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ORIGINAL ARTICLE

The effects of nanoemulsions based on citrus essential oils on the formation of biogenic amines in trout fillets stored at 4 ± 2°C

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Abstract

The influences of nanoemulsions based on citrus peel (orange, lemon, mandarin, and grapefruit) essential oils on the biogenic amine (BA) formation in rainbow trout (*Oncorhynchus mykiss*) fillets stored 4 ± 2°C were investigated. The results demonstrated that main BAs in the trout fillets were putrescine, cadaverine, spermidine, spermine, serotonin, tyramine, dopamine, and agmatine. It was determined that nanoemulsion application had generally suppressive effect on the formation of BAs during storage. It was also found that especially orange and lemon nanoemulsion groups were effective on histamine formation. Histamine values ranged from 0.00 to 4.08 in all groups during storage and the levels were below the limit values of FDA and EU throughout the storage period for all treatment groups. Similarly, Quality Index (QI) values of BAs were found to be below legal limits in all groups. Consequently, it was found that nanoemulsions prepared from essential oils kept the BA formation at a lower level during storage.

1 | INTRODUCTION

Biogenic amines (BAs) are low molecular weight organic bases with aliphatic, aromatic and heterocyclic structures that form in foods through the microbial decarboxylation of specific amino acids or transamination of aldehydes and ketones through amino acid transaminase (Bulushi, Poole, Deeth, & Dykes, 2009; Visciano, Schirone, Tofalo, & Suzzi, 2012). They are formed by the removal of the alpha-carboxyl group in the amino acid decarboxylation (Özoğul, 2004; Shalaby, 1996). The most important BAs such as histamine, tyramine, tryptamine, putrescine, and cadaverine are comprised of amino acids (FAA) histidine, tyrosine, tryptophane, ornithine, and lysine, respectively. Spermidine and spermine comprise of putrescine. FAA can naturally exist in foods, or come free through proteolysis (Visciano et al., 2012). Many amine compounds form by the enzymatic decarboxylation of amino acids present in fish meat. Substrate necessary for decarboxylase enzyme are free amino acids. For this reason, during the spoiling and decomposition process of the fish, amino acids are released through bacterial activity, amino acid decarboxylation activity and proteolysis activity, and thus resulting in the production of BAs (Özoğul, 2004). BAs are toxic, and cause sickness in humans and animals. Histamine and tyramine are the most important BAs in fish and fish products (EFSA, 2011). Presence of other amines like putrescine, cadaverine or spermidine, phenylethylamine, agmatine, and spermine can contribute to histamine toxicity. In addition, putrescine and cadaverine can react with nitrites to form toxic or carcinogen compounds like nitrosamines (Ruiz-Capillas & Jiménez-Colmenero, 2010). BAs and their metabolites are also important signal molecules that regulate peripheral nervous system and central nervous system (CNS) nerve cell functions in vertebrates and invertebrates (Farooqui & Farooqui, 2010). However, the level of BAs can have negative effects on humans thus they have to be monitored in food since consumption of foods with high toxic BA content can cause food poisoning. BA are not only important for their toxic effects, but also important indicators of the level of spoilage and freshness in foods (Halasz, Barath, Simon-Sarkadi, & Holzapfel, 1994). BAs can also show the hygiene of a process (EFSA, 2011). They are used to determine the quality levels in food industry in general and seafood in particular. Thus, they are a quality parameter frequently used to determine the quality level of rainbow trout (*Oncorhynchus mykiss*), a species widely cultivated in

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aquaculture and heavily consumed around the world (Erikson, Shabani, Beli, Muji, & Rexhepi, 2018; Křížek, Matějková, Vácha, & Dadáková, 2012; López et al., 2017; Matějková, Křížek, Vácha, & Dadáková, 2013).

Rainbow trout is one of the most important aquacultured fish species in around the world. The demand for rainbow trout has been significantly increasing over time. It was determined that the global aquaculture production of *O. mykiss* is around 814,000 tons in 2017 (FAO, 2018). For Turkey, this number is 103,192 tons (TUIK, 2019). For a species with such an economic importance, protecting against spoilage, preserving freshness, and long shelf life are quite essential.

New processing techniques and preservatives are being developed to preserve food products. One of the new techniques that stand out in recent years in food processing is the application of nanotechnological methods on food. Nanotechnology application on food has a much shorter history compared to other applications. By using nanotechnology in food, certain compounds can be designed and controlled at the molecular level. New products can be developed by adding nanostructures that carry different color, flavor, and nutritional characteristics, and by changing the sensory and mechanical characteristics of food. Nanocomposite packaging developed for product preservation and nanosensors to detect pathogens leading to improving shelf life can be new advances in food safety (Dag, 2014). An important variety of nanotechnology applications in food is nanoemulsions, which are transparent or semitransparent emulsions that contain nano-scale droplets. Droplets in the nanoemulsion have a vital role on microorganisms (Grassi, Coceani, & Magarotto, 2000). These droplets selectively bind to the bacterial cell wall or the viral surface that makes the lipid layer of the organism unstable (Gutierrez et al., 2008) and as a result destroy the prokaryotic cells (pathogens) without affecting the eukaryotic cells of the tissue (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). This nonspecific mechanism of action of nanoemulsions ensure that no resistant strains develop. Many studies show that nanoemulsions have a bactericidal effect on clinical pathogens like *Haemophilus influenzae*, *Bacillus cereus*, *Streptococcus pneumoniae*, and *Vibrio cholerae* (Fellows et al., 2001; Hamouda et al., 1999), alongside pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa* (Al-Adham, Khali, Al-Hmoud, Kierans, & Collier, 2000), and *Listeria monocytogenes* (Ferreira et al., 2010) that might be present in food products.

Seafood can spoil very quickly due to the effects of pathogenic bacteria and the structure of their muscles. The spoilage is caused initially by the enzymes in muscles, then by microbial enzymes. Even though this spoilage can be controlled with synthetic preservatives (Mahmoud et al., 2006), these are not preferred by consumers due to their negative effects on health (Tassou, Drosinos, & Nychas, 1995) and their flavor. For this reason, natural preservatives are preferred to keep the food fresh and bring it to consumers in a healthy manner. These days, one natural preservative recommended often is essential oils extracted from plants. It is known that the application of many essential oils, such as thyme, rosemary, or laurel have a positive effect on the preservation of seafood. However, despite their widespread presence around the world, the number of studies on the effect of citrus peels on seafood, especially BA levels is very limited (Amorati, Foti, & Valgimigli, 2013). In addition, scientific opinion of EFSA Panel on Biological Hazards states that for the quantitative risk assessment of BA and more information and data are needed on BAs including toxicity, concentration, and consumption of food (EFSA, 2011). Therefore, the effects of nanoemulsions based on orange, grapefruit, mandarin, and lemon essential oils on the formation of BAs in trout fillets were investigated.

2 | MATERIALS AND METHODS

2.1 | Preparation of nanoemulsions

Citrus peel essential oils used in this study (orange, lemon, mandarin, and grapefruit) were obtained from a local company in Adana (BIOMESI Bio-agrotechnology R&D, Adana, Turkey). Nanoemulsions containing citrus essential oils were prepared using a small modification of the method described by Joe et al. (2012). This method was originally developed by Hamouda et al. (1999). In the study, to create an oil phase in the oil nanoemulsion in water; 4% essential oil, 3% ethanol, and 3% surfactant-Tween 80, (GRAS = considered safe-harmless in general) were used. For the oil phase of 1,000 ml nanoemulsion, 40 ml of essential oil, 30 ml surfactant, and 30 ml ethanol were added to a beaker, mixed, and kept in the oven set to 86°C for 1 hr. The mix was removed from the oven and cooled down to room temperature, and then 900 ml of sterilized pure water was added to the cooled mixture to make up 90% of the total emulsion. The mixture was then placed in an ice-filled beaker and homogenized for 15 min in an ultrasonic homogenisator (Optic Ivymen System CY-500, Barcelona, Spain) for 15 min at 72 amplitude. The droplet size measurement of the nanoemulsions prepared was analyzed by METU Central Laboratory (Middle East Technical University, Ankara, Turkey) using Zeta Potential and Mobility Measurement device. Droplet size of nanoemulsions prepared using orange, grapefruit, mandarin, and lemon essential oils are 94.66, 81.05, 79.14, and 47.40 nm, respectively.

2.2 | Preparation of samples

An economically valuable and widely cultivated rainbow trout (*O. mykiss*) was used for this study. Trout were purchased from a fish farm in Mersin. After harvesting, the fish filled containers were placed in ice and brought to Çukurova University, Faculty of Seafood Processing Technology Laboratory within 2–3 hr. The samples were quickly filleted and separated into six groups: the control, tween 80, orange, mandarin, lemon, and grapefruit. In groups other than the control group, the nanoemulsion application was done using the immersing method. Fish fillets were immersed into nanoemulsions prepared using different oils and kept for 3 min. Nanoemulsion applied fillets were stored in the refrigerator (4 ± 2°C) on styrofoam plates covered with plastic wrap that was perforated to allow air

TABLE 1 Biogenic amine changes in trout fillets treated with/without nanoemulsions during cold storage (mg/100 g)

Days		Cadaverine	Putrescine	Spermine	Spermidine	Tryptamine	Tyramine	Histamine	Agmatine	Seratonin	Dopamine
0	Control	12.82 ± 0.63^{c}	10.49 ± 0.28^{a}	15.33 ± 0.47^{d}	15.50 ± 0.74^{b}	4.18 ± 0.27^{a}	9.13 ± 0.82^{d}	1.41 ± 0.01^{cd}	25.19 ± 1.09^{c}	7.47 ± 0.48^{b}	0.69 ± 0.06^{e}
	Tween80	17.16 ± 1.00^{a}	9.14 ± 0.52^{b}	19.00 ± 0.13^{b}	15.68 ± 1.20^{b}	3.69 ± 0.33^{b}	18.30 ± 0.63^{b}	1.81 ± 0.32^{ab}	22.16 ± 0.60^{c}	6.92 ± 0.25^{c}	68.45 ± 3.01^{a}
	Orange	8.66 ± 0.64^{d}	9.72 ± 0.94^{ab}	10.92 ± 0.87^{f}	6.35 ± 0.64^{d}	1.59 ± 0.21^{d}	6.25 ± 0.35^{e}	1.19 ± 0.09^{d}	43.86 ± 3.47^{a}	3.81 ± 0.26^{e}	17.42 ± 0.52^{d}
	Lemon	7.42 ± 0.53^{d}	5.48 ± 0.47^{c}	16.61 ± 0.32^{c}	14.02 ± 0.70^{bc}	2.49 ± 0.45^{c}	5.36 ± 0.29^{e}	1.98 ± 0.11^{a}	33.56 ± 2.40^{b}	5.66 ± 0.29^{d}	35.08 ± 1.92^{c}
	Mandarin	15.41 ± 1.38^{b}	3.01 ± 0.085^{d}	12.65 ± 0.75^{e}	12.41 ± 0.16^{c}	0.00 ± 0.00^{e}	19.48 ± 0.48^{a}	0.98 ± 0.02^{e}	13.35 ± 1.12^{d}	3.14 ± 0.16^{f}	15.67 ± 0.27^{d}
	Grapefruit	13.65 ± 0.62^{c}	8.87 ± 0.50^{b}	26.70 ± 0.82^{a}	32.43 ± 1.63^{a}	0.29 ± 0.02^{e}	13.57 ± 0.20^{c}	1.59 ± 0.27^{bc}	23.58 ± 0.33^{c}	8.17 ± 0.14^{a}	43.40 ± 2.49^{b}
2	Control	23.49 ± 0.51^{b}	16.54 ± 1.10^{a}	26.37 ± 0.96^{c}	32.90 ± 0.58^{c}	5.72 ± 0.34^{a}	0.45 ± 0.02^{d}	2.34 ± 0.12^{c}	22.84 ± 0.66^{b}	3.65 ± 0.06^{e}	54.59 ± 1.80^{b}
	Tween80	27.39 ± 0.93^{a}	10.52 ± 0.25^{d}	26.91 ± 0.50^{c}	44.48 ± 2.11^{a}	0.30 ± 0.00^{c}	25.86 ± 0.80^{a}	3.08 ± 0.48^{b}	4.58 ± 0.37^{f}	11.81 ± 0.45^{a}	13.45 ± 0.13^{d}
	Orange	21.72 ± 0.87^{c}	8.92 ± 0.75^{e}	15.48 ± 0.17^{e}	19.52 ± 0.82^{e}	0.39 ± 0.01^{c}	1.89 ± 0.03^{c}	4.08 ± 0.08^{a}	12.71 ± 0.77^{d}	3.81 ± 0.13^{e}	12.20 ± 1.07^{d}
	Lemon	0.00 ± 0.00^{d}	14.14 ± 0.75^{b}	23.65 ± 0.48^{d}	22.85 ± 0.38^{d}	0.00 ± 0.00^{d}	10.05 ± 0.50^{b}	3.27 ± 0.32^{b}	25.68 ± 0.33^{a}	5.30 ± 0.20^{d}	71.36 ± 2.64^{a}
	Mandarin	0.00 ± 0.00^{d}	11.16 ± 0.15^{cd}	32.46 ± 0.25^{a}	36.53 ± 0.16^{b}	1.90 ± 0.09^{b}	10.31 ± 0.15^{b}	3.01 ± 0.56^{b}	10.99 ± 0.73^{e}	8.18 ± 0.12^{c}	5.80 ± 0.23^{e}
	Grapefruit	0.00 ± 0.00^{d}	11.98 ± 0.72^{c}	31.41 ± 0.32^{b}	34.77 ± 0.99^{b}	0.51 ± 0.02^{c}	9.95 ± 0.20^{b}	1.57 ± 0.06^{d}	19.07 ± 0.29^{c}	9.15 ± 0.29^{b}	39.65 ± 1.66^{c}
4	Control	15.10 ± 0.97d	6.61 ± 0.23c	20.32 ± 0.61c	20.10 ± 0.07b	0.00 ± 0.00b	11.94 ± 0.05c	0.86 ± 0.06d	7.90 ± 0.45d	4.20 ± 0.19d	18.89 ± 0.81c
	Tween80	24.08 ± 0.94c	10.09 ± 0.82a	22.30 ± 0.44b	16.89 ± 0.31c	0.00 ± 0.00b	5.69 ± 0.03d	0.96 ± 0.04 cd	3.87 ± 0.24e	6.03 ± 0.05b	11.27 ± 0.80d
	Orange	29.95 ± 0.63b	5.67 ± 0.23d	23.59 ± 0.60a	18.96 ± 1.08b	0.00 ± 0.00b	0.22 ± 0.02e	1.07 ± 0.01c	9.03 ± 0.27c	3.38 ± 0.08e	5.94 ± 0.25e
	Lemon	30.91 ± 0.48b	2.95 ± 0.08f	14.14 ± 0.54e	11.38 ± 0.52d	0.52 ± 0.03a	10.85 ± 0.18c	1.80 ± 0.08a	25.37 ± 0.90a	2.29 ± 0.01f	65.31 ± 1.52a
	Mandarin	40.80 ± 0.16a	4.43 ± 0.39e	17.84 ± 0.76d	17.25 ± 0.65c	0.00 ± 0.00b	18.71 ± 0.38b	1.42 ± 0.06b	17.72 ± 0.64b	5.02 ± 0.15c	64.36 ± 1.12a
	Grapefruit	9.91 ± 0.16e	7.49 ± 0.19b	21.77 ± 0.49b	26.80 ± 1.54a	0.00 ± 0.00b	21.96 ± 1.41a	1.38 ± 0.10b	18.12 ± 0.79b	6.94 ± 0.10a	34.44 ± 0.04b
6	Control	17.10 ± 0.11bc	4.79 ± 0.55d	15.37 ± 0.67f	13.02 ± 0.43d	0.47 ± 0.03c	14.36 ± 0.29d	1.27 ± 0.05b	28.37 ± 0.21a	7.93 ± 0.22b	31.45 ± 1.26a
	Tween80	27.89 ± 0.92a	6.17 ± 0.14c	19.09 ± 0.52d	18.27 ± 1.16c	1.94 ± 0.09a	12.68 ± 0.34e	0.83 ± 0.03c	5.46 ± 0.47e	7.51 ± 0.22c	12.49 ± 0.54c
	Orange	20.08 ± 0.68b	7.58 ± 0.14b	16.99 ± 0.74e	12.74 ± 0.55d	0.00 ± 0.00d	7.49 ± 0.09f	1.25 ± 0.01b	18.96 ± 0.40b	3.40 ± 0.05e	9.98 ± 0.17d
	Lemon	12.86 ± 0.18d	10.20 ± 0.02a	26.76 ± 1.30a	26.14 ± 0.40a	0.00 ± 0.00d	31.59 ± 0.22b	2.43 ± 0.09a	17.08 ± 0.58c	9.42 ± 0.11a	12.67 ± 0.58c
	Mandarin	16.42 ± 0.89c	9.49 ± 0.69a	21.31 ± 0.39c	18.94 ± 0.23c	1.53 ± 0.05b	16.01 ± 0.04c	0.73 ± 0.07c	7.26 ± 0.32d	3.62 ± 0.08e	26.88 ± 1.18b
	Grapefruit	28.01 ± 1.40a	10.21 ± 1.05a	25.13 ± 0.71b	22.26 ± 1.42b	0.00 ± 0.00d	38.10 ± 0.65a	1.33 ± 0.07b	5.38 ± 0.50e	4.66 ± 0.18d	7.28 ± 0.67e
8	Control	29.63 ± 1.69c	8.16 ± 0.42c	24.47 ± 0.32c	13.39 ± 0.32c	0.00 ± 0.00c	50.90 ± 1.62b	0.17 ± 0.01e	14.67 ± 0.76b	5.68 ± 0.13b	130.78 ± 2.74b
	Tween80	28.74 ± 0.28c	7.11 ± 0.35d	21.41 ± 0.21d	14.61 ± 0.33c	0.70 ± 0.00b	55.92 ± 1.63a	1.01 ± 0.02b	15.15 ± 0.53b	3.31 ± 0.12d	5.68 ± 0.28f
	Orange	24.04 ± 1.03d	6.01 ± 0.61e	16.62 ± 0.51e	10.16 ± 1.02d	0.00 ± 0.00c	11.95 ± 0.03d	0.37 ± 0.05d	4.76 ± 0.98d	3.43 ± 0.00d	12.30 ± 0.20e
	Lemon	42.19 ± 0.70a	9.77 ± 0.70b	33.47 ± 1.97a	20.41 ± 0.45b	0.00 ± 0.00c	56.03 ± 1.07a	0.92 ± 0.07c	16.57 ± 0.54a	3.77 ± 0.19c	144.58 ± 4.84a
	Mandarin	41.90 ± 1.08a	11.54 ± 0.40a	26.42 ± 0.29b	23.95 ± 0.41a	0.00 ± 0.00c	43.61 ± 0.60c	1.10 ± 0.06a	11.93 ± 0.45c	7.56 ± 0.01a	54.24 ± 0.88c
	Grapefruit	39.70 ± 1.27b	12.10 ± 0.46a	27.54 ± 0.25b	24.70 ± 0.26a	1.05 ± 0.05a	2.71 ± 0.10e	1.11 ± 0.03a	16.99 ± 1.15a	3.50 ± 0.04d	46.25 ± 0.59d
10	Control	35.44 ± 0.31b	7.43 ± 0.44b	25.32 ± 0.56b	18.16 ± 0.59a	0.00 ± 0.00a	12.12 ± 0.13c	1.80 ± 0.11b	16.87 ± 1.00c	3.34 ± 0.10c	54.03 ± 0.98b
	Tween80	38.77 ± 1.22a	9.25 ± 0.40a	26.63 ± 0.19a	18.85 ± 0.58a	0.00 ± 0.00a	7.35 ± 0.79d	0.44 ± 0.01d	22.48 ± 1.29a	4.19 ± 0.08a	105.07 ± 2.91a
	Orange	24.11 ± 1.00e	4.39 ± 0.17d	15.60 ± 0.22 cd	6.97 ± 0.04d	0.00 ± 0.00a	13.84 ± 0.06c	0.18 ± 0.01e	18.79 ± 0.09b	1.99 ± 0.04e	16.86 ± 0.93c

(Continues)

TABLE 1 (Continued)

Days		Cadaverine	Putrescine	Spermine	Spermidine	Tryptamine	Tyramine	Histamine	Agmatine	Seratonin	Dopamine
	Lemon	33.33 ± 0.24c	4.70 ± 0.09d	13.60 ± 0.41e	8.86 ± 0.59c	0.00 ± 0.00a	12.26 ± 0.08c	1.64 ± 0.05b	18.91 ± 0.25b	3.75 ± 0.05b	6.67 ± 0.42d
	Mandarin	10.04 ± 0.06f	5.56 ± 0.18c	16.08 ± 0.22c	11.60 ± 0.12b	0.00 ± 0.00a	27.39 ± 0.08a	1.24 ± 0.03c	8.88 ± 0.11d	4.35 ± 0.20a	19.16 ± 0.72c
	Grapefruit	31.57 ± 0.27d	3.56 ± 0.24e	15.38 ± 0.45d	7.67 ± 0.40d	0.00 ± 0.00a	22.27 ± 0.22b	3.27 ± 0.20a	5.48 ± 0.46e	2.79 ± 0.10d	7.20 ± 0.34d
12	Control	14.96 ± 0.06d	9.72 ± 0.68a	16.40 ± 0.44a	15.02 ± 0.98a	0.29 ± 0.02a	69.47 ± 0.86a	2.87 ± 0.16b	20.27 ± 0.34a	4.71 ± 0.03a	9.87 ± 0.80d
	Tween80	30.59 ± 0.51c	5.75 ± 0.09b	9.18 ± 0.28e	9.16 ± 0.40b	0.00 ± 0.00b	40.31 ± 0.15b	3.34 ± 0.06a	1.65 ± 0.15f	2.66 ± 0.02d	18.00 ± 0.65c
	Orange	31.66 ± 0.62c	4.63 ± 0.15d	12.40 ± 0.55c	7.26 ± 0.51c	0.00 ± 0.00b	29.47 ± 0.82c	1.65 ± 0.04c	4.09 ± 0.10e	1.94 ± 0.09f	9.99 ± 0.64d
	Lemon	38.80 ± 0.47b	3.97 ± 0.07e	10.86 ± 0.63d	7.37 ± 0.15c	0.00 ± 0.00b	24.90 ± 0.29d	3.35 ± 0.16a	8.48 ± 0.48d	2.86 ± 0.04c	3.36 ± 0.32e
	Mandarin	38.45 ± 1.03b	4.85 ± 0.06 cd	14.45 ± 0.40b	9.72 ± 0.15b	0.00 ± 0.00b	21.12 ± 0.39e	0.00 ± 0.00d	15.23 ± 0.27b	3.83 ± 0.05b	133.04 ± 1.93a
	Grapefruit	44.30 ± 2.07a	5.31 ± 0.21bc	9.44 ± 0.21e	7.16 ± 0.43c	0.00 ± 0.00b	13.93 ± 0.07f	3.40 ± 0.05a	12.07 ± 0.81c	2.14 ± 0.03e	124.98 ± 3.70b
14	Control	34.45 ± 0.87c	9.24 ± 0.21b	12.71 ± 0.24e	12.63 ± 0.28b	0.00 ± 0.00a	8.72 ± 0.10f	2.56 ± 0.14a	4.47 ± 0.15e	2.58 ± 0.02d	15.50 ± 0.14c
	Tween80	38.88 ± 2.60b	11.30 ± 0.61a	26.72 ± 0.52a	22.63 ± 3.63a	0.00 ± 0.00a	18.8 ± 0.02d	2.37 ± 0.09b	6.39 ± 0.17d	6.31 ± 0.04a	6.47 ± 0.44d
	Orange	40.50 ± 2.68b	7.73 ± 0.34c	14.71 ± 0.36c	11.36 ± 0.48bc	0.00 ± 0.00a	16.16 ± 0.38e	1.59 ± 0.04d	18.06 ± 0.27b	2.57 ± 0.04d	13.67 ± 1.02c
	Lemon	26.51 ± 0.35d	4.94 ± 0.44e	10.07 ± 0.79f	8.74 ± 0.28c	0.00 ± 0.00a	26.13 ± 0.18a	1.23 ± 0.04e	16.81 ± 0.85c	2.96 ± 0.10c	55.14 ± 2.81b
	Mandarin	33.27 ± 0.47c	6.32 ± 0.11d	13.66 ± 0.44d	8.85 ± 0.66c	0.00 ± 0.00a	21.97 ± 0.45c	2.07 ± 0.05c	27.06 ± 0.91a	3.22 ± 0.04b	56.95 ± 1.69b
	Grapefruit	51.64 ± 1.78a	7.69 ± 0.39c	21.19 ± 0.26b	11.63 ± 0.53bc	0.00 ± 0.00a	22.80 ± 0.25b	2.09 ± 0.04c	16.01 ± 0.73c	2.97 ± 0.04c	64.07 ± 3.74a
16	Control	54.63 ± 1.54a	36.81 ± 0.36a	20.69 ± 0.33a	11.71 ± 0.61a	0.00 ± 0.00a	1.38 ± 0.03e	1.79 ± 0.01a	2.71 ± 0.06b	1.01 ± 0.04e	18.44 ± 0.82b
	Tween80	30.09 ± 1.97e	11.42 ± 0.83c	12.97 ± 0.18b	7.96 ± 0.51b	0.00 ± 0.00a	0.12 ± 0.01f	0.51 ± 0.07b	0.31 ± 0.01d	1.34 ± 0.03c	11.56 ± 0.52e
	Orange	42.06 ± 1.22b	13.08 ± 0.78b	9.67 ± 0.41c	5.51 ± 0.29c	0.00 ± 0.00a	9.11 ± 0.22a	0.00 ± 0.00d	0.28 ± 0.01d	1.23 ± 0.02d	14.84 ± 0.76d
	Lemon	42.55 ± 1.42b	5.67 ± 0.59d	13.70 ± 0.56b	8.86 ± 1.01b	0.00 ± 0.00a	4.59 ± 0.11c	0.00 ± 0.00d	0.28 ± 0.02d	4.00 ± 0.11a	27.78 ± 0.99a
	Mandarin	38.97 ± 0.69c	5.54 ± 0.41d	10.11 ± 0.74c	5.52 ± 0.33c	0.00 ± 0.00a	4.89 ± 0.02b	0.00 ± 0.00d	1.99 ± 0.06c	2.39 ± 0.04b	19.65 ± 0.89b
	Grapefruit	36.04 ± 0.75d	4.64 ± 0.23d	8.16 ± 0.74d	5.71 ± 0.15c	0.00 ± 0.00a	4.25 ± 0.12d	0.23 ± 0.02c	3.25 ± 0.25a	1.32 ± 0.00 cd	16.37 ± 0.17c

Note: Means sharing the same letter in the same column (a-e) are not significantly different ($P > 0.05$).
Abbreviations: Std, standard deviation; X, mean.

circulation, with two fillets per plate. For each day of analysis, three random plates (a total of six fillets) were chosen. BA analysis of these randomly selected samples were analyzed on Days 0, 2, 4, 6, 8, 10, 12, 14, and 16 of storage.

2.3 | Biogenic amine analysis

BAs were analyzed using a quick HPLC method (Özogul, Taylor, Quantick, & Özogul, 2002). Samples (2 ml) was taken from the extracted solution for benzoyl chloride derivatization. For that 1 ml 2 M sodium hydroxide and 40 µl benzoil chloride were added to it, and it was mixed using Vortex mixer for 30 s. The mixture was kept in room temperature (24°C) for 20 min. Benzolation process was stopped with the addition of 2 ml saturated sodium hydroxide, and then the solution was extracted twice using 2 ml diethyl ether. After mixing, the top organic phase was transferred to a clean tube and evaporated in nitrogen. The residue in the tube was dissolved in 1 ml acetonitrile, transferred to HPCL tubes and analyzed.

For BA analysis, a Shimadzu Prominence HPLC device (Shimadzu, Kyoto, Japan) with a SPD-M20A diode array detector, two channel gradient pump (Shimadzu LC-10AT), autosampler (SIL 20AC), column oven (CTO-20AC), valve unit (FCV-11AL) and communication bus module (CBM-20A). For BA analysis, a reverse phase Spherisorb 5 Si C18 pH-St, 250X4.6 mm column (Fenomenex, Macclesfield, Cheshire, UK) was used.

2.4 | Quality index

The quality index (QI) was determined by using formulas described by Mietz and Karmas (1977).

The formulae used was as follows:

Quality index (QI) = (histamine + putrescine + cadaverine)/(1 + spermidine + spermine).

2.5 | Statistical analyses

The data were reported as the mean and standard deviation in triplicates. A one-way analysis of variance (ANOVA) was run using the SPSS version 22 software (SPSS, Chicago, IL) and the Duncan's multiple range test comparisons at p value of <.05 were run to determine significant differences.

3 | RESULTS AND DISCUSSION

BA changes in trout fillets treated with/without nanoemulsion during cold storage are given in Table 1. The results demonstrated that the main BAs in the trout fillets during storage were putrescine, cadaverine, spermidine, spermine, serotonin, tyramine, dopamine, and agmatine. Many other studies reported similar results (Kulawik, Dordevic, Gambuś, Szczurowska, & Zając, 2018; Ozogul, Durmus, Kuley Boga, Uçar, & Ozogul, 2018; Wang et al., 2019).

3.1 | Cadaverin and putrescine

BAs like cadaverine and putrescine are very important to determine the freshness of food, especially seafood products. Putrescine and cadaverine production increases constantly in fish once the bacterial decomposition starts. For this reason, putrescine and cadaverine are important potential indicators for seafood quality (Fernández-Salguero & Mackie, 1987; Özogul, Polat, & Özogul, 2004). At the same time, it was reported that putrescine and cadaverine increase histamine toxicity (Lehane & Olley, 2000; Staruszkiewicz et al., 2004). Under normal circumstances, the human body possesses detoxification systems to take care of putresin and cadaverin. However, it is worth noting an additional toxicological risk associated with BAs (putrescine and cadaverine), which are involved in other kinds of food poisoning, such as the formation of nitrosamines, that are believed to be cancer-causing compounds (EFSA, 2011; Ruiz-Capillas & Jiménez-Colmenero, 2010). In this study, especially lemon, mandarin, and grapefruit citrus essential oils treatment groups showed a suppressive effect on cadaverine and putrescine compared to control group.

In this study, the highest and the lowest initial putrescine levels were found as 10.49 and 3.01 mg/100 g in the control and the mandarin group, respectively. Fluctuations were observed in the putrescine levels over the storage period. On the sixth day of storage, no statistical difference was observed (p > .05) among lemon, mandarin, and grapefruit groups, while statistically significant differences were observed among the other groups. The highest putrescine level was observed in the control group on day 16 of storage with 36.81 mg/100 g. Among the nanoemulsion treatment groups, while the highest putrescine level was found in the lemon group on Day 2 of storage with 14.14 mg/100 g, the lowest level was also observed in the lemon group with 2.95 mg/100 g on Day 4. Rezaei et al. (2007) investigated the change of BA in the storage of trout fillet in ice. They reported that the value of putrescine at the beginning of storage was 0.42 µg/g and at the end of storage it was 8.97 µg/g. The value of putrescine increased similarly in the present study.

Cadaverine levels in the control, tween 80, orange, lemon, mandarin, and grapefruit groups at the beginning of the storage were determined as 12.82, 17.16, 8.66, 7.42, 15.41, and 13.65 mg/100 g, respectively. At the end of the storage period, these levels were found as 54.63, 30.09, 42.06, 42.55, 38.97, and 36.04 mg/100 g, respectively. Baixas-Nogueras, Bover-Cid, Veciana-Nogués, and Vidal-Carou (2002) reported that cadaverine usually increased later than putrescine, but the levels at the end of the storage period are generally higher. In this study, cadaverine formation was found in all groups during storage. The highest cadaverine level among treatment groups was found in the grapefruit group on Day 14 of storage, with 51.64 mg/100 g. It was reported that the highest cadaverine level can be 10 mg/100 g as a sign of freshness in salmonids (Yamanaka,

Shiomi, & Kikuchi, 1989). In this study, the cadaverine levels were found to be higher than this legal limit. López et al. (2017) investigated the effects of nisin on BA production in vacuum packed trout fillets. And reported that the amount of cadaverine increased during storage, similar to the present study. BA formation in fish meat is dependent on fish species, microbial flora, and various parameters that affect bacterial growth during storage like temperature, packaging and antimicrobial agents (Halasz et al., 1994; Mackie, Pirie, Ritchie, & Yamanaka, 1997). These factors can lead to differences in the BA formation in fish meat.

3.2 | Spermidine and spermine

Spermidine and spermine are BAs that are naturally present in food. But spermidine and spermine have associated with food allergies (Kalač, 2014). Therefore it is important to monitor these BAs in foods. Veciana-Nogués, Mariné-Font, and Vidal-Carou (1997) reported that these BAs were unrelated to bacterial decomposition. Mackie et al. (1997) reported that the concentration of these BAs in fresh muscle was under 1 mg/100 g. However, Özogul et al. (2002) determined that this depends on the fish species, the free amino acids present in the tissue, and the conditions of exposure to decomposition bacteria. In this study, the lowest spermidine and spermine levels at the beginning of the storage were found to be 6.35 and 10.92 mg/100 g in the orange group, while the highest values were in the grapefruit group with 32.43 and 26.70 mg/100 g. During the storage period, statistical differences and fluctuations were observed among the groups. Among the nanoemulsion treatment groups, the highest spermidine level was found in the mandarin group on the second day of storage with 36.53 mg/100 g, while the highest spermine level was found in the same treatment group on the same day with 32.42 mg/100 g. Chytiri, Paleologos, Savvaidis, and Kontominas (2004) investigated the changes in BA during trout fillets of trout fillets. The amounts of spermine and spermidine in trout fillets were 2.52 and 4.93 mg/kg at the beginning of storage and 5.74 and 14.10 mg/kg at the end of storage, respectively. In the present study, an increase in spermine and spermidine values was not observed. This is considered to be the result of nanoemulsion effects.

3.3 | Tryptamine and tyramine

Tryptamine is formed by the decarboxylation of tryptophane. Even though the highest tryptamine level was found in the control group with 5.72 mg/100 g, on some days of the storage period, no tryptamine formation was observed for the other treatments. This may be related to the bacterial load in trout during chill storage. According to Hu, Huang, Li, and Yang (2012), the growth of mesophilic and psychrophilic bacteria positively associated with the production of amines such as putrescine, cadaverine, histamine and tyramine during chill storage. Similar results were reported by Özogul, Kuley, and Kenar (2011). Kim, Mah, and Hwang (2009) reported that tryptamine levels in fish ranged from ND (not detected) to 89.4 mg/kg. Tyramine is a BA that is produced by the microbial decarboxylization of tyrosine. Despite the different chemical structure of tyramine, its formation process is known to be similar to putrescine and especially cadaverine (Křížek, Dadáková, Vácha, Pelikánová, & Matějková, 2018). The highest recommended tyramine level in food is between 100 and 800 mg/kg (ten Brink, Damink, Joosten, & In't Veld, 1990). In this study, it was determined that the tyramine levels in fish muscles ranged from 0.12 to 55.92 mg/100 g. Over the storage period, statistically significant differences were observed between the control group and nanoemulsion treatment groups. The highest tyramine levels were found in the Tween80 and the control groups on the eighth day of storage. It was found that nanoemulsion treatment slowed tyramine formation compared to the control group. Ozogul et al. (2018) reported that emulsions prepared from commercial oils can be used to prevent the BA formation in sea bass fillets. Matějková et al. (2013) reported that the high-pressure application of Tryptamine and Tyramine values in vacuum packed trout varies during storage. Moreover, they reported that these values could not be determined on certain days of storage. These findings are similar to the present study.

3.4 | Histamine

Histamine is produced by the decarboxylation of the histidine by some microorganisms. Histamine in fish and other seafood products can cause scombroid poisoning. For this reason, the importance of estimating the histamine concentration in fish products is related to its effect on human health and food quality (Özogul et al., 2004). Bartholomew, Berry, Rodhouse, Gilbert, and Murray (1987) determined a limit dose for histamine. For each 100 g of fish, these limits were determined as follows: below 5 mg for safe to consume, 5–20 mg for possibly toxic, 20–100 mg for most probably toxic, and over 100 mg for toxic and dangerous to consume. European Union (EU) determined the legal histamine limit value as 10 mg, while Food and Drug Administration (FDA) determined this value as 5 mg (FDA, 1996). At the beginning of the storage, histamine levels for the control, tween 80, orange, lemon, mandarin, and grapefruit groups were found as 1.41, 1.81, 1.19, 1.98, 0.98, and 1.59 mg/100 g, respectively. During the entire storage period, in all groups, it was determined that the histamine amount did not exceed the limit value determined by FDA. Among the nanoemulsion treatment groups, the highest histamine amount was found in the orange group on the second day of storage with 4.08 mg/100 g. On the last day of storage, no histamine was detected in the orange, mandarin, and lemon treatment groups. Formation of histamine and other BAs depend on the time spent and temperature levels during storage, processing, and distribution (Chang, Kung, Chen, Lin, & Tsai, 2008). Rezaei et al. (2007) and Chytiri et al. (2004) reported that the amount of histamine could not be detected at the beginning of storage in trout, but increased histamine amounts toward the end of storage. In the present study, the amount of histamine, especially trout fillets treated

TABLE 2 Quality index (QI) of trout fillets treated with/without nanoemulsions during cold storage

Days	Control	Tween80	Orange	Lemon	Mandarin	Grapefruit
0	0.78 ± 0.04^{b}	0.79 ± 0.03^{b}	1.07 ± 0.04^{a}	0.47 ± 0.02^{c}	0.72 ± 0.06^{b}	0.40 ± 0.02^{d}
2	0.70 ± 0.02^{b}	0.57 ± 0.02^{c}	0.96 ± 0.03^{a}	0.37 ± 0.01^{d}	0.20 ± 0.01^{e}	0.20 ± 0.01^{e}
4	0.54 ± 0.03^{d}	0.87 ± 0.03^{c}	0.84 ± 0.01^{c}	1.35 ± 0.04^{a}	1.29 ± 0.04^{b}	0.38 ± 0.02^{e}
6	0.79 ± 0.02^{b}	0.91 ± 0.02^{ab}	0.94 ± 0.02^{a}	0.47 ± 0.01^{d}	0.65 ± 0.03^{c}	0.83 ± 0.16^{ab}
8	0.98 ± 0.04^{b}	1.00 ± 0.01^{b}	1.10 ± 0.11^{a}	0.96 ± 0.05^{b}	1.06 ± 0.02^{ab}	0.99 ± 0.03^{b}
10	1.00 ± 0.03^{d}	1.04 ± 0.03^{d}	1.22 ± 0.05^{c}	1.69 ± 0.08^{a}	0.59 ± 0.01^{e}	1.60 ± 0.08^{b}
12	0.85 ± 0.06^{e}	2.05 ± 0.08^{c}	1.84 ± 0.11^{d}	2.40 ± 0.08^{b}	1.72 ± 0.03^{d}	3.01 ± 0.22^{a}
14	1.76 ± 0.04^{a}	1.04 ± 0.02^{c}	1.84 ± 0.07^{a}	1.65 ± 0.09^{b}	1.77 ± 0.01^{a}	1.82 ± 0.07^{a}
16	2.79 ± 0.11^{b}	1.92 ± 0.03^{c}	3.41 ± 0.14^{a}	2.05 ± 0.08^{c}	2.68 ± 0.10^{b}	2.75 ± 0.12^{b}

Quality index (QI) = (histamine + putrescine + cadaverine)/(1 + spermidine + spermine).
Different lowercase letters (a–e) in the same line indicate significant differences ($p < .05$) among groups.

with nanoemulsion, decreased toward the end of storage. This showed that the application of nanoemulsions had a positive inhibitory effect on the concentration of histamine. However, low amount of histamine and other BAs in the trout used for the study shows that the fish is high quality. High histamine levels in food, and presence of other BAs like putrescine and cadaverine, which can enhance the toxicity of histamine, can cause food poisoning due to consumption of fish with high histamine content (Ozogul et al., 2018). Because, putrescine, cadaverine, spermine, spermidine in fish tissue can potentiate the toxic effect of histamine by inhibiting intestinal histamine metabolizing enzymes (Hungerford & Arefyev, 1992).

3.5 | Agmatine, serotonin, and dopamine

During storage, significant differences were observed in agmatine, serotonin, and dopamine levels for all groups, and fluctuations were found during storage period (Table 1). Serotonin and dopamine are a derivative of the amino acid tyrosine. In the neural system, dopamine roles as a neurotransmitter, a chemical released by neurons to transmit signals to other neurons (Ozogul et al., 2018). It was determined that the serotonin concentration in fish muscles decreased with nanoemulsion treatment at the beginning of the storage compared to the control group. It was found that the serotonin amount varied from 1.01 to 11.81 mg/100 g during the storage period. The highest level was found in the Tween 80 treatment group on the second day of storage. Dopamine levels in fish muscle varied between 0.69 and 144.58 mg/100 g. On the eighth day of storage, the highest dopamine amount was found in the lemon group followed by the control group. On the last day of storage, the highest dopamine amount among treatment groups was found in the mandarin group with 19.65 mg/100 g while the lowest was in the tween 80 group with 11.56 mg/100 g. Overall, it was found that the orange group was more effective on dopamine compared to the other groups.

The highest agmatine levels were found at the beginning of the storage in all groups. Throughout the storage period, there were fluctuations and a decrease in agmatine levels. At the end of the storage period, the highest agmatine level was found in the grapefruit (3.25 mg/100 g) and the control groups (2.71 mg/100 g) while the lowest agmatine level was in the orange (0.28 mg/100 g) and lemon (0.28 mg/100 g) treatment groups. Veciana-Nogues, Albala-Hurtado, Marine-Font, and Vidal-Carou (1996) reported that agmatine concentration increased at the earlier stage of decomposition but decreased afterward. Agmatine was recommended as a freshness indicator for squid and various types of calamari kept on ice (Prester, 2011). Özogul and Özogul (2006) found that agmatine levels in vacuum-packed sardines increased over the storage period, reaching 2.32 mg/100 g at the end of the storage period (15 days).

3.6 | Quality index

It was reported that QI should be used to determine for food quality and human health concern in fish and fish products. QI changes in trout fillets treated with/without nanoemulsions are shown in (Table 2). QI values for the control, tween80, orange, lemon, mandarin, and grapefruit were found to be in the range of 0.54–2.79, 0.57–2.05, 0.84–3.41, 0.37–2.40, 0.20–2.68, and 0.20–3.01. Fluctuations were observed in QI values in all groups over the storage period. Although the highest QI levels were found in the orange and the control groups on the last day of storage, the lowest QI levels among nanoemulsion treatment groups was observed in the mandarin and grapefruit groups. Mieltz and Karmas (1977) reported that the acceptable QI level for fish is 10. In this study, throughout the storage period, QI values were found to be below this acceptable limit value. Ozogul et al. (2018) studied the effects of nanoemulsions prepared from commercial oils on the BA formation in vacuum packed sea bass fillets stored at $2 \pm 2°C$ and reported that on the last day (Day 18) of storage period the control, sunflower seed oil, soybean oil, corn oil, and olive oil emulsion groups exceeded the QI limit value, while canola group did not exceed the limit value.

4 | CONCLUSION

BA levels showed an increase during storage; remarkable was the accumulation of cadaverine, putrescine, and dopamin in all the treatment groups. Compared to the control group, some nanoemulsion treatment was found to prevent BA formation. It was determined that especially lemon and orange treatment groups were effective on putrescine and histamine formation. However, for the other BA groups, the treatment groups were insufficient compared to the control group. For this reason, to ensure consumer health, the use of nanoemulsions prepared from citrus essential oils would be an option to prevent toxic compound like BAs in seafood.

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CONFLICT OF INTEREST

The author declare that they have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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