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Fish spoilage bacterial growth and their biogenic amine accumulation: Inhibitory effects of olive by-products

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ABSTRACT

The antimicrobial effects of olive by-products (olive leaf extract, olive cake, and black water) on foodborne pathogens and fish spoilage bacteria isolated from anchovy, mackerel, and sardine were investigated. Total polyphenol contents in olive by-products were determined by the Folin–Ciocalteu procedure and their chemical composition was also evaluated by gas chromatography-mass spectrometry. The minimum inhibitory concentrations of olive by-product were performed using the broth microdilution method. Their impact on bacterial growth and biogenic amine production were also monitored in anchovy infusion decarboxylase broth. The total phenol content of olive cake and black water were 14.9 and 20.9 mg gallic acid/g extract, respectively. The major compounds were ethyl oleate (52.3%) and squalene (22.8%) in olive cake and palmitic acid (12.2%), phenanthrene (11.9%), and linoleic acid (11.4%) in olive leaf, while black water consisted of 51.1% squalene and 17.5% oleic acid ethyl ester. The minimum inhibitory concentration of olive leaf ranged from 0.78 to 25 mg/mL. Bacterial strains were more sensitive to olive leaf than other olive by-products. Bacterial load in anchovy infusion decarboxylase broth did not always correlate well with biogenic amine production. The effect of olive leaf, olive cake, and black water on biogenic amine accumulation varied depending on specific bacterial strains and biogenic amine. Olive cake and olive leaf generally had a stronger effect on reducing histamine accumulation by bacteria. Therefore, the results showed the potential effect of olive by-products in preventing or reducing the accumulation of histamine, which may beneficially affect human health.

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Introduction

One-fourth of the world's food supplies and 30% of landed fish are lost through microbial activity alone.[1] Fish live in a microbe-rich environment and are vulnerable to invasion by pathogenic or opportunistic microorganisms.[2] Microbial growth and metabolism is a major cause of fish spoilage, which includes production of biogenic amines, such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes, and ketones, with unpleasant and unacceptable off-flavors.[1,3,4] Biogenic amines can be useful in estimating the freshness or degree of spoilage of fish because these compounds are found at very low levels in fresh fish, and their formation is associated with bacterial spoilage.[5] Emborg et al.[6] found that *Photobacterium phosphoreum* dominated the spoilage microflora of modified atmosphere-packed salmon and produced less than 20 mg/kg histamine prior to sensory spoilage. *Proteus mirabilis* and *Enterobacter cloacae* which were dominantly found in spoiled sardine, were reported as strong amine producers.[7] Takahashi et al.[8] identified *Morganella*

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morganii, Proteus vulgaris, Photobacterium damselae, and *Raoultella planticola* as histamine forming bacteria from different type of fish. *Alcaligenes, Flavobacterium, Acinetobacter, Shewanella*, and *Pseudomonas* were the predominant amine-forming bacteria during the ice storage of fish and shrimp.[9]

Biogenic amines are biologically active nitrogenous compounds of low molecular weight, mainly formed by the decarboxylation of amino acids.[10] Biogenic amines are important due to the risk of food intoxication and to serve as chemical indicators of fish spoilage.[11] Scombroid fish poisoning results from eating fish in the Scombroidae family that have been spoiled. However, non-scombroid fishes such as mahi-mahi, blue fish, amberjack, herring, sardine, and anchovy have also been implicated in histamine fish poisoning.[12]

Different bacteria capable of decarboxylating amino acids have been isolated from fish muscle.[13] A mesophilic bacterial count of log 6–7 colony formic unit (cfu) g^{-1} has been found to be associated with about 5 mg histamine 100 g^{-1} fish, the U.S. Food and Drug Administration (FDA) maximum allowable histamine level.[14]

Biogenic amine formation in food has been prevented primarily by limiting microbial growth through chilling and freezing, and/or the use of hydrostatic pressures, irradiation, or controlled atmosphere packaging. The control of water activity and/or NaCl concentration may influence the microbiota composition and lead to differences in biogenic amine content.[15] Once formed, histamine is difficult to destroy as it even survives retorting.[16] The control of biogenic amine formation has mainly focused on controlling the growth of biogenic amines forming bacteria.[17] Histamine is heat stable[18] and is not detectable organoleptically even by trained panellists.[19]

Olive mill and olive processing residues are attractive sources of natural antimicrobials and antioxidants. The Mediterranean area has 97% of the world's total olive production producing 95% of the world' s olive oil.[20] This industry generates large amounts and different varieties of wastes. These wastes retain many potentially interesting compounds.[21] Olive oil extraction produces two major waste streams, namely olive pomace (olive cake [OC] or "prina") and vegetation water (or black water [BW]).[22] Olive mill waste contains large amounts of aromatic compounds, which are responsible for its phytotoxic and antimicrobial effects.[23] Olive leaves (OLs) have been used as a remedy against various diseases. OL have been reported to have antioxidant capacity, antimicrobial activity, anti-HIV properties, a vasodilator effect, and a hypoglycaemic effect.[24,25] The major physiologically active compounds of OL are hydroxytyrosol, tyrosol, caffeic acid, *p*-coumaric acid, vanillic acid, vanillin, oleuropein, luteolin, diosmetin, rutin, verbascoside, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside.[26] In the past few years the demand for OL for use in foodstuffs, food additives, and functional foods has increased.[25] Recently, research has focused on the antimicrobial and antioxidant properties of olive by-products. Phenolic compounds as well as olive mill waste extracts were evaluated *in vitro* for their antimicrobial activity against Gram-positive (*Streptococcus pyogenes* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). The results suggest that specific fractions of olive mill waste augmented with natural phenolic ingredients might be used as a source of bioactive compounds to control pathogenic bacteria.[27] Pathogenic bacteria have been considered as the primary causes of human foodborne diseases. Control of foodborne pathogens is particularly important in reducing risk of foodborne diseases for food safety. Therefore, the aim of the study was to assess chemical composition of olive by-products; to investigate their effects on bacterial growth and biogenic amine production by fish spoilage bacteria isolated from anchovy and five common foodborne pathogen and one non-pathogen reference strains (*Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Yersinia enterocolitica* NCTC 11175, and *Salmonella* Paratyphi A NCTC13) and to determine the potential use of olive by-products in fish preservation.

Material and methods

Bacterial isolation from fish

Fresh anchovy, mackerel, and sardine were obtained in October 2013 from a local fish market in Adana, Turkey. They had been stored in ice for 4 h post-capture on arrival at the laboratory. The fish were immediately gutted, beheaded, and filleted, without skin removal. They were vacuum packaged and stored at 4°C until they became spoiled. When an off-odor started to develop in the fish (within about 2 weeks), the sampling was taken. Spoiled fish muscles were aseptically weighed (about 10 g) and mixed with 90 mL of Ringer solution and then mixed well using a Stomacher (IUL, Barcelona, Spain) for 3 min. Further decimal dilutions were made and then 0.1 mL of each dilution likely to be within counting range was pipetted onto the surface of plate count agar plate in triplicate and spread over the surface. They were incubated for 2 days at 30°C. Each of the individually selected bacterial colonies was streaked several times on the agar plate using a sterile loop to obtain pure colonies. Isolates were identified according to the manufacturer's instructions for the API 20E and API 20NE strip system (BioMereux, France). The inoculated strip was incubated for 16–24 h and the colour reactions were noted as either positive or negative. The result obtained were analysed using the APILAB PLUS software (BioMereux).

The four common foodborne pathogen strains purchased were *Staphylococcus aureus* (ATCC29213) and *Klebsiella pneumoniae* (ATCC700603), which were purchased from the American Type Culture Collection (Rockville, MD, USA), and *Yersinia enterocolitica* (NCTC 11175) and *Salmonella* Paratyphi A (NCTC13) which were obtained from the National Collection of Type Cultures (London, UK). Non-pathogenic reference *Escherichia coli* (ATCC25922) strain was also used. Nutrient broth (Merck 1.05443.0500, Darmstadt, Germany) was used for propagation of all bacterial cultures.

Olive oil by-products

OL, OC, and BW (olive-mill wastewater, BW) were obtained from four different olive oil manufacturer during October 2013 in Hatay, Turkey. OC were steam distilled for 4 h in a flask of a steam distillation apparatus (Electrotermal, Staffordshire UK) and filtered using Whatman filtration paper (Whatman GmbH, Dassel, Germany). Extraction of OL was done according to method of Chen et al.[28] Prior to extraction with ethanol, the OL were steam-distilled for 4 h in a flask of a steam distillation unit to remove essential oils. Then, steam-distilled leaves were dried at the room temperature until completely drying.

Gas chromatography-mass spectrometry (GC-MS) analysis of olive by-products

GC-MS analyses were done using a Perkin Elmer Clarus 500 capillary gas chromatography (Waltham, MA, USA) directly coupled to the mass spectrometer system (Perkin Elmer Clarus). An SGE non-polar fused silica capillary column (60 m × 0.25 mm, ID; BPX5 0.25 um, Perkin Elmer, Shelton, CT, USA) was used under the following conditions: oven temperature programmed from 60°C for 10 min to 250°C at 4°C min^{-1}, and the final temperature kept for 10 min; injector temperature was 220°C; helium was the carrier gas, and flow rate was 1.5 mL min^{-1}. The volume of injected sample was 1 µL of diluted oil in hexane; a splitless injection technique was used; ionization energy was 70eV in the electronic ionization (EI) mode; ion source temperature was 200°C; the scan mass range was m/z 35–425 and the interface line temperature was 250°C. The constituents of olive by-products were identified and calculated in relation to the retention time of a series of alkanes (C4-C28) used as the reference and the similarity of their mass spectra with those gathered in the NIST-MS and WILEY-MS libraries, or reported in the literature were used.

Total phenol content

Total phenol content was done using a Folin–Ciocalteau method[29] with minor modifications. Results are reported at mg gallic acid equivalent (GAE)/g of (dry weight) sample.

Minimum inhibition concentration

The determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) was done as described according to the Clinical and Laboratory Standards Institute's methods.[30] The final concentrations of the olive by-products were 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, and 0.19 mg/mL distilled water.

Culture media and bacterial extraction for biogenic amine analysis

Fish infusion broth was prepared according to method of Okuzumi et al.[31] with minor modifications. Two hundred fifty grams of anchovy flesh was homogenised with 2 volumes of water (w/v), steamed at 100°C for 1 h and filtered. The filtrate was enriched with 1% glucose and 0.5% NaCl. To allow bacteria to decarboxylate amino acids, 3 mg pyridoxal HCl was added into each infusion broth before autoclaving. Nutrient broth was used for propagation of bacterial cultures. Bacterial strains were incubated at 37°C for 24 h. The suspension was adjusted to match the 0.5 McFarland turbidity standard using phosphate buffer saline, after which 0.5 mL of these bacterial cultures (~10^6 cfu/mL) was removed and put into 9 mL of the anchovy infusion decarboxylase broth (AIDB). BW, OC, or OL (50 mg/mL) were also added (0.5 mL) into the AIDB. The control was the absence of any of the by-product materials. After that, samples were incubated at 37°C for 72 h.

For extraction of bacterial cultures, 5 mL of the AIDB containing the bacterial strains were removed and placed in separate bottles and then 2 mL trichloroacetic acid was added. They were centrifuged at 3000 × *g* for 10 min using a Hettich 32R centrifuge (Tuttlingen, Germany) and then filtered through a Whatman filter paper. After that, 4 mL of bacterial supernatant was taken for derivatization of the biogenic amines obtained from each of the bacterial strains.

Biogenic amine analysis

All biogenic amine standards were purchased from Sigma-Aldrich (Munich, Germany). The mobile phase consisted of acetonitrile and high-performance liquid chromatography (HPLC) grade water for the amine analyses. Preparation of standard amine solutions and derivatisation of biogenic amines were done according to the method of Kuley and Ozogul.[32] Biogenic amine analysis was done using the method of Özogul[33] and measured as milligrams of amines per liter of broth. The confirmation of biogenic amine production was done using a rapid HPLC method with a reversed-phase column by using a gradient elution program. The same analytic method and conditions were used for ammonia and trimethylamine (TMA) separation.

Statistical analysis

The mean value and standard deviation were calculated from the data obtained from the four samples for each treatment. Duncan's multiple comparison test was used to determine the significance of differences at $p < 0.05$. All statistics were done using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Result and discussion

Chemical compositions and total phenol content of olive by-products

Table 1 shows the chemical profile of OL, OC, and BW which included 41, 24, and 21 different compounds identified, respectively. Ethyl oleate (52.3%) and squalene (22.8%) were the major compounds in OC, while BW had 51.2% squalene and 17.5% oleic acid ethyl ester. The main natural source of squalene is shark liver oil, but it has also been reported to be present in olive oil by-product.[34,35] The main components of OL were palmitic acid (12.2%), phenanthrene (11.9%), and linoleic acid (11.4%). Keskin et al.[36] identified cyclotrisiloxane hexamethyl (37.0%), cyclotetrasiloxane octamethyl (15.2%), and cyclopentasiloxane decamethyl (14.6%) as major constituents of OL by GC-MS. Hayes et al.[37] found six major polyphenolic compounds (oleuropein, verbascoside, luteolin-7-O-glucoside, apigenin-7-O-glucoside, hydroxytyrosol, and tyrosol) in OL extract: using HPLC coupled with photo diode array detection.

In this study, the total phenol content of OC, BW, and OL were 14.9, 20.8, and 21.3 mg GA/g extract, respectively. Luo[38] reported that total phenols of OL were between 19 to 26 mg caffeic acid

Table 1. Chemical composition of olive by-products that were identified.

OL*		BW		OC	
Compounds	%	Compounds	%	Compounds	%
Pyridine	1.20	Isopinocampheol	0.01	Isopropyl linoleate	0.03
Cyclotetradecane	1.98	Eicosadiene	1.37	Pentadecanol	0.04
3-pyridinecarboxylic acid	1.34	Octadecanal. 2-bromo-	1.50	Cetylol	0.78
2-buten-1-one[x]	5.39	Cyclohexadecane	1.80	Methyl stearate	0.64
3-buten-1-one[y]	1.68	Heptadecanol	1.45	Octadecenoic acid ethyl ester	0.83
Cis-nerolidol	2.49	Elaidinsaeure	0.27	Ethyl palmitate	3.02
9-Hydroxyfluorene	1.21	Pentanol	0.94	Eicosadiene	0.23
Palmitic acid	12.2	Octadecenoic acid ethyl ester	0.20	Oleyl alcohol	0.82
Cyclopentaphenanthrene	1.98	Oelsauere	0.08	Linoleic acid ethyl ester	2.91
1-methylphenanthrene	2.87	Oleyl alcohol	0.21	Octadecenoıc acid methyl ester	8.90
Dicarbonitrile-biphenyl	2.30	Octadecenoic acid methyl ester	4.43	Methyl oleate	0.47
Cyclopropane carboxamide	1.35	Ethyl linoleate	5.44	Trıacontanoic acid methyl ester	0.12
Pyrene	4.70	Ethyl oleate	7.52	Ethyl oleate	52.3
Linoleic acid	11.4	Squalene	51.1	Octadecenal	2.07
Stearic acid	4.17	Oelsauere	0.23	Squalene	22.8
Phenanthrene	11.9	Arachidic acid	0.40	Stenol	0.52
2-pentadecanone	4.20	Tetracosanol	3.49	Oelsauere	0.98
Heptadecane	2.97	Docosene	0.15	Octadecanal	0.99
N-heptanal	1.73	Heptadecanol	0.15	Hexadecal vinyl ether	0.92
Hexacosane	5.56	Oleic acid	0.36	Heptadecanol	0.34
Tricosane	2.47	Hexadecenoic acid	0.33	Trıcosenyl formate	0.29
1-phenanthrenecarboxylic acid	1.01	Monoolein	1.06		
Emetine	1.07	Oleic acid ethyl ester	17.5		
Cyclohexadecane	1.13				
Alpha-farnesene	0.97				
Octadecane	1.86				
Caryophyllene	0.79				
Promecarp	1.29				
Ethyldimethylbenzene	0.96				
Decane	0.81				
Cyclopentane	0.55				
Acenophthylene	0.52				
Dodecane	0.45				
Dibenzofuran	0.63				
Fluorene	0.59				
Butyloctylphthalate	0.97				
Pentadecane	0.52				
2-pentene	0.41				
6-octenal	0.37				

[x]2-buten-1-one, 1-(2,6,6-trimetyl-1,3-cyclohexadien-1-yl).
[y]3-buten-1-one, 4-(2,6,6-trimetyl-1-cyclohexen-1-yl).
*OC: olive cake, BW: black water, OL: olive leaf extract.

equivalent/g dry matter for all extracts obtained using 12 different treatments (e.g., extraction temperature, ethanol concentration, solvent–solid ratio). A lower total phenol content for ethanol extracts from OL (2.7% caffeic acid) was reported by Lafka et al.[25] Total phenolic compound content of the OC extracts varied significantly for all heat treatments (25–70°C) and ranged from 2.2 to 4.4 mg GA/g.[39] Phenolic compounds of OC were mainly caffeic acid (1730 mg/100 g) and vanillic acid (1290 mg/100 g).[40]

Antimicrobial activity of olive by-products on bacteria

The identified strains from spoiled fish were *Enterobacter cloacae, Serratia liquefaciens, Proteus mirabilis, Photobacterium damseale, Pseudomonas luteola, Pantoea* spp., *Vibrio vulnificus, Stenotrophomonas maltophila, Acinetobacter lwoffii, Pasteurella* spp., and *Citrobacter* spp. MIC and MBC of olive by-products is shown in Table 2. Significant differences were observed among bacterial growth ($p < 0.05$). OC was highly effective against *A. lwoffii* and *Pantoea* spp. compared to other bacteria. BW showed bactericidal activity.[41] MIC value of BW ranged from 12.5 to 50.0 mg/mL. BW was the most effective against *Pantoea* spp., *Ser. Liquefaciens*, and *V. vulnificus*. Since total phenol content of BW was higher than OC, the sensitivity of bacteria to BW was higher compared to OC except for *A. lwoffii* and *Citrobacter* spp. The least susceptible organisms to olive by-products were *Steno maltophilia, Pasteurella* spp., and *Y. enterocolitica*.

In a recent study, Leouifoudi et al.[42] found that no correlation had been observed between antimicrobial activity and the polyphenol content of olive mill wastewater extracts and OC extracts. In our work, OL had lower MIC and MBC compared to BW, although the total phenolic content in OL and BW was almost similar with OC. This may have been due to differences in the type and concentrations of compounds present in the extracts used.[43]

Bacterial strains that were more sensitive to OL than other olive by-products were *Phot. damseale and Citrobacter* spp. with corresponding MIC value of 0.78 and 1.56 mg/mL. Among the foodborne pathogens, *E. coli, S.* Paratyphi A, *Staph. aureus, Y. enterocolitica*, and *K. pneumoniae* are the most important pathogens that lead to foodborne diseases. OL had significant effect on reducing *E. coli, S.* Paratyphi A, and *Staph. aureus* growth with MIC value of 3, 6, and 12.5 mg/mL, respectively.

Table 2. Minimum inhibition concentration (MIC) and minimum bactericidal concentrations (MBC) of olive by-products against bacteria (mg/mL).

	OC*		BW		OL	
Bacteria	MIC	MBC	MIC	MBC	MIC	MBC
Fish spoilage bacteria						
Ent. cloacae	50.0	50.0	25.0	50.0	12.5	25.0
Ser. liquefaciens	50.0	>50.0	25.0	25.0	6.25	12.5
Prot. mirabilis	50.0	>50.0	50.0	50.0	6.25	25.0
Phot. damseale	50.0	>50.0	25.0	50.0	0.78	3.12
Pseu. luteola	50.0	>50.0	50.0	>50.0	3.12	25.0
Pantoea spp.	12.5	25.0	12.5	50.0	25.0	25.0
V. vulnificus	50.0	50.0	25.0	25.0	6.25	25.0
Steno. maltophila	50.0	>50.0	50.0	>50.0	25.0	25.0
A. lwoffii	6.25	25.00	50.0	>50.0	6.25	25.0
Pasteurella spp.	50.0	50.0	50.0	>50.0	25.0	25.0
Citrobacter spp.	25.0	50.0	50.0	>50.0	1.56	3.12
Reference strains						
E. coli ATCC25922	50.0	>50.0	50.0	>50.0	3.12	25.0
S. Paratyphi A NCTC13	50.0	>50.0	50.0	>50.0	6.25	25.0
Staph. aureus ATCC29213	50.0	>50.0	50.0	>50.0	12.5	50.0
Y. enterocolitica NCTC 11175	50.0	>50.0	50.0	>50.0	25.0	50.0
K. pneumoniae ATCC700603	50.0	>50.0	25.0	50.0	25.0	50.0

*OC: olive cake, BW: black water, OL: olive leaf extract.

MBC values of OL against *Phot. damseale* and *Citrobacter* spp. were similar (3.12 mg/mL). OL showed similar effects against *Pseu. luteola* and *E. coli*, with MIC value of 3.12 mg/mL. MBC values of OC and BW against bacteria tested were generally above 25 mg/mL. *E. coli* and *K. pneumoniae* were found to be the most susceptible organism, followed by *Staph. aureus* to OL extract,[44] although Pereira et al.[45] reported that the least susceptible organism were *B. subtilis, P. aeruginosa, K. pneumoniae, Staph. aureus*, and *E. coli.*

There was no significant differences in inhibition concentration of OL against *Ser. liquefaciens, Prot. mirabilis, V. vulnificus, A. lwoffii*, and *S.* Paratyphi A. OL aqueous extracts showed good antimicrobial effect and the highest inhibition of 11.5 mm against *Salmonella typhimurium* PTCC 1639.[46] Mission and Frantoio olive fruit extract showed broad spectrum antibacterial activity against *Staph. aureus, Bacillus subtilis, E. coli*, and *Pseu. aeruginosa*; whereas individual biophenols (hydroxytyrosol, luteolin, and oleuropein) characterized in olive mill waste showed more limited activity.[47] At low concentrations OL extracts showed high antibacterial activity namely against Gram-positive (*B. cereus, B. subtilis*, and *Staph. aureus*) and Gram-negative bacteria (*Pseu. aeruginosa, E. coli*, and *K. pneumoniae*), which suggested that it was a potential effect antibacterial, particularly as a source of phenolic compounds.[45] Sudjana et al.[48] found that OL was most active against *Campylobacter jejuni, Helicobacter pylori*, and *Staph. aureus*, with MICs as low as 0.31–0.78% (v/v). In the current study, MIC of OL on *Staph. aureus* was found to be 12.5 mg/mL.

Bacterial growth and biogenic amine production in anchovy infusion broth

Table 3 shows the total bacterial count in anchovy decarboxylase broth. Significant differences in bacterial counts were observed among groups ($p < 0.05$). Bacterial growth in control fish infusion broth ranged from 4.38 log cfu/mL for *K. pneumoniae* to 5.98 log cfu/mL for *Steno. maltophilia*. The lowest bacterial growth was observed for *Prot. mirabilis* (3.26 log cfu/mL) and *Pontea* spp. (3.54 log cfu/mL) in the presence of OL and OC, respectively. The antibacterial effect of olive by-products depended on bacterial strains. The presence of olive oil by-products in anchovy infusion broth significantly reduced *Phot. damseale, Ser. liquefaciens*, and *Prot. mirabilis* growth. *Pasteurella* spp. and *Citrobacter* spp. growth was also inhibited by OL, although there was no effect of OL on most of the fish spoilage bacteria as well as *E. coli* and *Y. enterocolitica*. BW also had a significant effect on

Table 3. Microbial growth in anchovy decarboxylase broth.

Bacterial strains	C	OL	OC	BW
Fish spoilage bacteria				
Ent. cloacea	5.68 ± 0.05^{a*}	5.68 ± 0.10^{a}	5.68 ± 0.06^{a}	5.82 ± 0.08^{a}
Ser. liquefaciens	5.68 ± 0.32^{a}	4.64 ± 0.44^{b}	4.70 ± 0.05^{b}	4.76 ± 0.16^{b}
Prot. mirabilis	5.68 ± 0.32^{a}	3.26 ± 0.10^{c}	4.70 ± 0.23^{b}	4.67 ± 0.05^{b}
Phot. damselae	5.68 ± 0.04^{a}	4.48 ± 0.00^{b}	5.43 ± 0.39^{a}	5.48 ± 0.07^{a}
Pseu. luteola	5.68 ± 0.05^{a}	5.68 ± 0.07^{a}	5.68 ± 0.35^{a}	5.11 ± 0.39^{a}
Pontea spp.	5.13 ± 0.44^{a}	5.13 ± 0.23^{a}	3.54 ± 0.31^{c}	4.64 ± 0.09^{b}
V. vulnificus	5.98 ± 0.05^{a}	5.20 ± 0.10^{c}	5.61 ± 0.06^{b}	5.61 ± 0.10^{b}
Steno. maltophilia	5.98 ± 0.07^{a}	5.98 ± 0.16^{a}	5.68 ± 0.05^{b}	5.98 ± 0.04^{a}
Pasteurella spp.	5.68 ± 0.09^{a}	4.60 ± 0.30^{b}	4.61 ± 0.04^{b}	4.91 ± 0.23^{b}
Citrobacter spp.	5.68 ± 0.27^{a}	4.91 ± 0.12^{b}	5.39 ± 0.03^{ab}	5.12 ± 0.12^{b}
A. lwoffii	5.68 ± 0.08^{a}	5.68 ± 0.07^{a}	5.04 ± 0.04^{b}	5.68 ± 0.10^{a}
Reference strains				
E. coli ATCC25922	5.39 ± 0.20^{a}	5.39 ± 0.07^{a}	5.05 ± 0.02^{b}	5.11 ± 0.05^{b}
S. Paratyphi A NCTC13	5.68 ± 0.32^{a}	5.01 ± 0.13^{b}	4.97 ± 0.22^{b}	4.92 ± 0.09^{b}
Staph. aureus ATCC29213	5.68 ± 0.04^{a}	4.78 ± 0.04^{b}	5.68 ± 0.06^{a}	5.68 ± 0.07^{a}
Y. enterocolitica NCTC 11175	5.68 ± 0.04^{a}	5.68 ± 0.26^{a}	4.58 ± 0.03^{c}	5.21 ± 0.03^{b}
K. pneumoniae ATCC700603	5.68 ± 0.03^{a}	4.38 ± 0.23^{b}	5.52 ± 0.03^{a}	5.68 ± 0.17^{a}

*Data are expressed as mean value of three samples.
Mean value ± standard deviation (log cfu/ mL).
C: control, OC: olive cake, BW: black water, OL: olive leaf extract.
$^{a–c}$Indicate significant differences ($p < 0.05$) between control and specific treated group in the same row.

reducing the growth of *S*. Paratyphi A. The presence of OC in fish infusion broth showed 1–1.6 log reduction in *Pontea spp*. and *Y. enterocolitica* growth. OL 0.6% (w/v) water extract showed complete destruction of *Escherichia coli* cells.[44] In fish infusion broth, *E. coli* count was significantly changed in the presence of OC and BW. Sudjana et al.[48] reported that OL did not have a broad-spectrum action, showing appreciable activity only against *H. pylori, C. jejuni*, and *Staph. aureus*.

Ammonia and biogenic amine production by bacteria isolated from spoiled fish and reference strains are shown in Table 4 and Table 5, respectively. Significant differences in ammonia and biogenic amine were observed among bacteria ($p < 0.05$). The highest ammonia production was observed for *Staph. aureus*, at 117 mg/L. Lower ammonia production (78.9 mg/L) was found with *Staph. aureus* in histidine decarboxylase broth.[49]

The effect of OL, OC, and BW on ammonia and biogenic amine accumulation varied depending on specific bacterial strains and biogenic amine. OL was the most effective oil by-product for reducing ammonia production by bacteria such as *Phot. damselae, Pontea* spp., *V. vulnificus, Steno. maltophilia*, and *Pasteurella* spp. OC also generally suppressed ammonia accumulation. However, significant increases were observed for *Ent. cloacae* and *S*. Paratyphi A, although the total viable count in fish infusion broth was statistically similar to the control.

Enterobacteriaceae growth and *Pseudomonas* counts were responsible for the formation of biogenic amines but also the other bacterial groups contributed in the formation of biogenic amines in the examined fish products.[50] In the current study, the bacteria isolated from fish were capable of producing biogenic amine mainly TMA, dopamine, serotonin, agmatine, and tyramine. The highest putrescine and cadaverine production were observed for *A. lwoffii* (65.0 mg/L) and *Ser. liquefaciens* (61.9 mg/L), respectively. *Ser. liquefaciens* produced higher putrescine (>1000 mg/L) in histidine and tyrosine enrichment broths.[51] *Enterobacteriaceae* belonging to the genera *Citrobacter, Klebsiella, Escherichia, Proteus, Salmonella*, and *Shigella* are associated with production of considerable amounts of putrescine, cadaverine, and histamine in fish and meat products or, more generally, in spoiled food.[12,15,52–54]

Higher levels of cadaverine (380–430 mg/L) production were reported with *Ser. liquefaciens* isolated from cold-smoked salmon.[55] Greif et al.[56] found that *Ent. cloacae* inoculated with 5×10^3 cfu/cm^3 in glucose, tryptone and yeast autolysate (GTY) broth only produced putrescine from ornithine, and the highest putrescine concentrations (3210 mg/L) were measured at the NaCl concentration of 0.5% and pH 6 (5480 mg/L). In the present study, putrescine production by *Ent. cloacae* was as low as 2.24 mg/L. Putrescine production by *Ser. liquefaciens* and *Prot. mirabilis*, did not change significantly in the presence of OL and OC, although significant inhibition of putrescine production was observed for *A. lwoffii, V. vulnificus, Pontea* spp., and *E. coli* ($p < 0.05$). A thirty-seven-fold decreases in putrescine accumulation by *A. lwoffii* occurred in the presence of olive by-products in fish infusion broth. Although fish infusion broth with OL included lower bacterial loads, putrescine production by *Ent. cloacae, Pasteurella* spp., *Citrobacter* spp., and *K. pneumoniae* were higher with addition of OL compared to control. This might be due to the possible presence of biogenic amines in olive by-products. Garcia and Garcia[57] determined the content of biogenic amines in different commercial preparations of table olives. Concentrations of amines in packed table olives were less than 60 mg of total biogenic amines per kilogram of fruit. The highest concentrations of putrescine (50 mg/kg) were found in untreated natural black olives.

The FDA[58] identified some potential species of fish with histamine poisoning potential such as amberjack, anchovy, bluefish, bonito, oilfish, herring, jack, jobfish, mackerel, mahi-mahi, marlin, sardine, saury, shad, trevally, and tuna. The fish spoilage bacteria produced histamine in the range of 0.99 mg/L with *Ser. liquefaciens* to 9.66 mg/L with *A. lwoffii* in anchovy infusion broth. *M. morganii* isolated from albacore produced the highest level of histamine, 5.25 mg/L, at 25°C in the stationary phase in tuna fish infusion broth[59] Gokdogan et al.[49] reported that histamine production was highest for *E. coli*, whereas *Staph. aureus* did not produce histamine in histidine decarboxylase broth. Among the Gram-negative bacteria, *Klebsiella* spp. was characterized as the lowest histamine producer, followed by *Aeromonas* spp. and *Pseudomonas* spp. However, in the present study, histamine production by

Table 4. Biogenic amine production by bacteria isolated from spoiled anchovy in the presence of olive oil by-products (mg/L).

	AMN	PUT	CAD	SPD	TRPT	2-PHN	SPN	HİS	SER	TYR	TMA	DOP	AGM	
Ent. cloacae	20.5 ± 1.13^{*abc}	2.24 ± 0.21^{c}	0.77 ± 0.02^{b}	1.34 ± 0.07^{b}	0.12 ± 0.01^{c}	0.19 ± 0.02^{b}	1.10 ± 0.10^{d}	2.30 ± 0.22^{c}	3.55 ± 0.30^{d}	7.52 ± 0.71^{c}	11.2 ± 1.15^{c}	2.07 ± 0.19^{b}	8.78 ± 0.07^{b}	C
	24.7 ± 1.39^{b}	7.74 ± 0.52^{a}	0.48 ± 0.09^{c}	2.32 ± 0.19^{a}	0.79 ± 0.01^{b}	0.29 ± 0.01^{b}	1.41 ± 0.03^{c}	5.65 ± 0.11^{a}	7.41 ± 0.42^{c}	11.67 ± 0.14^{b}	23.6 ± 1.51^{b}	0.63 ± 0.02^{b}	9.67 ± 0.02^{a}	OL
	39.0 ± 0.05^{a}	3.70 ± 0.04^{b}	0.77 ± 0.02^{b}	2.07 ± 0.12^{a}	0.76 ± 0.07^{b}	0.26 ± 0.01^{b}	2.09 ± 0.08^{b}	2.61 ± 0.01^{c}	6.00 ± 0.19^{b}	13.0 ± 0.07^{a}	61.1 ± 2.48^{a}	13.5 ± 0.76^{a}	9.17 ± 0.18^{bc}	OC
	22.3 ± 1.59^{bc}	2.88 ± 0.04^{c}	1.03 ± 0.11^{a}	1.30 ± 0.05^{b}	1.58 ± 0.09^{a}	3.60 ± 0.21^{a}	2.39 ± 0.03^{a}	3.91 ± 0.12^{b}	13.7 ± 0.84^{a}	6.38 ± 0.29^{d}	3.10 ± 0.15^{d}	13.9 ± 1.12^{d}	8.41 ± 0.41^{c}	BW
Ser. liquefaciens	79.5 ± 3.97^{a}	19.0 ± 0.90^{a}	61.9 ± 3.57^{b}	0.44 ± 0.02^{b}	0.04 ± 0.00^{a}	1.85 ± 0.11^{c}	0.38 ± 0.02^{b}	0.99 ± 0.95^{a}	12.1 ± 0.54^{b}	5.33 ± 0.30^{b}	0.50 ± 0.01^{c}	26.4 ± 2.04^{ab}	7.52 ± 0.80^{b}	C
	59.2 ± 3.06^{b}	18.1 ± 0.59^{a}	56.7 ± 0.26^{bc}	0.25 ± 0.04^{c}	0.02 ± 0.00^{a}	1.35 ± 0.01^{c}	0.38 ± 0.04^{b}	0.74 ± 0.03^{a}	8.60 ± 0.50^{c}	3.83 ± 0.05^{c}	0.60 ± 0.02^{b}	30.9 ± 0.87^{a}	8.64 ± 0.51^{ab}	OL
	39.2 ± 0.80^{d}	17.3 ± 0.79^{a}	51.5 ± 2.55^{c}	0.15 ± 0.01^{d}	0.00 ± 0.00^{a}	3.64 ± 0.37^{b}	0.58 ± 0.04^{a}	0.23 ± 0.01^{a}	7.31 ± 0.71^{c}	21.6 ± 0.76^{a}	0.10 ± 0.01^{d}	24.0 ± 1.68^{b}	9.68 ± 0.28^{a}	OC
	47.9 ± 1.13^{c}	9.80 ± 0.10^{b}	70.8 ± 3.70^{a}	0.71 ± 0.01^{a}	0.02 ± 0.02^{a}	12.9 ± 1.22^{a}	0.51 ± 0.03^{a}	0.63 ± 0.04^{a}	25.9 ± 2.01^{a}	4.92 ± 0.15^{bc}	13.2 ± 0.03^{a}	22.5 ± 2.22^{b}	4.70 ± 0.43^{c}	BW
Prot. mirabilis	59.9 ± 5.03^{a}	4.77 ± 0.44^{b}	0.59 ± 0.00^{b}	1.05 ± 0.04^{c}	0.20 ± 0.01^{c}	0.15 ± 0.01^{d}	0.99 ± 0.00^{c}	2.32 ± 0.11^{a}	12.3 ± 0.97^{a}	4.84 ± 0.31^{b}	2.99 ± 0.14^{d}	6.01 ± 0.51^{b}	3.04 ± 0.25^{b}	C
	62.0 ± 4.81^{a}	4.68 ± 0.10^{b}	0.64 ± 0.05^{b}	1.38 ± 0.03^{b}	0.88 ± 0.00^{a}	0.35 ± 0.00^{b}	1.64 ± 0.05^{b}	1.37 ± 0.10^{b}	4.58 ± 0.24^{c}	2.06 ± 0.04^{c}	93.3 ± 5.45^{a}	2.74 ± 0.38^{c}	0.53 ± 0.02^{d}	OL
	56.5 ± 0.86^{a}	4.87 ± 0.18^{b}	1.04 ± 0.06^{a}	1.79 ± 0.03^{d}	0.52 ± 0.03^{b}	0.24 ± 0.02^{c}	2.06 ± 0.04^{a}	2.31 ± 0.01^{a}	7.46 ± 0.54^{b}	9.60 ± 0.22^{a}	21.3 ± 1.18^{c}	3.36 ± 0.21^{c}	1.84 ± 0.13^{c}	OC
	32.6 ± 0.85^{b}	6.46 ± 0.22^{a}	0.58 ± 0.01^{b}	1.14 ± 0.09^{c}	0.23 ± 0.03^{c}	0.53 ± 0.03^{a}	0.36 ± 0.02^{d}	1.10 ± 0.10^{c}	8.14 ± 0.59^{b}	2.18 ± 0.01^{c}	44.5 ± 4.35^{b}	14.8 ± 0.39^{a}	3.70 ± 0.15^{a}	BW
Phot. damselae	42.2 ± 2.42^{a}	1.01 ± 0.07^{b}	1.07 ± 0.12^{b}	2.42 ± 0.12^{a}	0.44 ± 0.07^{a}	0.28 ± 0.03^{c}	2.94 ± 0.06^{b}	4.33 ± 0.35^{a}	8.89 ± 0.49^{b}	16.0 ± 0.75^{b}	72.7 ± 5.36^{b}	15.1 ± 0.90^{b}	9.12 ± 0.93^{a}	C
	21.0 ± 1.58^{c}	0.46 ± 0.04^{c}	0.35 ± 0.01^{d}	1.33 ± 0.05^{c}	0.46 ± 0.01^{a}	0.67 ± 0.06^{b}	1.70 ± 0.02^{c}	1.65 ± 0.10^{b}	6.06 ± 0.36^{c}	7.01 ± 0.07^{c}	36.8 ± 2.26^{c}	10.4 ± 0.34^{c}	5.85 ± 0.33^{b}	OL
	30.4 ± 1.80^{b}	3.75 ± 0.37^{a}	1.33 ± 0.05^{a}	1.99 ± 0.02^{b}	0.56 ± 0.07^{a}	1.18 ± 0.02^{a}	5.18 ± 0.08^{a}	4.81 ± 0.36^{a}	13.1 ± 1.11^{a}	24.6 ± 1.17^{a}	90.4 ± 3.68^{a}	31.9 ± 2.92^{a}	6.19 ± 0.73^{b}	OC
	38.0 ± 3.11^{a}	0.56 ± 0.02^{bc}	0.57 ± 0.03^{c}	2.46 ± 0.09^{a}	0.00 ± 0.00^{b}	0.13 ± 0.01^{d}	1.38 ± 0.58^{c}	1.45 ± 0.02^{b}	2.47 ± 0.05^{d}	7.41 ± 0.57^{c}	7.01 ± 0.20^{d}	8.95 ± 0.51^{c}	6.78 ± 0.44^{b}	BW
A. lwoffii	57.6 ± 3.41^{a}	64.9 ± 5.79^{a}	8.04 ± 0.81^{a}	3.48 ± 0.17^{a}	1.18 ± 0.03^{b}	2.64 ± 0.11^{a}	2.40 ± 0.24^{ab}	9.66 ± 0.36^{a}	15.57 ± 0.73^{b}	11.5 ± 0.85^{ab}	14.5 ± 0.74^{a}	25.4 ± 1.60^{c}	5.35 ± 0.19^{b}	C
	60.4 ± 4.68^{a}	1.68 ± 0.05^{b}	0.82 ± 0.05^{b}	3.08 ± 0.09^{b}	1.70 ± 0.11^{a}	2.64 ± 0.06^{a}	3.22 ± 0.31^{a}	3.79 ± 0.14^{b}	6.12 ± 0.29^{c}	12.8 ± 0.85^{a}	16.2 ± 1.40^{a}	31.5 ± 2.24^{c}	14.5 ± 0.00^{a}	OL
	24.3 ± 0.68^{c}	1.91 ± 0.11^{b}	0.64 ± 0.01^{b}	2.77 ± 0.08^{c}	0.32 ± 0.00^{c}	0.64 ± 0.01^{c}	2.30 ± 0.13^{b}	2.84 ± 0.19^{c}	26.2 ± 1.65^{a}	9.67 ± 0.02^{b}	7.78 ± 0.05^{b}	60.3 ± 3.75^{a}	15.3 ± 1.24^{a}	OC
	45.5 ± 3.22^{b}	1.72 ± 0.15^{b}	0.86 ± 0.03^{b}	3.10 ± 0.08^{b}	1.02 ± 0.16^{b}	1.59 ± 0.20^{b}	3.09 ± 0.44^{ab}	3.60 ± 0.20^{b}	5.58 ± 0.39^{c}	12.9 ± 1.10^{a}	6.90 ± 0.12^{b}	47.4 ± 1.18^{b}	14.5 ± 0.17^{b}	BW
Pseu. luteola	47.6 ± 1.22^{a}	1.69 ± 0.09^{b}	0.75 ± 0.04^{a}	2.60 ± 0.21^{b}	4.14 ± 0.28^{b}	3.15 ± 0.06^{b}	2.19 ± 0.09^{b}	4.97 ± 0.21^{b}	34.7 ± 1.04^{a}	10.4 ± 0.26^{a}	120.6 ± 6.85^{a}	41.9 ± 3.98^{a}	12.1 ± 1.07^{a}	C
	42.0 ± 3.82^{b}	2.09 ± 0.01^{a}	0.65 ± 0.01^{b}	3.52 ± 0.36^{a}	5.09 ± 0.14^{a}	4.36 ± 0.01^{a}	3.52 ± 0.49^{a}	6.31 ± 0.36^{a}	1.86 ± 0.05^{c}	10.6 ± 12.24^{a}	86.7 ± 5.82^{b}	28.1 ± 2.69^{c}	11.2 ± 0.45^{ab}	OL
	40.8 ± 0.71^{c}	1.50 ± 0.06^{c}	0.78 ± 0.03^{a}	1.74 ± 0.09^{c}	3.71 ± 0.05^{bc}	3.00 ± 0.07^{b}	2.63 ± 0.14^{b}	4.46 ± 0.56^{b}	42.5 ± 6.1^{a}	7.23 ± 0.46^{a}	97.4 ± 4.32^{b}	39.8 ± 1.05^{b}	10.4 ± 0.18^{b}	OC
	41.8 ± 0.15^{bc}	0.72 ± 0.01^{d}	0.25 ± 0.02^{c}	1.99 ± 0.04^{c}	3.32 ± 0.34^{c}	2.53 ± 0.14^{c}	1.27 ± 0.04^{c}	3.16 ± 0.09^{c}	14.6 ± 1.10^{b}	19.7 ± 0.94^{a}	0.38 ± 0.06^{c}	28.7 ± 2.62^{c}	6.77 ± 0.42^{c}	BW
Pontea spp.	33.0 ± 1.73^{b}	9.22 ± 0.50^{a}	4.92 ± 0.45^{a}	1.82 ± 0.12^{c}	0.05 ± 0.00^{c}	0.33 ± 0.01^{d}	0.99 ± 0.10^{d}	1.30 ± 0.01^{d}	13.9 ± 0.72^{b}	8.50 ± 2.84^{ab}	1.77 ± 0.07^{c}	23.5 ± 4.62^{b}	6.08 ± 0.42^{b}	C
	24.8 ± 1.98^{c}	5.77 ± 0.50^{b}	4.23 ± 0.25^{ab}	2.37 ± 0.10^{b}	0.37 ± 0.01^{b}	0.81 ± 0.03^{a}	1.96 ± 0.19^{c}	4.96 ± 0.08^{b}	3.42 ± 0.48^{d}	3.26 ± 0.11^{c}	16.5 ± 0.61^{b}	15.4 ± 0.61^{c}	1.26 ± 0.06^{c}	OL
	25.0 ± 1.81^{c}	4.45 ± ± 0.26^{c}	2.48 ± 0.12 ^{c}	1.47 ± 0.16^{c}	0.44 ± 0.02^{a}	0.69 ± 0.00^{b}	2.69 ± 0.05^{a}	2.81 ± 0.08^{c}	9.46 ± 0.28^{c}	12.4 ± 0.22^{a}	62.5 ± 6.04^{a}	47.1 ± 3.25^{a}	10.7 ± 0.97^{a}	OC
	37.0 ± 2.01^{a}	4.23 ± 0.20^{c}	3.65 ± 0.11^{b}	2.89 ± 0.28^{a}	0.43 ± 0.05^{ab}	0.57 ± 0.06^{c}	2.37 ± 0.03^{b}	5.12 ± 0.34^{a}	18.0 ± 0.83^{a}	7.19 ± 0.40^{bc}	57.1 ± 4.44^{a}	9.40 ± 0.63^{c}	5.23 ± 0.02^{b}	BW
V.vulnificus	37.6 ± 0.46^{b}	1.05 ± 0.04^{a}	0.84 ± 0.04^{a}	3.85 ± 0.22^{a}	0.36 ± 0.02^{a}	0.40 ± 0.04^{a}	2.22 ± 0.19^{b}	2.76 ± 0.24^{a}	3.56 ± 0.25^{c}	8.22 ± 0.75^{b}	9.29 ± 0.54^{c}	28.5 ± 1.84^{b}	6.02 ± 0.51^{c}	C
	23.7 ± 1.89^{c}	0.66 ± 0.05^{b}	0.40 ± 0.04^{c}	2.53 ± 0.13^{b}	0.26 ± 0.26^{a}	0.26 ± 0.01^{b}	1.27 ± 0.13^{c}	1.91 ± 0.09^{b}	3.17 ± 0.08^{c}	3.34 ± 0.24^{c}	18.9 ± 1.12^{b}	7.86 ± 0.42^{c}	0.96 ± 0.08^{d}	OL
	22.6 ± 2.19^{c}	0.62 ± 0.02^{b}	0.77 ± 0.04 ^{a}	1.77 ± 0.13^{c}	0.32 ± 0.01^{a}	0.11 ± 0.01^{c}	3.66 ± 0.26^{a}	2.95 ± 0.03^{a}	7.37 ± 0.62^{b}	14.3 ± 1.01^{a}	19.2 ± 1.00^{b}	41.0 ± 2.00^{a}	14.4 ± 0.63^{a}	OC
	49.7 ± 2.00^{a}	0.45 ± 0.01^{c}	0.57 ± 0.05^{b}	1.86 ± 0.04^{c}	0.00 ± 0.00^{a}	0.28 ± 0.00^{b}	1.21 ± 0.01^{c}	2.10 ± 0.05^{b}	17.8 ± 1.78^{a}	6.92 ± 0.57^{b}	47.3 ± 1.75^{a}	36.7 ± 3.05^{a}	9.10 ± 0.10^{b}	BW
Steno. maltophilia	38.5 ± 2.93^{b}	1.55 ± 0.11^{c}	0.43 ± 0.01^{c}	2.42 ± 0.10^{b}	0.33 ± 0.04^{c}	0.39 ± 0.03^{b}	1.17 ± 0.05^{c}	1.61 ± 0.02^{d}	11.9 ± 0.48^{a}	10.1 ± 0.03^{b}	153.8 ± 3.51^{a}	47.6 ± 1.37^{a}	10.5 ± 0.78^{b}	C
	24.5 ± 0.87^{d}	1.99 ± 0.18^{b}	1.38 ± 0.05^{a}	2.25 ± 0.21^{b}	0.40 ± 0.02^{c}	0.54 ± 0.03^{a}	1.93 ± 0.08^{c}	2.47 ± 0.19^{c}	3.86 ± 0.16^{c}	5.55 ± 0.26^{c}	9.99 ± 0.24^{d}	8.51 ± 0.37^{d}	13.9 ± 1.09^{a}	OL
	36.1 ± 2.88^{b}	1.42 ± 0.02^{c}	1.09 ± 0.05^{ab}	2.67 ± 0.25^{b}	0.64 ± 0.00^{b}	0.35 ± 0.01^{b}	4.71 ± 0.42^{a}	5.11 ± 0.29^{a}	6.24 ± 0.26^{b}	16.6 ± 0.06^{a}	50.6 ± 3.13^{b}	42.0 ± 3.55^{b}	15.6 ± 1.14^{a}	OC
	42.1 ± 3.68^{a}	2.48 ± 0.07^{a}	0.89 ± 0.26^{b}	3.73 ± 0.21^{a}	1.20 ± 0.11^{a}	0.52 ± 0.05^{a}	3.48 ± 0.38^{b}	4.45 ± 0.01^{b}	5.34 ± 0.39^{b}	15.9 ± 1.21^{a}	33.7 ± 3.45^{c}	16.6 ± 0.01^{c}	11.1 ± 0.74^{b}	BW
Pasteurella spp.	37.9 ± 1.09^{b}	0.80 ± 0.07^{b}	0.60 ± 0.01^{c}	2.46 ± 0.08^{b}	0.35 ± 0.03^{b}	0.17 ± 0.01^{c}	3.62 ± 0.35^{c}	5.99 ± 0.27^{a}	36.2 ± 1.83^{a}	20.0 ± 0.76^{b}	127.2 ± 3.12^{a}	34.5 ± 2.58^{a}	11.4 ± 0.84^{a}	C
	22.0 ± 0.90^{d}	15.8 ± 1.21^{a}	4.41 ± 0.17^{a}	3.92 ± 0.16^{b}	2.43 ± 0.17^{a}	0.23 ± 0.01^{c}	10.33 ± 1.00^{b}	6.46 ± 0.19^{a}	9.10 ± 0.29^{b}	23.8 ± 1.25^{a}	62.9 ± 1.64^{c}	32.8 ± 2.47^{a}	7.88 ± 0.04^{b}	OL
	30.1 ± 1.22^{c}	3.02 ± 1.62^{b}	1.09 ± 0.05^{b}	2.48 ± 0.61^{b}	0.62 ± 0.03^{b}	0.91 ± 0.09^{a}	3.59 ± 0.09^{c}	3.99 ± 0.02^{b}	9.15 ± 0.78^{b}	15.9 ± 0.96^{c}	15.1 ± 1.07^{d}	19.8 ± 1.40^{b}	11.8 ± 1.18^{a}	OC
	54.8 ± 4.71^{a}	0.36 ± 0.01^{b}	4.36 ± 0.22^{a}	12.12 ± 1.01^{a}	0.64 ± 0.20^{b}	0.47 ± 0.10^{b}	22.19 ± 1.62^{a}	2.12 ± 0.16^{c}	7.17 ± 0.32^{b}	6.31 ± 0.01^{d}	96.5 ± 2.60^{b}	30.6 ± 1.00^{a}	8.35 ± 0.49^{b}	BW

(*Continued*)

Table 4. (Continued).

	AMN	PUT	CAD	SPD	TRPT	2-PHN	SPN	HİS	SER	TYR	TMA	DOP	AGM	
Citrobacter spp.	23.2 ± 2.05^{b}	1.50 ± 0.16^{c}	0.81 ± 0.05^{b}	1.44 ± 0.10^{c}	0.26 ± 0.02^{c}	0.40 ± 0.04^{b}	1.83 ± 0.09^{c}	3.01 ± 0.09^{bc}	19.3 ± 0.80^{a}	11.2 ± 0.63^{b}	53.4 ± 3.48^{a}	32.5 ± 2.17^{b}	12.9 ± 0.71^{b}	C
	24.3 ± 0.41^{b}	4.45 ± 0.48^{a}	1.73 ± 0.07^{a}	1.93 ± 0.01^{b}	0.57 ± 0.08^{b}	0.41 ± 0.02^{b}	6.18 ± 0.20^{a}	7.87 ± 0.12^{a}	18.3 ± 0.83^{a}	24.2 ± 0.88^{a}	27.8 ± 1.98^{b}	35.1 ± 1.71^{b}	25.3 ± 1.47^{a}	OL
	29.4 ± 2.43^{b}	2.59 ± 0.09^{b}	0.59 ± 0.10^{c}	0.03 ± 0.00^{d}	0.10 ± 0.00^{d}	0.01 ± 0.00^{c}	3.72 ± 0.10^{b}	2.68 ± 0.25^{c}	19.2 ± 1.77^{a}	12.2 ± 0.70^{b}	11.7 ± 0.24^{c}	40.7 ± 3.38^{a}	13.9 ± 0.02^{a}	OC
	44.2 ± 3.18^{a}	0.55 ± 0.05^{d}	0.69 ± 0.04^{bc}	3.32 ± 0.04^{a}	0.73 ± 0.04^{a}	1.68 ± 0.01^{a}	1.39 ± 0.14^{d}	3.46 ± 0.33^{b}	19.0 ± 1.37^{a}	8.39 ± 0.28^{c}	6.49 ± 0.81^{c}	24.6 ± 0.99^{c}	6.75 ± 0.02^{c}	BW

Table 5. Biogenic amine production by common foodborne pathogens and non-pathogen *E. coli* strain in the presence of olive oil by-products (mg/L).

	AMN	PUT	CAD	SPD	TRPT	2-PHN	SPN	HİS	SER	TYR	TMA	DOP	AGM	Groups
E. coli	27.5 ± 1.09^{a}	1.60 ± 0.09^{a}	0.82 ± 0.06^{b}	1.01 ± 0.06^{ab}	0.00 ± 0.00^{b}	4.06 ± 0.16^{b}	1.97 ± 0.04^{a}	4.23 ± 0.43^{a}	27.1 ± 1.25^{b}	29.4 ± 2.11^{a}	99.6 ± 8.82^{a}	13.6 ± 0.62^{b}	9.26 ± 0.76^{b}	C
ATCC25922	29.6 ± 0.70^{a}	1.28 ± 0.09^{b}	1.06 ± 0.08^{a}	1.12 ± 0.03^{a}	0.23 ± 0.09^{a}	5.64 ± 0.08^{a}	1.43 ± 0.08^{b}	2.29 ± 0.01^{b}	31.4 ± 1.05^{a}	22.4 ± 1.17^{b}	19.6 ± 0.37^{c}	26.2 ± 1.18^{a}	17.7 ± 0.21^{a}	OL
	28.4 ± 1.33^{a}	0.73 ± 0.03^{c}	0.51 ± 0.05^{c}	0.55 ± 0.03^{c}	0.00 ± 0.00^{b}	3.94 ± 0.26^{b}	0.83 ± 0.03^{c}	2.28 ± 0.24^{b}	20.7 ± 2.25^{c}	21.6 ± 2.03^{bc}	35.4 ± 3.33^{b}	15.0 ± 0.50^{b}	10.5 ± 1.07^{b}	OC
	22.3 ± 1.48^{b}	1.63 ± 0.17^{a}	0.41 ± 0.04^{c}	0.89 ± 0.05^{b}	0.00 ± 0.00^{b}	2.97 ± 0.06^{c}	0.58 ± 0.02^{a}	1.53 ± 0.11^{c}	7.15 ± 0.60^{d}	17.6 ± 0.31^{c}	10.1 ± 0.53^{c}	29.4 ± 2.43^{a}	7.17 ± 0.35^{c}	BW
S. Paratyphi A	29.9 ± 0.68^{c}	1.06 ± 0.02^{b}	0.33 ± 0.17^{b}	0.73 ± 0.05^{b}	0.15 ± 0.01^{b}	3.91 ± 0.28^{a}	0.65 ± 0.07^{b}	1.16 ± 0.10^{b}	3.10 ± 0.14^{c}	10.5 ± 0.85^{c}	0.99 ± 0.01^{c}	2.28 ± 0.02^{c}	6.61 ± 0.62^{b}	C
NCTC13	43.2 ± 4.17^{a}	2.10 ± 0.05^{a}	0.60 ± 0.03^{a}	1.29 ± 0.08^{a}	0.49 ± 0.02^{a}	3.57 ± 0.33^{ab}	0.94 ± 0.05^{a}	1.44 ± 0.11^{a}	13.28 ± 0.41^{a}	10.2 ± 0.49^{c}	3.14 ± 0.29^{c}	7.56 ± 0.24^{a}	7.40 ± 0.47^{a}	OL
	41.2 ± 2.78^{ab}	1.14 ± 0.01^{b}	0.20 ± 0.01^{b}	0.42 ± 0.02^{c}	0.03 ± 0.00^{c}	2.68 ± 0.23^{c}	0.15 ± 0.01^{c}	0.31 ± 0.00^{c}	5.82 ± 0.09^{b}	12.8 ± 0.81^{b}	27.7 ± 2.52^{a}	1.49 ± 0.14^{d}	4.45 ± 0.44^{c}	OC
	34.7 ± 2.93^{bc}	0.73 ± 0.04^{c}	0.28 ± 0.00^{b}	0.37 ± 0.02^{c}	0.02 ± 0.00^{c}	2.94 ± 0.12^{bc}	0.13 ± 0.01^{c}	0.32 ± 0.02^{c}	1.40 ± 0.03^{d}	17.5 ± 0.51^{a}	7.35 ± 0.25^{b}	3.30 ± 0.15^{B}	4.11 ± 0.01^{c}	BW
Staph.aureus	117.06 ± 9.48^{a}	10.12 ± 0.66^{a}	1.06 ± 0.04^{a}	0.45 ± 0.01^{b}	0.58 ± 0.02^{b}	0.17 ± 0.02^{b}	1.01 ± 0.03^{c}	56.08 ± 5.05^{b}	3.56 ± 0.33^{c}	5.02 ± 0.03^{b}	81.6 ± 3.65^{a}	3.01 ± 0.30^{b}	1.18 ± 0.00^{c}	C
ATCC29213	32.3 ± 0.20^{c}	2.67 ± 0.04^{c}	1.03 ± 0.07^{a}	0.40 ± 0.01^{b}	0.47 ± 0.04^{c}	0.21 ± 0.03^{b}	2.09 ± 0.20^{a}	5.87 ± 0.01^{c}	5.84 ± 0.43^{b}	11.7 ± 1.13^{a}	8.05 ± 0.40^{c}	4.20 ± 0.24^{a}	7.33 ± 0.49^{a}	OL
	23.2 ± 1.31^{c}	2.52 ± 0.16^{c}	0.51 ± 0.00^{c}	0.45 ± 0.01^{b}	0.26 ± 0.01^{d}	0.00 ± 0.00^{c}	1.25 ± 0.01^{bc}	10.31 ± 0.26^{c}	15.7 ± 0.69^{a}	6.71 ± 0.54^{b}	3.21 ± 0.20^{c}	2.08 ± 0.03^{c}	4.12 ± 0.05^{b}	OC
	54.3 ± 3.39^{b}	7.53 ± 0.10^{b}	0.83 ± 0.01^{b}	1.40 ± 0.08^{a}	0.73 ± 0.02^{a}	0.40 ± 0.00^{a}	1.42 ± 0.07^{b}	82.60 ± 2.63^{a}	14.5 ± 1.09^{a}	3.25 ± 0.01^{c}	52.7 ± 4.82^{b}	3.58 ± 0.49^{b}	3.82 ± 0.20^{b}	BW
Y. enterocolitica	73.0 ± 4.46^{b}	5.05 ± 0.35^{b}	0.40 ± 0.03^{c}	0.60 ± 0.04^{b}	0.10 ± 0.00^{c}	0.83 ± 0.07^{a}	0.99 ± 0.00^{c}	4.08 ± 0.21^{b}	14.1 ± 1.11^{b}	4.16 ± 0.27^{c}	15.7 ± 1.51^{c}	20.3 ± 1.18^{b}	3.70 ± 0.22^{c}	C
NCTC 11175	88.4 ± 3.03^{a}	6.88 ± 0.50^{a}	1.95 ± 0.13^{a}	0.74 ± 0.17^{b}	0.31 ± 0.01^{b}	0.20 ± 0.00^{b}	2.36 ± 0.10^{a}	3.88 ± 0.13^{b}	4.30 ± 0.40^{c}	9.38 ± 0.16^{b}	25.3 ± 1.22^{b}	36.6 ± 2.80^{a}	7.93 ± 0.02^{a}	OL
	26.2 ± 1.13^{c}	2.82 ± 0.26^{c}	1.18 ± 0.06^{b}	0.58 ± 0.03^{b}	0.37 ± 0.03^{a}	0.25 ± 0.04^{b}	1.95 ± 0.10^{b}	2.37 ± 0.17^{c}	5.08 ± 0.37^{c}	9.64 ± 0.34^{b}	18.1 ± 0.84^{c}	24.6 ± 1.68^{b}	6.14 ± 0.35^{b}	OC
	73.6 ± 2.59^{b}	7.11 ± 0.16^{a}	1.37 ± 0.05^{b}	1.23 ± 0.05^{a}	0.34 ± 0.02^{a}	0.86 ± 0.03^{a}	1.12 ± 0.01^{c}	6.35 ± 0.01^{a}	20.1 ± 1.45^{a}	18.3 ± 0.74^{a}	29.5 ± 1.31^{a}	14.2 ± 0.38^{c}	5.56 ± 0.05^{b}	BW
K. pneumoniae	46.7 ± 8.50^{a}	1.11 ± 0.45^{c}	0.35 ± 0.17^{a}	1.17 ± 0.01^{b}	0.23 ± 0.14^{b}	7.44 ± 0.13^{a}	0.09 ± 0.01^{c}	0.33 ± 0.11^{c}	5.54 ± 0.82^{c}	20.7 ± 1.70^{a}	40.7 ± 3.51^{b}	12.9 ± 0.20^{b}	5.25 ± 2.05^{b}	C
ATCC700603	29.6 ± 1.21^{b}	5.81 ± 0.34^{a}	0.62 ± 0.05^{a}	1.49 ± 0.03^{b}	0.17 ± 0.00^{b}	0.99 ± 0.06^{d}	0.89 ± 0.07^{b}	2.13 ± 0.12^{b}	8.00 ± 0.05^{b}	11.5 ± 0.84^{c}	9.18 ± 0.36^{c}	10.9 ± 0.08^{c}	8.98 ± 0.27^{a}	OL
	23.3 ± 2.64^{b}	1.22 ± 0.03^{c}	0.43 ± 0.02^{a}	5.50 ± 0.59^{a}	2.59 ± 0.34^{a}	1.44 ± 0.03^{c}	8.28 ± 0.37^{a}	4.27 ± 0.36^{a}	5.58 ± 0.05^{c}	10.3 ± 1.00^{c}	58.2 ± 2.72^{a}	28.6 ± 0.92^{a}	5.57 ± 0.39^{b}	OC
	25.2 ± 0.92^{b}	4.14 ± 0.21^{b}	0.49 ± 0.09^{a}	1.44 ± 0.14^{b}	0.07 ± 0.00^{b}	2.53 ± 0.15^{b}	0.82 ± 0.00^{b}	4.48 ± 0.39^{a}	12.2 ± 1.13^{a}	15.4 ± 0.51^{b}	6.94 ± 0.11^{c}	5.75 ± 0.50^{d}	5.84 ± 0.34^{b}	BW

*Data are expressed as mean value of three samples.
Mean value ± standard deviation.
AMN: ammonia, PUT: putrescine, CAD: cadaverine, SPD: spermidine, TRPT: tryptamine, PHEN: 2-phenylethyl amine, SPN: spermine, HIS: histamine, SER: serotonin, TYR: tyramine, TMA: trimethylamine, DOP: dopamine, AGM: agmatine, C: control group, OL: olive leaf extract, OC: olive cake, BW: black water.
$^{a-d}$Indicate significant differences ($p < 0.05$) between control and treated group in a row.

Staph. aureus was the highest (56.1 mg/L) compared to other bacteria. OL and OC resulted in 9.5- and 5.4-fold lower histamine accumulation by *Staph. aureus* in fish infusion broth, respectively. Significant inhibition effects on histamine accumulation by *A. lwoffi* was observed in olive by-products, although similar microbial loads were found among groups except for OC, which was lower than the other groups. Significant increases in histamine production were observed with OC for *Steno. maltophilia, Pontea* spp., and *K. pneumoniae*. Apart from *K. pneumoniae*, OC significantly inhibited histamine production by foodborne pathogens. OL was also effective in reducing histamine accumulation by *V. vulnificus, A. lwoffii, Phot. damselae*, and *Prot. mirabilis*. BW increased histamine production by *Staph. aureus, Y. enterocolitica*, and *K. pneumoniae*, while significant decreases were observed in histamine production by *E. coli* and *S.* Paratyphi A in the presence of BW in fish infusion broth.

Tyramine production was the highest by *E. coli* (29.4 mg/L) and *K. pneumoniae* (20.7 mg/L) and *Pasteurella* spp. (20.0 mg/L). *Pseudomonas oryzihabitans, Chryseobacterium indologenus*, and *V. vulnificus* showed the highest tyramine accumulation in tyrosine decarboxylase broth with values of 1650, 774, and 188 mg/L, respectively.[51] In the current study, lower tyramine production was observed with *V. vulnificus* and *Pseu. luteola* in AIDB with values of 8.22 and 10.4 mg/L, respectively. All olive by-products tested significantly decreased tyramine production by *E. coli* and *K. pneumoniae*. OL significantly reduced tyramine production by *Pontea* spp., *V. vulnificus, Ser. liquefaciens, Prot. mirabilis, Phot. damselae*, and *Steno. maltophilia*. Tyramine production also decreased with *Prot. mirabilis, Ent. cloacae, Phot. damselae, Pasteurella* spp., and *Citrobacter* spp., by adding BW into fish infusion broth. However, tyramine accumulation by most of fish spoilage bacteria was significantly higher in fish infusion broth that contained OC compared with the control.

TMA levels are used to assess microbial deterioration leading to fish spoilage. *Shewanella putrifaciens, Aeromonas* spp., *psychrotolerant Enterobacteriacceae, P. phosphoreum*, and *Vibrio* spp. Can obtain energy by reducing trimethyl amine oxide (TMAO) to TMA creating the ammonia-like off-flavours.[1,2] TMA was the most accumulated amine and mainly produced by *Steno. Maltophilia* (154 mg/L) and *Pseu. Luteola* (121 mg/L) in fish infusion broth. Olive by-products had a significant impact on reducing TMA production by *Pseu. Luteola, Steno. Maltophilia*, and *Citrobacter spp*., although considerable increases were observed in TMA production with *Pontea* spp., *V. vulnificus*, and *Prot. Mirabilis* in the presence of olive by-products. TMA production by *K. pneumoniae* was suppressed with OL but increased with OC.

Conclusions

In conclusions, fish spoilage bacteria and reference strains were more sensitive to OL than other olive by-products, followed by OC. Bacterial load in fish infusion broth did not always correlate well with biogenic amine production. Histamine production by fish spoilage bacteria apart from *Pontea* spp., *Steno. maltophilia*, and *Ent. cloacae* was significantly inhibited by olive by-products (mainly BW and OL), whereas OC was generally more effective in reducing histamine accumulation by foodborne pathogens apart from *K. pneumoniae*. Significant stimulation effects of BW on histamine production by *Staph. aureus* was also found. Tyramine accumulation by most of the fish spoilage bacteria tested considerably was reduced in the presence of OL. OL followed by BW seemed to be more effective in reducing histamine and tyramine production by fish spoilage bacteria than OC. The result of the study suggested that olive by-products could be considered as food additives in the future to improve food safety. However, more research is needed to confirm these findings and to determine the exact effects of olive by-products in foods.

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