5.67±0.37, 5.49± 0.17 and 4.21±0.07 respectively) in 500 GIR group on day 12, while in control the respective score was 3.06±0.18, 3.63± 0.17, 2.35±0.18 and 2.85±0.11 on same day.

DISCUSSION

pH index can be evaluated as an indicator of spoilage in aquatic products. On day 1 this value increased steadily in 750 RE group by the 16th day which is in parallel with some studies (Özogul *et al.,* 2016). Growth of bacteria due to increased pH in the fish was reported (Jay *et al.,* 2005). When the bacterial growth was investigated, expectedly the increased pH was compatible with bacterial growth. More precisely, bacteria related to spoilage consume molecular compounds with low weight such as amino acids which are available in fish muscle and consequently, the accumulation of alkaline ammonia components and eventually, increasing of pH can occur (Campos *et al.,* 2005). In this study, obtained lower pH values in fish muscle immersed in 500 GIR, 250 BHA, 500 BHA and 1500 RE could be due to inhibitory influences of rosemary on microbial growth, which delays nitrogenous compounds production; in addition, irradiated rosemary had a more positive effect in this regard. Similar results were found by Özogul *et al.* (2016; 2017) in rosemary and herb oils nanoemulsion. Moreover, Özyurt *et al.* (2009) affirmed that if pH values were above 7.1, they can be considered as a spoilage indicator in fish fillet. In this sense, in our research degradation has started after the 8th day of storage.

Considering PV as an indicator of peroxides and hydroperoxides formed in the initial stages of lipid oxidation, it is broadly used for the evaluation of oxidative rancidity in lipids (Olafsdottir *et al.,* 1997). At this study, the initial PV of untreated fillets was approximately 2.1 indicating the freshness of fish samples in line with other data reported for herring (*Clupea harengus*): 0.8–1.2 (Smith *et al.,* 1980) and < 1 meq/kg for the farmed turbot (*Psetta maxima*) (Aubourg *et al.,* 2005). Furthermore, several studies indicated that PV value in rainbow trout fillet gradually increased during storage (Özogul *et al.,* 2017) which is in agreement with the current study. This factor on day 16 in 500 BHA and 500 GIR groups was significantly lower than control. Similar results in rainbow trout muscle treated with rosemary were reported and treated samples in comparison to other natural antioxidants had the lowest PV value (Özogul *et al.,* 2017). What is more, some investigators have studied the effect of adding natural extracts of wild marjoram (*Majorana syriaca*) on tuna (*Thunnus albacares*) fillet and this plant improved PV and TBA factors, probably due to the presence of phenolic compounds and flavonoids (Al‐Bandak *et al.,* 2009). In this

Table 4. Sensory analysis scores (texture, colour, odour, overall score) during 16-day storage (4 °C) of rainbow trout fillet immersed in different forms and concentrations of rosemary. Values are means ± SEM of four samples

		Control	750 RE	1500 RE	250 GIR	500 GIR	250 BHA	500 BHA
Texture	Day 1	9.96±0.07 A	10±0 A	9.89±0.14 A	9.78±0.18 A	9.96±0.08 A	9.92±0.07 A	9.88±0.09 A
	Day 4	7.1±0.13 Bc	7.46±0.13 Bbc	7.63±0.18 Bb	7.42±0.26 Bbc	7.98±0.24 Ba	7.35±0.18 Bbc	7.21±0.29 Bbc
	Day 8	5.13±0.11 Cd	6.07±0.18 Cb	6.21±0.18 Cb	6.1±0.24 Cb	6.74±0.25 Ca	5.49±0.18 Cc	5.13±0.25 Cd
	Day 12	3.06±0.18 Dd	4.21±0.17 Db	4.49±0.18 Db	4.28±0.3 Db	5.6±0.24 Da	3.35±0.18 Dcd	3.56±0.3 Dc
	Day 16	1.21±0.18 Ed	2.24±0.17 Ec	2.63±0.15 Eb	2.42±0.36 Ebc	3.56±0.52 Ea	2.17±0.24 Ec	2.24±0.23 Ec
Colour	Day 1	9.92±0.14 A	9.78±0.08 A	9.96±0.07 A	9.85±0.16 A	9.95±0.1 A	9.88±0.07 A	9.84±0.05 A
	Day 4	7.78±0.18 B	7.63±0.17 B	7.56±0.11 B	7.81±0.13 B	7.67±0.18 B	7.74±0.24 B	7.56±0.42 B
	Day 8	5.78±0.18 Cc	5.81±0.13 Cc	5.92±0.17 Cc	6.49±0.18 Cb	6.92±0.16 Ca	5.85±0.11 Cc	5.96±0.44 Cc
	Day 12	3.63±0.17 Dd	3.78±0.12 Dd	4.46±0.31 Dc	5.06±0.18 Db	5.67±0.37 Da	3.42±0.29 Dd	3.49±0.11 Dd
	Day 16	1.78±0.17 Ee	1.89±0.14 Ede	2.46±0.31 Ec	3.24±0.24 Eb	4.28±0.25 Ea	2.06±0.18 Ede	2.14±0.2 Ecd
Odour	Day 1	9.92±0.08 A	9.66±0.07 A	9.85±0.12 A	9.85±0.16 A	9.81±0.17 A	9.92±0.14 A	9.88±0.07 A
	Day 4	7.35±0.17 Bc	7.42±0.17 Bab	7.53±0.13 Bab	8.17±0.24 Ba	8.07±0.13 Ba	7.78±0.17 Bb	7.63±0.17 Bab
	Day 8	4.92±0.17 Cc	5.06±0.18 Cbc	5.21±0.18 Cbc	6.63±0.18 Ca	6.53±0.14 Ca	5.32±0.24 Cb	5.24±0.17 Cb
	Day 12	2.35±0.18 De	3.1±0.14 Dd	3.99±0.12 Dc	5.1±0.13 Db	5.49±0.17 Da	2.99±0.12 Dd	3.14±0.26 Dd
	Day 16	1.21±0.18 Ed	1.42±0.27 Ecd	2.1±0.19 Eb	3±0.12 Ea	3.1±0.13 Ea	1.64±0.16 Ec	2.21±0.18 Eb
Overall score	Day 1	9.92±0.14 A	9.85±0.12 A	9.88±0.07 A	9.92±0.14 A	9.96±0.07 A	9.96±0.07 A	9.89±0.14 A
	Day 4	7.49±0.18 Bab	7.31±0.24 Ba	7.53±0.14 Bab	7.49±0.24 Bab	7.71±0.12 Bab	7.56±0.18 Bab	7.63±0.15 Bb
	Day 8	5.15±0.26 Cc	5.22±0.2 Cbc	5.35±0.26 Cbc	5.49±0.18 Cb	5.92±0.18 Ca	5.17±0.18 Cbc	5.31±0.17 Cbc
	Day 12	2.85±0.11 Db	2.89±0.14 Db	2.93±0.17 Db	2.94±0.18 Db	4.21±0.07 Da	2.85±0.12 Db	3.07±0.18 Db
	Day 16	1.24±0.13 Ebc	1.14±0.11 Ed	1.35±0.17 Ebc	1.49±0.18 Ec	2.56±0.24 Ea	1.35±0.17 Ebc	2.03±0.13 Eb

Dietary treatments: 750 RE and 1500 RE (immersed in 750 and 1500 ppm rosemary extract), 250 GIR and 500 GIR (immersed in 250 and 500 ppm gamma irradiated rosemary); 250 BHA and 500 BHA (immersed in 250 and 500 BHA). Means with common letter labels are not significantly different ($P>0.05$). Different capital letters (A–E) within the same column (different storage day) indicate significant differences ($P<0.05$) and different lowercase letters (a–e) within the same row (different concentrations and forms) indicate significant differences ($P<0.05$).

way, positive influence of rosemary in our work, especially in the form of irradiated rosemary can be associated with the fact that rosemary is a rich source of phenolic compounds and flavonoids (Bai *et al.,* 2010).

Bacterial spoilage and the action of endogenous enzymes usually produce TVB-N so it is might be the reason of TVB-N increase (Özogul *et al.,* 2004). TVB-N content was found to be an acceptable indicator of freshness in a variety of fish species such as sardine (*Sardina pilchardus)* (Özogul *et al.,* 2004) and European eel *(Anguilla Anguilla*) (Özogul *et al.,* 2005). Considering the current results, it can be said that TVB-N experienced a gradual increase from initial value in control. At the end of the 16th day, the lowest TVB-N level was observed in 500 GIR and 500 BHA treatment groups. This is attributed to the role of rosemary, in other words, the control treatment in our study led to quicker deterioration of fish compared to fillets treated with rosemary extract, nanopowder and BHA. Similar results were reported for sea bass *(Dicentrarchus labrax*) treated with commercial oils (sunflower, canola, corn, olive, soybean, and hazelnut oils) (Özogul *et al.,* 2016). It should be noted that variation in TVB-N value can be changed by fish non-protein nitrogen content, which in turn depends on different factors such as size of fish, fish feeding types, season of catching, and microbial activity in the fish tissue as well as environmental factors (Özogul *et al.,* 2004).

Additionally, a gradual increase in FFA content was observed in all treatments as a result of the frozen storage time. Whittle *et al.* (1990) have observed FFA formation during a first stage of storage due to endogenous enzyme (namely, lipases and phospholipases) activity. After that, microbial activity should be crucial, so that FFA formation is more likely due to bacterial enzyme activity. According to the current results, a partial inhibitory effect of different forms of rosemary on the endogenous enzyme activity can be probably seen in the first stage (days 2–6); furthermore, the role of rosemary on decreasing microbial activity in second stage would lead to a lower FFA concentration in the rainbow trout muscle. Lipid hydrolysis developed at a slower rate in treated groups, especially in the rosemary group. Similar results were reported by Özogul *et al.,* (2017) who found that FFA values of the rainbow trout fillets treated with nanoemulsion based on sunflower oil remained lower than those in the control group.

In the present study, lower concentration of FFA in 500 GIR group was observed on the 12th day, whereas other groups showed no significant difference. Other investigators reported significant positive effect of rosemary in declining FFA in rainbow trout fillet (Özogul *et al.,* 2017). Moreover, Özogul *et al.* (2016) reported that treating fish muscle with natural oils based nanoemulsion led to a decrease in FFA supposedly due to FFA forming complexes with the sarcoplasmic and myofibrillar muscle proteins (Reddy *et al.,* 1992). This could explain the lower FFA in fish fillet from GIR groups.

TBA is an indicator that is broadly investigated in order to measure the secondary lipid oxidation in meat products (Shahidi *et al.,* 1992). Checking the extent of lipid hydrolysis is crucial due to the fact that high incidence of lipid hydrolysis can result in lipid oxidation and protein denaturation (Miyashita & Takagi, 1986). Low levels of TBA in treated samples with rosemary can be attributed either to a decreased bacterial population or reduced

capacity of bacteria for oxidative deamination of nanoprotein nitrogen compounds or both of them (Manju *et al.,* 2007). This decrease in TBA value in samples treated with rosemary compared to control in the present study correspond to other works which have tested other natural antioxidants (Özogul *et al.,* 2016), grape seed extract (Pazos *et al.,* 2005) and green tea with grape seed extract (Yerlikaya & Gokoglu, 2010). The initial value in present research was lower than those reported for rainbow trout (Özogul *et al.,* 2017) but higher than those for European eel (Özogul *et al.,* 2005). In addition, TBA in the present research was acceptable until day 12 for 500 GIR group (2.01) when compared with other studies having evaluated chemical, microbial and sensory indices of rainbow trout (Özogul *et al.,* 2017). Also, it should be said that TBA of fish samples treated with 500 GIR was significantly lower vs the other treatments.

To sum up, in the current study, rosemary demonstrated positive effects on chemical factors especially in the form of nanopowder. In this sense, concentrations increased considerably with storage time for all treatments as following order: C>RE>GIR>BHA.

Bacteria in living fish are generally present in the skin and gut, but are prevented from entering the muscle. Once fish dies and autolysis begins, bacteria can enter and decompose the muscle (Aberoumand, 2010). PTC and TVC on the 1st day indicated acceptable quality of the samples in the current research while initial microbial count of rainbow trout muscle was found to be between 3 and 4 in previous works and went up with increasing storage time (Frangos *et al.,* 2010). However, some researchers suggested that rosemary, especially in the form of nanopowder, effectively delayed the growth of TVC and PTC on trout fillets which was attributed to antibacterial effects of rosemary extract and rosemary nanoemulsion (Özogul *et al.,* 2016). On the other hand, Giménez *et al.,* (2004) reported that treating gilthead sea bream *(Sparus aurata)* fillets with rosemary extract had no effect on the bacterial measurements.

From the results of sensory evaluation, it can be said that decomposition was less remarkable in fillets dipped in RE and especially in the GIR groups, meaning that shelf-life was extended by 4 days at 4 °C. Similar results were observed for sea bass and sea bream treated with herbal nanoemulsions (Özogul *et al.,* 2016).

Furthermore, at this investigation, the rejection of rainbow trout fillets on the 12th day followed the increase in PTC, TVC, TBA, FFA, TVB-N, PV and pH in 500 GIR group. In the other study groups however, the above-mentioned factors showed unacceptable values by the 8th day. In this regard, Refsgaard *et al.* (2000) reported that FFA formation had a strong direct relationship with lack of acceptability, which in our study was observed as well. In addition, extending product shelf-life of carp fillets by 8 days at 5 °C dipped in 1% carvacrol and thymol extract were reported (Mahmoud *et al.,* 2004).

From the obtained results, it is noticeable that extending shelf-life of muscle, delaying deterioration and more importantly, bringing a pleasant flavour, texture and odour to fish fillet resulted from application of rosemary especially in the form of nanopowder. Similar results were found when it was applied exogenously (Giménez *et al.,* 2004) or used as a dietary supplement (Hernández *et al.,* 2014), whereas no study has conducted in order to investigate the effect of the different

concentrations and forms of this plant and assay sensory attributes over 16 days. Thus, our data have demonstrated that rosemary, especially gamma-irradiated rosemary had a strong effect on decreasing bacterial counts, lipid oxidation and off-shelf life, consequently extending the shelf-life of the fish.

CONCLUSION

The results of the present study showed that there was an obvious relation among chemical (pH, PV, TVB-N, FFA, TBA), microbial (PTC, TVC) and sensory (texture, colour, odour, overall score) parameters of rainbow trout fillets stored at 4 °C after being dipped with rosemary and BHA. Rosemary had positive effects same as BHA, when compared with the control group. Among different forms of rosemary, the following trend in effectiveness was reported: gamma-irradiated > BHA > extract.

Furthermore, the present work indicates that treating samples with gamma-irradiated rosemary can be used to protect fish fillet from oxidation during storage, delaying post mortem spoilage. However, in order to obtain a greater effect, additional studies should be designed to determine the optimal concentration of the gamma-irradiated rosemary as a natural preservative agent used to supplement the diets and test its influences in fish physiology and biochemistry.

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