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Application of atomized hydrosols to rainbow trout fillets as an easy preservative

Gökkuşağı alabalığı filetolarına kolay bir koruyucu olarak atomize hidrosollerin uygulanması

Emre Yavuzer[1,*], Mehmet Yetişen[2], Dilek Yaprak Uslu[3]

[1,2] *Niğde Ömer Halisdemir University, Faculty of Engineering, Department of Food Engineering, 51240, Niğde, Türkiye*
[3] *Niğde Ömer Halisdemir University, Vocational School of Social Sciences, Cookery Department, 51240, Niğde, Türkiye*

Abstract
In this study, a storage box including an ultrasonic atomizer was obtained with a 3D printer, and atomized hydrosols of thyme and lavender were applied to the fish meat at regular intervals under refrigeration conditions (3±1℃) for 6 days. The effects of application with atomized hydrosols on lipid oxidation (malondialdehyde levels), peroxide value (PV), total volatile basic nitrogen (TVB-N) level, microbiologic level, color changes, and texture parameters were investigated. The results of the study showed that aerosolizing hydrosol droplets on the meat protected the quality significantly as the storage period increased and that the ultrasonic atomizer could be used effectively in the preservation of food quality.

Keywords: 3D printer, Fish meat, Atomized hydrosols, Lipid oxidation

Öz
Bu çalışmada, 3D yazıcı ile ultrasonik atomizör içeren bir saklama kutusu imal edilmiş ve atomize kekik ve lavanta hidrosolleri altı gün boyunca soğutma koşulları altında (3±1℃) balık etine belirli aralıklarla uygulanmıştır. Atomize hidrosollerle uygulamanın lipid oksidasyonu (malondialdehit seviyeleri), peroksit değeri (PV), toplam uçucu bazik azot (TVB-N) seviyesi, mikrobiyolojik seviye, renk değişiklikleri ve tekstür parametreleri üzerindeki etkileri araştırılmıştır. Çalışma sonuçları, depolama süresi arttıkça et üzerine hidrosol damlacıklarının aerosolize edilmesinin kaliteyi önemli ölçüde koruduğunu ve ultrasonik atomizörün gıda kalitesinin korunmasında etkili bir şekilde kullanılabileceğini göstermiştir.

Anahtar kelimeler: 3D yazıcı, Balık eti, Atomize hidrosoller, Lipid oksidasyonu

1 Introduction

The atomization of liquid is the process of ejecting small droplets in a gas environment after their disruption from a liquid layer [1-4]. In previous processes for atomizing liquids, mechanical atomizers have generally been used [5, 6]. However, the ultrasonic atomizer is more useful due to its structure, which is economical and can be connected to electronic circuits. Ultrasonic atomization is known as the process of removing fine droplets from a liquid layer exposed to ultrasound power greater than 20 kHz [3, 7]. Although there are many theories about the working logic of the ultrasonic atomizer [3, 8], according to the connection theory, the formation of droplets is caused by the interaction between random hydraulic shock and capillary waves produced by cavitation disorder [9].

Oxidative and microbial degradation of aquatic products are the most important problems in terms of storage of the product in processing technology. Depending on these deteriorations, undesirable changes in taste, smell, and color of the product may result in reduced shelf life [10, 11]. Since thyme and lavender oils are widely used in the food and pharmaceutical industries, the hydrosols of these plants appear as by-products.

In the present study, the reason for using these hydrosols to form microdroplets with an ultrasonic atomizer was the knowledge of their antimicrobial effects of them. For example, thyme oil has serious effects on some Salmonella species [12], resistant *Listeria monocytogenes* strains [12, 13] or *Enterococcus* spp [14], to name only a few of those in the literature. The lavender hydrosol is reported to contain linalool (24.52-39.2%), borneol (5.8-14.3%), cis-linalool oxide (18.2-25.0%) [15], whilst thyme hydrosol has carvacrol (48.30%) and thymol (17.55%) [16]. Additionally, our previous study [17] showed that some hydrosols have significant antimicrobial effects on foodborne spoilage and pathogenic microorganisms. However, there are no relevant studies in the literature on the effects of micro droplets of hydrosols obtained with an ultrasonic atomizer on the quality changes of fish meat. In this study, a fish storage box containing an ultrasonic atomizer was developed to increase the storage quality. The effect of this newly developed storage box on the freshness performance of fish meat during storage was calculated by sensory, chemical, and microbiological analysis. The ability to integrate the operating data obtained with a model box into larger storage environments in the industry will provide serious benefits for healthy food storage.

2 Materials and methods

2.1 Preparation of hydrosols

2.1.1 Compounds analysis of plant hydrosols

The components of the hydrosols of the thyme and lavender plants used in the study were determined by a gas

* Sorumlu yazar / Corresponding author, e-posta / e-mail: emreyavuzer@gmail.com (E. Yavuzer)
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chromatography-Mass spectrometry (GC-MS) device in Kırşehir Ahi Evran University Central Laboratory. The method given by [18] was applied to determine the components of thyme hydrosol. The method determined by [19] was used to specify the components of lavender hydrosol.

2.1.2 Ultrasonic atomizer and producing storage box with 3D printer

"Feellife Donut" brand portable humidifier was used as an ultrasonic atomizer in the study. The atomizer used in the study can work with 220 volts by connecting to an adapter, USB, or Arduino microcontroller. To obtain the 3D printed storage box, 1.75 mm white PLA filament and a standard 3D printer were used. Figure 1 shows the storage box with an ultrasonic atomizer. Approximately 1 kg (330 meters) of PLA filament was used for the construction of the 25x25x20cm box.

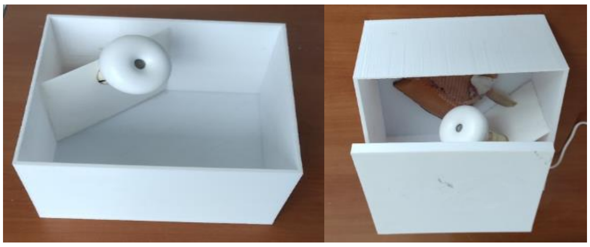

Figure 1. Storage box with ultrasonic atomizer

2.1.3 Electronic component used for color measurement

In the study, a TCS3200 color sensor (Figure 2) connected to an Arduino Uno was used to make color measurements. Color measurements were recorded on storage days for each group, as we did in our previous study [20].

Figure 2. TCS3200 color sensor used to determine the rainbow trout's meat color

2.1.4 Fish materials and storage conditions

The trout used in the study were obtained from a trout production facility (EZG Corp. Hirfanlı Dam/Turkey) located 20 minutes from our university laboratory. Harvested fish were delivered to the laboratory in polystyrene boxes filled with ice. The average weight of the fish was 280 ±23 g. In aseptic conditions, the fish were filleted and instantly placed in 3D-printed boxes for the control group and boxes including an ultrasonic atomizer for the test group. With the help of an Arduino microcontroller, the ultrasonic atomizer was able to spray hydrosol for 5 minutes every half hour. All data were prepared using the fish muscle that was stored at 3±1 °C without ice for each analysis at 0, 1, 2, 3, 4, 5, and 6th days of storage.

2.1.5 Physicochemical analysis

The TBARS (mg malonaldehyde/kg), TVB-N (mg/100g), and PV (meq O2 kg-1) values of the rainbow trout were analyzed during storage according to the methods given by [21-23] respectively.

2.1.6 Sensory analysis

Sensory analysis was carried out according to the Quality Index Method [24], which we described in detail in our previous [17] study. During the sensory analysis, experienced panelists performed mouthwash and taste changes with diet biscuits during the transition between the groups.

2.1.7 Microbiological analysis

Triplicate samples were taken for total viable count (TVC / 2 days at 30 °C) and psychrotrophic count (PTC / 10 days at 6 °C).

3 Results and discussion

The components and ratios of the hydrosols used in the study are given in Table 1. The major compounds in thyme hydrosol were carvacrol and acetic acid, 58.72% and 10.58%, respectively. For lavender hydrosol, the highest determined compound was a-terpineol with 17.46%.

Table 1. Components of lavender and thyme hydrosols used in the study

Components of lavender hydrosol	RT	%	Components of thyme hydrosol	RT	%
Linalool-3,7-oxide	6.28	0.08	Hydroxyacetone	13.62	0.51
1,8-cineole	8.73	0.64	LINALOOL OXIDE	19.44	0.26
Cis-ocimene	9.42	0.85	Acetic acid	20.41	10.58
Delta 3-carene	9.88	0.33	2-Methyltetrahydro-2-furanol	21.05	0.87
Cis-ocimene	10.08	5.84	Formic acid	23.67	0.28
3-octanol	13.86	0.10	a-butyrolactone	26.00	1.83
Linalool oxide	15.13	2.19	1-BORNEOL	28.03	0.23
Acetic acid	15.67	2.30	Epoxylinalol	29.96	0.17
Trans Linalool oxide	15.87	1.96	4-Methylmannose	33.62	0.41
Camphor	16.98	0.43	Geranic oxide	35.83	0.63
Linalool	17.77	6.97	Terpinol	38.68	1.14
Linalyl acetate	17.95	4.36	Thymol	40.69	1.26
trans-Caryophyllene	18.86	0.24	Carvacrol	41.33	58.72
α-terpineol	21.39	17.46			
Epoxylinalool	22.81	1.10			
Betulin	23.68	0.31			
Farnesol	24.88	2.05			
7-Octene-2,6-diol, 2,6-dimethyl	29.56	16.77			
Terpinol	35.29	11.15			
D-carvone	39.33	2.58			

The fact that trout is rich in mono and polyunsaturated fatty acids makes it crucial as a functional food. But this richness makes the fish meat vulnerable to lipid oxidation. For this reason, analyzing PV is important to capture lipid oxidation. PV changes of the groups treated with different atomized hydrosols and stored at 3±1 °C are given in Figure 3. PV values for all groups increased during storage, and there were statistically significant differences between the groups ($p < 0.05$). The initial PV value of this study (2.57 meq O_2/kg^{-1}) was close to the initial PV value (2.85 meq O_2 kg^{-1}) of our previous study [17], where we stored trout on ice-containing extract. From the 2nd storage day to the 6th storage day, the PV values of atomized hydrosols of thyme and lavender were significantly lower than the control. The literature accepts the PV value between 10 and 20 meq O_2/kg^{-1} as the upper limit for foodstuffs [25]. In this study, all test groups exceeded the level (<10) except for atomized thyme hydrosol. In the present study, it was seen that testing groups with atomized plant hydrosols showed a slower lipid oxidation rate than the control, so the results of this study indicate that the use of the atomized hydrosol is effective at delaying lipid peroxidation in rainbow trout.

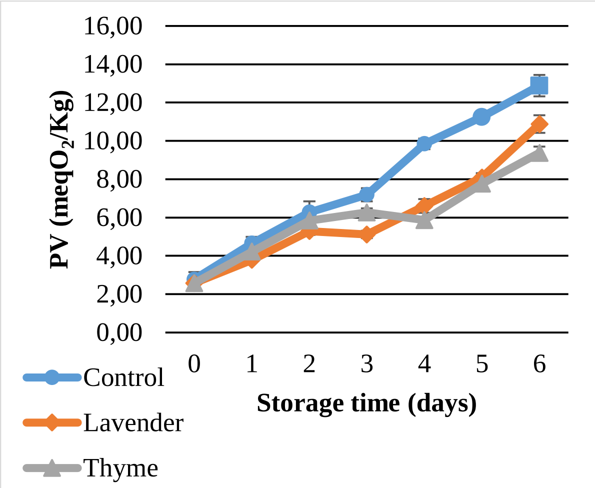

Figure 3. Peroxide value (PV) changes during storage in fish meat sprayed with different hydrosols with an ultrasonic atomizer

The TBARS value is a good indicator for predicting the degree of secondary oxidation and determining the quality of edible lipids. [26]. The effects of atomized hydrosols on the TBARS parameter are given in Figure 4. The beginning TBARS value of the samples was 0.6 mg malonaldehyde/kg and increased in all groups during the storage. In our previous studies [17, 27] on the storage of trout, the maximum TBARS value was found to be 2.08 mg malonaldehyde/kg. In the current study, TBARS values did not rise to an extremely high level, although the fishes were not stored on ice. On the 4th, 5th and 6th days of storage, the TBARS values of the control and lavender groups were significantly ($p<0.05$) higher than the thyme groups.

In the study, the initial TVB-N value was recorded as 19.09 mg/100 g. The TVB-N value contains ammonia, trimethylamine (TMA), and dimethylamine (DMA), which cause them to increase through bacterial or enzymatic degradation [28].

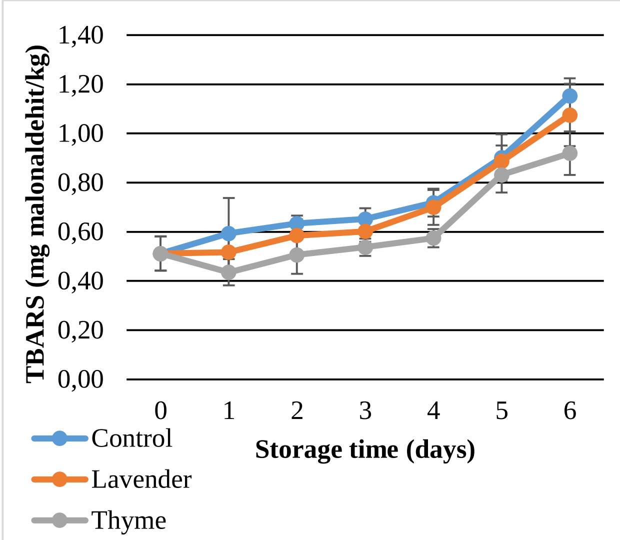

Figure 4. Thiobarbituric acid-reactive substances (TBARS) changes during storage in fish meat sprayed with different hydrosols with an ultrasonic atomizer

In the study, the atomized thyme hydrosol group remained at significantly ($p<0.05$) lower levels from the second storage day to the end of storage (Figure 5). Apart from this, the atomized lavender hydrosol group also had a significantly lower ($p<0.05$) TVB-N value compared to the control group. When we compare the effects of thyme and lavender hydrosols in terms of TVBN with the control group, it is seen that their effects are statistically significant ($p<0.05$). According to these results, it is concluded that bacterial or enzymatic degradation occurs less. Finally, the use of thyme and lavender hydrosols can be considered to have a protective effect on the sample.

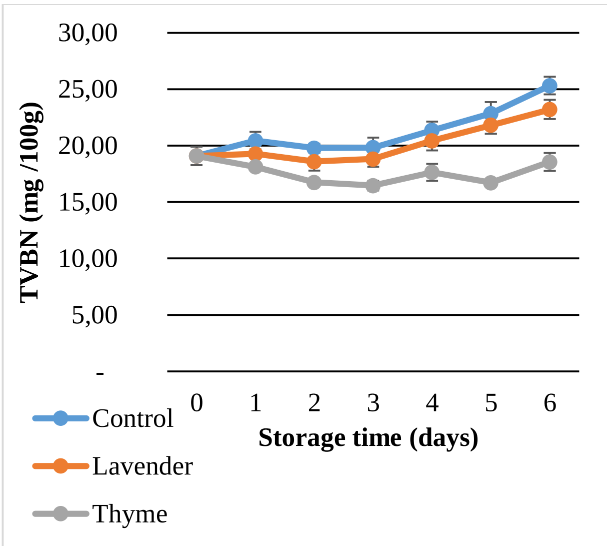

Figure 5. Total volatile basic nitrogen (TVB-N) changes during storage in fish meat sprayed with different hydrosols with an ultrasonic atomizer

The sensory analysis performances of fish atomized with plant hydrosols during storage are given in Table 2. In terms of texture, water, odour, color, brightness, and state of meat fragmentation, all test groups had significantly ($p<0.05$) lower sensory scores than the control group during storage. On the other hand, the sensory parameters of atomized thyme hydrosol were recorded at lower levels than those of the lavender group during storage. When the sensory results were evaluated together with the microbiological and chemical results, it was observed that all tested groups gave highly effective results as preservation materials.

Table 2. Evaluation of sensory quality of raw trout meat

	Storage days	Control	Lavender	Thyme
	0	0.00	0.00	0.00
	1	0.57 ± 0.19^{a}	0.00	0.00
	2	1.29 ± 0.49^{a}	0.43 ± 0.19^{a}	0.50 ± 0.00^{a}
Texture	3	2.00 ± 0.00^{a}	1.00 ± 0.00^{a}	0.93 ± 0.19^{a}
	4	3.00 ± 0.00^{by}	2.07 ± 0.19^{ax}	2.00 ± 0.00^{bx}
	5	3.00 ± 0.00^{by}	2.29 ± 0.76^{b}	2.14 ± 0.24^{c}
	6	3.00 ± 0.00^{by}	3.00 ± 0.00^{by}	3.00 ± 0.00^{by}
	0	0.57 ± 0.19^{a}	0.00	0.00
	1	0.36 ± 0.24^{a}	0.36 ± 0.24^{a}	0.36 ± 0.24^{a}
	2	0.86 ± 0.24^{ab}	0.50 ± 0.00^{a}	0.57 ± 0.19^{a}
Water	3	1.57 ± 0.53^{b}	0.93 ± 0.13^{a}	0.86 ± 0.24^{ab}
	4	2.43 ± 0.53^{c}	1.86 ± 0.38^{b}	1.71 ± 0.49^{bc}
	5	2.86 ± 0.38^{dy}	2.14 ± 0.38^{cxy}	2.00 ± 0.0^{cxy}
	6	3.00 ± 0.00^{by}	2.86 ± 0.38^{dy}	2.43 ± 0.53^{c}
	0	0.00	0.00	0.00
	1	0.71 ± 0.27^{a}	0.00	0.00
	2	1.86 ± 0.38^{a}	0.50 ± 0.00^{a}	0.71 ± 0.27^{a}
Odour	3	2.14 ± 0.38^{b}	1.07 ± 0.19^{b}	1.14 ± 0.24^{a}
	4	3.00 ± 0.00^{by}	1.93 ± 0.19^{bxy}	1.86 ± 0.38^{ax}
	5	3.00 ± 0.00^{by}	2.43 ± 0.53^{by}	2.0 ± 0.58^{by}
	6	3.00 ± 0.00^{by}	2.86 ± 0.38^{dy}	2.86 ± 0.38^{dy}
	0	0.00	0.00	0.00
	1	0.50 ± 0.0^{a}	0.00	0.00
	2	1.00 ± 0.00^{a}	0.79 ± 0.27^{a}	0.71 ± 0.27^{a}
Color	3	1.43 ± 0.53^{b}	1.14 ± 0.38^{ab}	1.00 ± 0.00^{a}
	4	2.57 ± 0.53^{by}	1.71 ± 0.49^{abxy}	1.57 ± 0.53^{ax}
	5	2.86 ± 0.38^{cq}	2.14 ± 0.38^{bx}	1.93 ± 0.19^{byz}
	6	3.00 ± 0.00^{by}	2.43 ± 0.53^{by}	2.00 ± 0.0^{cxy}
	0	0.00	0.00	0.00
	1	0.36 ± 0.24^{a}	0.21 ± 0.27^{a}	0.00
	2	0.93 ± 0.19^{a}	0.36 ± 0.24^{a}	0.36 ± 0.24^{a}
Brightness	3	1.71 ± 0.49^{a}	1.21 ± 0.27^{ab}	1.14 ± 0.24^{a}
	4	3.00 ± 0.00^{by}	1.86 ± 0.38^{bx}	1.79 ± 0.39^{ax}
	5	3.00 ± 0.00^{by}	2.29 ± 0.49^{cxy}	2.14 ± 0.38^{bx}
	6	3.00 ± 0.00^{by}	3.00 ± 0.00^{by}	2.29 ± 0.49^{cxy}
	0	0.00	0.00	0.00
	1	0.43 ± 0.19^{a}	0.00	0.00
	2	1.57 ± 0.53^{a}	0.50 ± 0.00^{a}	0.57 ± 0.19^{a}
State of meat fragmentation	3	2.14 ± 0.38^{a}	1.29 ± 0.49^{a}	1.07 ± 0.19^{a}
	4	3.00 ± 0.00^{by}	1.93 ± 0.19^{by}	1.71 ± 0.39^{by}
	5	3.00 ± 0.00^{by}	$2,00\pm0,29^{by}$	$1,93\pm0,45^{by}$
	6	3.00 ± 0.00^{by}	2.86 ± 0.38^{cq}	2.43 ± 0.53^{by}
	0	0.00	0.00	0.00
	1	0.00	0.50 ± 0.00^{a}	0.36 ± 0.24^{a}
	2	0.00	0.86 ± 0.24^{a}	0.50±0.00a
Hydrosol odor	3	0.00	0.79 ± 0.27^{a}	0.64 ± 0.24^{a}
	4	0.00	0.43 ± 0.19^{by}	0.57 ± 0.53^{by}
	5	0.00	$0,29\pm0,27^{by}$	$0,43\pm0,53^{by}$
	6	0.00	0.36 ± 0.24^{a}	0.79 ± 0.27^{a}

Values in a same column followed by different letters (a. b. c) indicate significant differences ($p<0.05$) during storage periods. Values in a same row followed by different letters (x. y. z.) indicate significant differences in the parameter with respect to the plant treatment

The RGB (red, green, and blue) changes measured by the color sensor connected to the Arduino microcontroller are given in Table 3. The decrease in sensory meat score in fish meat and the decrease in RGB values obtained by Arduino showed parallelism with each other. On the 4th, 5th, and 6th days of storage, the atomized thyme hydrosol group, which gave better color value than the control and lavender groups, had some differences according to sensor perceptions. The atomized thyme hydrosol group was more effective on these storage days according to the sensorial analyses, but thyme and lavender achieved similar color quality on the last storage day according to sensor values. This showed that machine sensory perception can make more sensitive measurements than human sensory perception.

Table 3. Changes in the color values of trout given by Arduino during chilling storage

Days	**Meat Color (RGB) of Control x̄±Sd**	**Meat Color (RGB) of Lavender x̄±Sd**	**Meat Color (RGB) of Thyme x̄±Sd**
0	16 ± 0.27^{ax} / 24 ± 0.68^{ax} / 26 ± 0.21^{ax}	16 ± 0.27^{ax} / 24 ± 0.68^{ax} / 26 ± 0.21^{ax}	16 ± 0.27^{ax}/ 24 ± 0.68^{ax} / 26 ± 0.21^{ax}
1	12 ± 0.81^{bz} / 18 ± 0.61^{bz}/ 23 ± 0.40^{by}	13 ± 0.81^{by} / 21 ± 0.17^{by} / 25 ± 0.63^{bxy}	14 ± 0.31^{bx} / 23 ± 0.48^{bx} / 26 ± 0.65^{ax}
2	11 ± 0.25^{cy} / 16 ± 0.61^{cy} / 20 ± 0.41^{cy}	12 ± 0.51^{cx} / 18 ± 0.61^{cx} / 23 ± 0.48^{cx}	12 ± 0.48^{cx} / 18 ± 0.37^{cx} / 23 ± 0.24^{bx}
3	10 ± 0.12^{dz} / 15 ± 0.67^{dy} / 19 ± 0.43^{dz}	11 ± 0.45^{dy} / 17 ± 0.57^{dx} / 21 ± 0.25^{dy}	12 ± 0.26^{dx} / 17 ± 0.37^{dx} / 22 ± 0.42^{cx}
4	9 ± 0.40^{ez} / 14 ± 0.23^{ez} / 19 ± 0.72^{ex}	10 ± 0.50^{ey} / 15 ± 0.36^{ey} / 19 ± 0.35^{ex}	11 ± 0.20^{ex} / 16 ± 0.63^{ex} / 19 ± 0.72^{dx}
5	8 ± 0.28^{fy}/ 13 ± 0.54^{fz} / 19 ± 0.41^{ex}	9 ± 0.51^{fx} / 15 ± 0.77^{ey} / 19 ± 0.61^{ex}	9 ± 0.21^{fx} / 16 ± 0.71^{ex} / 19 ± 0.23^{dx}
6	8 ± 0.32^{fx} / 12 ± 0.34^{gx} / 18 ± 0.23^{fx}	8 ± 0.32^{gx} / 12 ± 0.34^{fx} / 18 ± 0.23^{fx}	$8\pm0.32g^{ax}$ / 12 ± 0.34^{fx} / 18 ± 0.23^{ex}

Values (for red, green and blue separately) in a same column followed by different letters (a. b. c. d. e. f. g) indicate significant differences ($p<0.05$) during storage periods. Values in the same line followed by different letters (x.y.z.) show significant differences with other groups according to the color name of the same parameter

The microbiological changes of the tested fish during storage are given in Table 4. In terms of TVC data, all groups tested during storage performed much better than the control group. From the 2nd storage day to the end of storage, the thyme and lavender groups received significantly ($p<0.05$) lower TVC than the control group. According to our previous study [17] trout that was stored on ice, the TVC value was found to be 10.78 log cfu/g^{-1} on the 18th day of storage. In the present study, the highest TVC value was 9.03 log cfu/g^{-1} in the control group on the 6th day of storage. Although the control and lavender groups exceeded 7 log cfu/g^{-1}, which is the undesired level for TVC after the 5th day, the thyme group did not reach this level.

Table 4. Changes in the total viable count of trout meat during storage

Days	Control x̄±Sd	Lavender x̄±Sd	Thyme x̄±Sd
0	2.13±0.09^{aX}	2.13±0.09^{aX}	2.13±0.09^{aX}
1	3.87±0.08^{bZ}	2.77±0.08^{bY}	2.09±0.10^{aX}
2	4.25±0.03^{cZ}	2.88±0.09^{bY}	2.12±0.08^{aX}
3	6.16±0.07^{dZ}	3.21±0.06^{cY}	2.33±0.10^{bX}
4	6.89±0.04^{eZ}	5.28±0.09^{dY}	3.98±0.05^{cX}
5	9.16±0.08^{fZ}	7.02±0.04^{eY}	5.75±0.04^{dX}
6	9.03±0.10^{fZ}	7.42±0.02^{fY}	6.82±0.04^{eX}

Different letters (a. b. c. d. e. f.) in the same column and different letters (X. Y. Z) in the same row show significant differences ($p < 0.05$)

4 Conclusion

Previous studies showed that thyme and lavender oils are natural antimicrobial and antioxidant agents for food preservation. But the hydrosols from them are generally unused waste material after the oil extraction process. In this study, it was discovered that adding atomized hydrosols to fish meat reduced the number of bacteria significantly ($p < 0.05$) while having no negative effects on the senses. The application of hydrosol drops made a significant contribution to the treatment groups having lower levels of TVB-N, TBARS and PV. The results of the study showed that the use of hydrosols remaining after oil extraction from plants as spray material can be used in industry due to their cheap and effective results.

Conflict of interest

The authors declare that there is no conflict of interest.

Similarity rate (iThenticate): %15

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