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Original article

The effectiveness of garlic extracts on biogenic amine formation by foodborne pathogens and fish spoilage bacteria

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Summary Impacts of aqueous and ethanolic extracts of garlic were investigated in suppressing bacterial growth and biogenic amine (BA) formation by selected foodborne pathogens (*Candida albicans*, *Salmonella paratyphi* A, *Escherichia coli* and *Staphylococcus aureus*) and fish spoilage bacteria (*Enterococcus faecalis*, *Photobacterium damselae* and *Pseudomonas luteola*). The spread-plate method was used to monitor bacterial growth in histidine decarboxylase broth (HDB), whereas the rapid high-performance liquid chromatographic (HPLC) method was used for BA analysis. Bacterial growth and their ammonia and BA production were monitored using HDB. The results showed that bacterial growth on HDB was in the range from 9.13, for *P. luteola*, to 9.54 log CFU (colony-forming units) mL^{-1}, for *S. aureus* and *C. albicans*. The presence of garlic extracts in HDB resulted considerably in lowering bacterial growth and BA formation ($P < 0.05$). The highest inhibitory activities of ethanolic and water garlic extracts were obtained for Gram-positive *S. aureus* with 1.4 and 1.5 logarithmic reduction on bacterial growth, followed by Gram-negative *Salmonella* Paratyphi A and *E. coli*. Application of garlic extracts, mainly ethanolic ones, showed a significant inhibitory effect on bacterial ammonia production, with 4-100-fold lower ammonia accumulation ($P < 0.05$). Bacteria produced all tested BAs, mainly dopamine, agmatine and tryptamine. The highest levels of histamine and tyramine (61.99 and 36.45 mg L^{-1}) were produced by *S. aureus*. In the presence of aqueous or ethanolic garlic extracts, putrescine production by *E. faecalis* was around 110-fold lower than that of the control group. Results revealed that both garlic extracts are potent antimicrobials that can control the growth of foodborne pathogens and their harmful BA formation.

Keywords Antimicrobials, foodborne pathogens, garlic extracts, histamine, tyramine.

Introduction

Nitrogenous substances known as 'biogenic amines' (BAs) are produced in food by amino acid transaminases transaminating aldehydes and ketones or with decarboxylation of amino acids by decarboxylase-producing microorganisms (Visciano *et al.*, 2012; Özogul & Özogul, 2019). Foods could naturally be contaminated by microbes (especially by those with the capability to decarboxylate amino acids) during their preparation and distribution. Histidine decarboxylase activity will occur when foods are contaminated by histidine-producing microbes, such as *Clostridium perfringens*, *Escherichia coli*, *Enterobacter planticola*, *Proteus mirabilis*, *Salmonella* spp., *Klebsiella pneumoniae*, *Shigella* spp., *Serratia* spp., *Citrobacter freundii*, *Raoultella* spp., *Vibrio* spp., *Acinetobacter lowffi*,

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Plesiomonas shigelloides, *Pseudomonas* spp., *Photobacterium* spp., *Morganella morganii* and *Hafnia alvei* (Altafini *et al*., 2022; Oktariani *et al*., 2022; Ramona *et al*., 2023). The nature of foods and the microorganisms therein significantly affect the amount and variety of BA accumulation (Özogul *et al*., 2021). Food products most likely to contain high concentrations of BAs include fermented foodstuffs like vegetables, beer, cheese, wine, fish and meat and their derivatives, dairy products, etc. (Linares *et al*., 2011). The techniques that determine the amount of BA in food products are crucial for quantifying their intake. These methods include liquid chromatographic (LC) techniques like high or ultra-high-performance liquid chromatographic (HPLC or UHPLC) are often preferred. Detectors such as mass spectrometric, fluorimetric and UV–vis absorption spectrometric can be combined with LC systems (Angulo *et al*., 2020; Tırıs *et al*., 2023). The most important BAs in foods concerning their amounts and toxicological properties are cadaverine, putrescine, tryptamine, 2-phenylethylamine, histamine and tyramine (Gardini *et al*., 2016). Depending on the consumer's sensitivity, these amines might have hazardous consequences including headache, vomiting, diarrhoea and palpitations (Barbieri *et al*., 2019). While the upper limit of histamine for fish products and fish is suggested to be 100 mg/kg by European legislation (Directive 91/493/EEC, 2005), it is recommended to be 50 mg/kg by the US Food and Drug Administration (FDA, 2001; Misra *et al*., 2017). BAs synthesised by bacterial decarboxylase enzymes in foods do not decrease with the application of cooking or other technological processes such as freezing or cold storage; they may even increase during storage (Ramona *et al*., 2023). Therefore, it is of the most importance to control microbial growth in foods in order to prevent the formation of BAs above acceptable limits (Gardini *et al*., 2016; Ceylan *et al*., 2017; Meral *et al*., 2019; Burgut *et al*., 2020).

Due to the detrimental consequences of synthetic preservatives on the environment and public health (Singh *et al*., 2021), the use of natural alternatives to synthetic preservatives in food are commonly preferred by consumers and manufacturers (Pisoschi *et al*., 2018). Therefore, plant extracts and their essential oils with pharmaceutical properties can be used as a reliable alternative to synthetic preservative food additives. Recent research on the food industry's use of essential oils (EOs) and their ability to suppress foodborne pathogens has focused a lot of attention on plant extracts as a possible substitute for traditional antimicrobials (Youssef *et al*., 2021; Al-Mijalli *et al*., 2022; El-Sayed *et al*., 2022).

Garlic (*Allium sativum*) is an important plant frequently used in cooking as a spice and flavouring. Moreover, it includes protein, carbohydrate and high sulphur and sulphur-based ingredients (Bocate *et al*., 2021). Among these organic sulphur-based compounds, allicin, known as diallyl thiosulfate, is an essential component of garlic's biological activity and possesses antibacterial qualities (Rakshit *et al*., 2023). According to reports, the essential oil extracted from garlic by hydro-distillation techniques possesses antibacterial and antifungal properties (Li *et al*., 2016; El-Sayed *et al*., 2017; Chen *et al*., 2018). However, there are some disadvantages to using distillation techniques to extract the garlic essential oil, as some highly reactive sulphur compounds in the garlic volatile fraction, such as allicin, are thermally unstable and can destroyed during thermal distillation (El-Sayed *et al*., 2017; Yazgan *et al*., 2022). Since these garlic organosulfur compounds are very unstable and have low bioavailability, their presence depends on the processing and extraction method in the preparation of garlic supplements. The solvent extraction procedure results in a concentration of particular compounds such as allicin, ajoene and various aliphatic sulphides, which have antibacterial properties, rather than providing a pure compound. Different solvent extracts of garlic such as distilled water, methanol and ethanol, have been found to inhibit various plant pathogens and fungi under different pH conditions (Chen *et al*., 2018; Huang *et al*., 2023). Furthermore, it has been reported that the extraction of garlic with aqueous and ethanol will provide a product rich in allicin (Bhatwalkar *et al*., 2021).

The greatest inhibitory impact of garlic ethanol extracts on the BAs production in fermented anchovy (*Engraulis japonicus*) and Korean salted (Myeolchi-jeot) has been found by Mah *et al*. (2009). There is not much research on the impact of garlic extract on the production of histamine and other BAs, despite numerous *in vivo* and *in vitro* studies on the antioxidant and antibacterial properties of garlic extract in food matrixes. Thus, the aim of this research was to investigate the impact of garlic aqueous and ethanol extract in suppressing BA formation by food-related pathogens (*Candida albicans*, *Staphylococcus aureus* ATCC29213, *Salmonella paratyphi* A NCTC13 and *Escherichia coli* ATCC25922) and fish spoilage bacteria (*Enterococcus faecalis*, *Photobacterium damselae* and *Pseudomonas luteola*). This study gains importance in the prevention of foodborne undesirable microorganisms and BAs that pose a risk to human health with natural antimicrobials.

Materials and methods

Preparation of garlic extracts

The two different extracts (aqueous and ethanolic) of garlic bulbs were prepared as described in detail by

Yazgan *et al.* (2022). Garlic was purchased at a local market in Adana, Türkiye. Garlic bulbs were peeled and weighed (*ca.* 20 g). Then, using a laboratory mixer, garlic bulbs were crushed. It was filled with 120 mL water or 120 mL of ethanol (Merck, 1.00983, Darmstadt, Germany), and the liquid was blended for 5 min to create a slurry. The slurries were placed in dark at ambient temperature (25 °C) and stored for 60 min. Then, the slurries with distilled water or ethanol were centrifuged for 20 min at 4 °C at 3000 g Furthermore, a filter paper (Whatman No.1, Maidstone, UK) was used to filter the supernatants, and the water and ethanol garlic extracts were kept in a refrigerator (4 °C) in the dark condition for about 1 day and then used for analysis.

Microorganisms

Spoilage bacteria (*Enterococcus faecalis*, *Photobacterium damselae* and *Pseudomonas luteola*) were obtained from stock cultures in the microbiology laboratory of the Seafood Processing Technology department at Cukurova University in Adana, Türkiye. Polymerase chain reaction targeted 16S rRNA gene region with 8F and 519R primers was used for detection and identification of *E. faecalis*, *P. damselae* and *P. luteola* isolated from spoiled fish (Yazgan *et al.*, 2019). *Staphylococcus aureus* ATCC29213, *Salmonella* Paratyphi A NCTC13, *Escherichia coli* ATCC25922 and *Candida albicans* ATCC10231 were obtained from the National Collection of Type Cultures (London, UK) and the American Type Culture Collection (Rockville, MD, USA). Nutrient broth (Merck 1.05443.0500) was used to cultivate all bacterial cultures.

Ammonia and BAs analysis

Ammonia and BAs production by bacteria was monitored using histidine decarboxylase broth (HDB) (Klausen & Huss, 1987). Foodborne pathogen and fish spoilage microorganism cultures were incubated for 2–3 days at 37 °C in nutrient broth. For the bacterial culture processes, 0.5 mL of bacterial cultures (~10^8 CFU mL^{-1}) standardised to 0.5 McFarland turbidity using a densitometer (DEN-1, BioSan, Warren, MI, USA) were extracted and mixed with 9 mL of the HDB. After bacterial inoculation, extracts at a dosage of 2% (v/v) were transferred to the HDB. The control group consisted of HDB without any extract. The sample tubes were incubated for 72 h at 37 °C, and then the samples were derivatised. All samples were taken in triplicate on the same day for the experiment.

Derivatisation procedures of ammonia and BAs

The amine was derivatised according to the method of Özogul *et al.* (2002). Briefly, 1 µL of 2% benzoyl chloride and 2 M sodium hydroxide were mixed with 50 µL of standard amine solution (4 mL for extracted bacterial culture). After 20 min of room-temperature storage for the reaction mixture, 2 mL of sodium chloride was added to the reaction mixture, and 4 mL of diethyl ether was used for extraction. The uppermost organic layer was then removed using a nitrogen flow, and the residue was dissolved in 500 µL of acetonitrile before being injected into HPLC. The BAs analysis was performed using the Shimadzu Prominence HPLC (Shimadzu, Kyoto, Japan) system, which includes a valve unit FCV-11AL with a communication bus module (CBM-20A), a column oven (CTO-20AC), two dual gradient pumps (Shimadzu LC-10AT), an SPD-M20A diode array detector, and an auto-sampler (SIL 20AC). The analysis of ammonia was done using the same analytical technique.

Chromatographic separation

Using a continuous gradient elution technique with HPLC-grade water (eluant B) and acetonitrile (eluant A) at a flow rate of 1.2 mL min^{-1}, chromatographic separation was achieved. The oven temperature was 30 °C. A full separation occurred in less than 20 min. The injection volume was 10 µL. A wavelength of 254 nm was used for detection. A calibration curve is established for every BA in the 0–50 mg mL^{-1} range. Correlation coefficient (r) of the peak area against amine standard concentrations for each compound was calculated after injecting five replicates of each standard solution of amine. For each benzylated amine, the correlation coefficient (r^2) on the curve was more than 0.99.

Monitoring bacterial growth in HDB

Total viable bacteria in HDB were monitored using plate count agar (PCA) (Fluka 70152; Steinheim, Switzerland). Each bacterial culture grown in HDB medium was diluted (about 10^{-10}) and 0.1 mL of the dilution (10^{-6}–10^{-10}) inoculated into Petri dishes with PCA, using the spread-plate technique. The Petri plates were incubated at 37 °C for 72 h and the results were expressed as the logarithm of total viable colony-forming units per millilitre of broth log (average ± standard deviation), log (CFU mL^{-1}). The determination of total viable counts was made in triplicate for each sample.

Statistical analysis

The analysis of variance and Duncan's multiple range tests were used to find the significance of the differences ($P < 0.05$). All statistics were conducted using

Table 1 Bacterial growth in histidine decarboxylase broth (log CFU mL^{-1})

Microorganisms	Control	Ethanolic extract	Water extract
Salmonella Paratyphi A	9.38 ± 0.15[a]*	8.11 ± 0.02[b]	8.04 ± 0.07[b]
Escherichia coli	9.42 ± 0.08[a]	8.18 ± 0.03[b]	8.04 ± 0.03[b]
Staphylococcus aureus	9.54 ± 0.02[a]	8.17 ± 0.01[b]	8.03 ± 0.08[b]
Pseudomonas luteola	9.13 ± 0.16[a]	8.22 ± 0.03[b]	8.19 ± 0.06[b]
Photobacterium damselae	9.25 ± 0.01[a]	8.11 ± 0.022[b]	7.95 ± 0.02[c]
Enterococcus feacalis	9.17 ± 0.00[a]	8.13 ± 0.03[b]	8.18 ± 0.04[b]
Candida albicans	9.54 ± 0.02[a]	8.18 ± 0.08[b]	8.01 ± 0.01[c]

*Mean (n = 3) ± Standard deviation. [a–c]Display significant differences ($P < 0.05$) between control and treated group in a column.

IBM SPSS Statistics for Windows version 28.0.1.0 (142) (IBM Corporation, Amonk, NY, USA).

Results and discussion

Bacterial growth in HDB

All tested microbes show an increase in cell density following 72 h of incubation, although the cell density was found to be lower than that of control. Table 1 shows the inhibition effects of garlic extracts on bacterial growth. Bacterial growth in HDB ranged between 9.13 and 9.54 log CFU mL^{-1} for the control group. Significant differences were observed between control and garlic extract-treated groups ($P < 0.05$). The presence of garlic extracts in HDB considerably resulted in lower bacterial and fungal growth ($P < 0.05$). Consistent with this study, in our previous study, aqueous garlic extract was found to have bactericidal and bacteriostatic effects against foodborne pathogens such as *Salmonella* Paratyphi A NCTC13 and *S. aureus* ATCC29213 with values higher than 50 mg mL^{-1} (Yazgan *et al.*, 2022). Apart from *P. damselae* and *C. albicans*, the impact of both extracts on bacterial growth was statistically similar. The highest inhibitory activities of ethanolic and water garlic extracts were obtained for Gram-positive *S. aureus* with 1.4 and 1.5 logarithmic reduction on bacterial growth, followed by Gram-negative *Salmonella* Paratyphi A and *E. coli*. Similarly, Benmeziane *et al.* (2018) reported that Gram-positive bacteria are more susceptible to the action of garlic aqueous extract and garlic essential oil than Gram-negative bacteria. Gram-negative bacteria have an outer cell membrane that is extremely permeable to big glycopeptide molecules. Gram-positive bacteria have a hydrophilic porous cell wall that allows for greater penetration of bioactivity substances due to the absence of an outer membrane and periplasm (Tavares *et al.*, 2020). These variations in the cell membrane give the cell distinct characteristics, especially in how it reacts to drugs (Mai-Prochnow *et al.*, 2016). *C. albicans* is an opportunistic fungus (yeast) which is responsible for causing skin/mucous membrane and invasive (life-threatening) infections (Spampinato & Leonardi, 2013). Fresh garlic extract showed good antifungal activity against *C. albicans* in its planktonic, adherent and sessile phases (Shuford *et al.*, 2005). Likewise, these results, in the current study the presence of garlic aqueous and ethanolic extracts in HDB resulted in 1.5 and 1.4 log lower *C. albicans* viable counts.

Wolde *et al.* (2018) found similar inhibitory effect of garlic aqueous and ethanol extracts against *S. aureus*. The presence of ethanolic or aqueous extracts in HDB resulted in about 1.4 log reduction in the growth of *S. aureus*. The lowest inhibition effect of extracts was observed for *P. luteola*, with 0.9 log reduction on bacterial growth. *S. aureus* and *E. coli* were completely inhibited by 10 and 15 mg mL^{-1} of crude extract of garlic (Abiy & Berhe, 2016). Wolde *et al.* (2018) found that *E. coli* was more susceptible than *S. aureus* to the garlic extracts. Allicin is a typical sulphur-containing ingredient present in garlic and exerts antimicrobial properties against both Gram-positive and Gram-negative bacteria (Abiy & Berhe, 2016; Nakamoto *et al.*, 2020). Ajoenes, allyl sulphides, glycosides, tannins, alkaloids, saponins and flavonoids are other phytochemicals present in garlic that act as antibacterial agents (Bhatwalkar *et al.*, 2021; Yunus & Suwondo, 2021).

Ammonia and BAs production by microorganisms

Figure S1 shows a typical HPLC chromatogram obtained for the identification and quantification of BAs. Table 2 displays ammonia and BA production by foodborne pathogens and fish spoilage bacteria. Although all foodborne pathogens have been observed to produce less than 85 mg L^{-1} of ammonia, significant differences in ammonia production have been detected between pathogenic species ($P < 0.5$). Garlic extracts significantly reduced ammonia formation by foodborne pathogen bacteria; however, garlic ethanolic extracts were more effective on *S.* Paratyphi A, *E. coli* and *C. albicans* (Table 2).

It was reported that ammonia production by single bacterial isolates was in the range of 2.04 mg L^{-1} (*Corynebacterium* spp.) and 139.47 mg L^{-1} (*Morganella morganii*) (Özogul & Özogul, 2007). Moreover, significantly higher ammonia productions in HDB ranging from 2527 to 4122 mg L^{-1} by foodborne pathogens were reported by Özogul *et al.* (2022). In the current study, *Pseudomonas luteola* was the main fish spoilage bacteria, accumulating the uppermost level of ammonia (146.51 mg L^{-1}) (Table 2). Fish spoilage bacteria formed AMN between 69 and

Table 2 Ammonia and biogenic amine production by microorganisms in the presence or absence of garlic extracts

	AMN	PUT	CAD	SPD	TRP	SPN	2-PHEN	DOP	SER	AGM	Groups
Foodborne pathogen											
Salmonella Paratyphi A	36.12 ± 2.21[a]*	12.47 ± 0.13[a]	7.92 ± 0.15[a]	0.38 ± 0.03[a]	6.11 ± 0.48[a]	0.81 ± 0.01[a]	8.27 ± 0.21[a]	699.64 ± 4.34[a]	13.31 ± 0.39[a]	494.91 ± 15.01[a]	C
	2.47 ± 0.24[c]	1.88 ± 0.16[b]	1.04 ± 0.08[b]	0.17 ± 0.09[b]	5.88 ± 0.41[a]	0.01 ± 0.00[b]	0.50 ± 0.00[b]	5.23 ± 0.24[c]	0.08 ± 0.01[c]	34.22 ± 0.18[c]	GEE
	9.52 ± 0.08[b]	2.36 ± 0.48[b]	0.81 ± 0.02[b]	0.32 ± 0.01a[b]	2.52 ± 0.16[b]	0.12 ± 0.00[c]	0.31 ± 0.00[b]	17.40 ± 0.35[b]	1.99 ± 0.06[b]	103.54 ± 2.42[b]	GWE
Escherichia coli	49.69 ± 1.60[a]	28.62 ± 1.24[a]	15.76 ± 0.46[a]	18.72 ± 0.94[a]	127.52 ± 3.63[a]	3.37 ± 0.18[a]	15.11 ± 1.13[a]	559.29 ± 38.48[a]	16.58 ± 0.62[a]	974.28 ± 31.13[a]	C
	1.88 ± 0.16[c]	5.93 ± 0.43[b]	1.13 ± 0.03[c]	0.02 ± 0.00[b]	14.11 ± 0.10[c]	0.17 ± 0.01[c]	0.20 ± 0.01[c]	16.09 ± 0.09[c]	0.07 ± 0.01[c]	36.47 ± 0.28[c]	GEE
	29.37 ± 1.03[b]	29.37 ± 1.03[a]	7.71 ± 0.21[b]	1.80 ± 0.31[b]	46.36 ± 1.87[b]	2.68 ± 0.33[b]	5.06 ± 0.02[b]	416.54 ± 10.71[b]	6.33 ± 0.34[b]	620.76 ± 14.06[b]	GWE
Staphylococcus aureus	33.31 ± 2.07[a]	50.64 ± 2.86[a]	64.51 ± 3.01[a]	76.58 ± 5.74[a]	144.94 ± 7.80[a]	6.10 ± 0.11[a]	2.91 ± 0.04[a]	654.00 ± 34.94[a]	57.82 ± 3.03[a]	621.52 ± 28.96[a]	C
	8.06 ± 0.26[b]	21.49 ± 0.56[b]	9.90 ± 0.36[b]	2.11 ± 0.05[b]	52.46 ± 0.27[b]	0.66 ± 0.01[b]	2.99 ± 0.18[a]	674.29 ± 0.73[a]	0.98 ± 0.03[b]	384.75 ± 2.39[b]	GEE
	5.51 ± 0.49[b]	1.85 ± 0.16[c]	0.85 ± 0.07[c]	0.12 ± 0.02[b]	2.28 ± 0.10[c]	0.22 ± 0.11[c]	1.31 ± 0.01[b]	46.45 ± 1.94[b]	2.92 ± 0.11[b]	92.02 ± 1.84[c]	GWE
Candida albicans	83.81 ± 1.27[a]	9.13 ± 0.34[a]	16.27 ± 0.16[a]	9.77 ± 0.48[a]	6.74 ± 0.35[a]	14.02 ± 0.75[a]	52.27 ± 0.00[a]	278.26 ± 6.47[a]	18.87 ± 1.08[a]	938.25 ± 25.32[a]	C
	2.11 ± 0.08[b]	0.44 ± 0.02[b]	1.24 ± 0.27[b]	0.05 ± 0.00[b]	0.00 ± 0.00[c]	0.19 ± 0.01[b]	0.00 ± 0.00[b]	129.79 ± 0.76[b]	2.32 ± 0.08[b]	86.34 ± 1.03[b]	GEE
	3.78 ± 0.51[b]	0.66 ± 0.02[b]	1.21 ± 0.09[b]	0.16 ± 0.00[b]	1.54 ± 0.00[b]	0.22 ± 0.00[b]	0.11 ± 0.00[b]	16.33 ± 0.41[c]	0.41 ± 0.02[b]	106.11 ± 0.69[b]	GWE
Fish Spoilage bacteria											
Pseudomonas luteola	146.51 ± 5.67[a]	0.81 ± 0.03[a]	3.99 ± 0.17[a]	6.25 ± 0.47[a]	66.96 ± 3.28[a]	10.32 ± 0.98[a]	37.93 ± 0.75[a]	508.02 ± 24.95[a]	3.38 ± 0.19[a]	933.57 ± 8.07[a]	C
	2.01 ± 0.15[b]	0.36 ± 0.09[c]	0.81 ± 0.06[c]	0.18 ± 0.03[b]	3.31 ± 0.10[c]	0.33 ± 0.02[b]	0.00 ± 0.00[b]	49.84 ± 0.95[b]	1.36 ± 0.02[c]	158.07 ± 0.54[c]	GEE
	1.40 ± 0.08[b]	0.60 ± 0.02[b]	1.57 ± 0.06[b]	0.44 ± 0.02[b]	12.04 ± 0.62[b]	0.36 ± 0.01[b]	0.59 ± 0.02[b]	60.18 ± 4.39[b]	2.37 ± 0.05[b]	201.48 ± 0.84[b]	GWE
Photobacterium damselae	86.27 ± 2.98[a]	4.28 ± 0.26[a]	11.62 ± 0.03[a]	12.11 ± 1.07[a]	94.63 ± 0.00[a]	1.08 ± 0.00[a]	37.15 ± 0.14[a]	986.50 ± 26.45[a]	41.27 ± 2.51[a]	1032.32 ± 13.50[a]	C
	3.23 ± 0.24[b]	0.46 ± 0.01[b]	0.79 ± 0.02[b]	0.41 ± 0.01[b]	5.10 ± 0.23[b]	1.15 ± 0.09[a]	0.00 ± 0.00[b]	160.22 ± 0.29[b]	3.26 ± 0.22[b]	239.40 ± 1.85[b]	GEE
	4.69 ± 0.09[b]	0.28 ± 0.02[b]	0.72 ± 0.07[b]	0.13 ± 0.00[b]	0.66 ± 0.05[c]	0.50 ± 0.02[b]	0.00 ± 0.00[b]	7.77 ± 0.32[c]	0.26 ± 0.06[b]	123.64 ± 9.96[c]	GWE
Enterococcus feacalis	34.84 ± 0.37[a]	83.79 ± 5.55[a]	67.76 ± 1.13[a]	86.46 ± 4.40[a]	0.89 ± 0.01[b]	24.42 ± 1.32[a]	106.72 ± 0.04[a]	1090.55 ± 0.04[a]	52.88 ± 1.18[a]	1491.63 ± 127.68[a]	C
	5.62 ± 0.27[a]	0.74 ± 0.05[b]	1.99 ± 0.01[b]	0.18 ± 0.00[b]	4.27 ± 0.27[a]	0.65 ± 0.02[b]	0.22 ± 0.02[c]	91.19 ± 0.02[b]	2.62 ± 0.40[b]	133.26 ± 1.07[c]	GEE
	19.91 ± 0.82[b]	0.73 ± 0.09[b]	0.73 ± 0.03[b]	0.56 ± 0.00[b]	1.18 ± 0.03[b]	0.60 ± 0.03[b]	1.33 ± 0.03[b]	16.52 ± 0.03[c]	2.53 ± 0.04[b]	413.38 ± 11.93[b]	GWE

2-PHEN, 2-phenylethylamine; AGM, agmatine; AMN, ammonia; C, Control group; CAD, cadaverine; DOP, dopamine; GEE, garlic ethanol extract; GWE, Garlic water extract; PUT, putrescine; SER, serotonin; SPD, spermidine; SPN, spermine; TRP, tryptamine.

*Mean ($n = 3$) ± Standard deviation, [a–c]Display significant differences ($P < 0.05$) between control and treated group in a row.

550 mg L^{-1} in various growth medium (Houicher *et al*., 2013; Kuley *et al*., 2019; Özkütük, 2022). On the other hand, bacteria isolated from spoiled fish accumulated AMN lower than 80 mg L^{-1} (Kuley *et al*., 2017). Application of garlic extract, mainly ethanolic one, showed a significant inhibitory effect on ammonia production by bacteria, with 4–100-fold lower ammonia accumulation ($P < 0.05$) (Table 2). Garlic extracts exert a wide spectrum of antimicrobial impacts on the proliferation of several Gram-negative and Gram-positive bacteria (Khan *et al*., 2014). Among the available bioactive compounds, allicin seems to be the natural constituent associated with the antimicrobial properties of garlic (Zhou *et al*., 2016).

Bacteria capable of BAs formation include species of *Streptococcus*, *Escherichia*, *Salmonella*, *Enterobacter*, *Shigella*, *Leuconostoc*, *Lactobacillus* and *Clostridium perfringens* (Özogul & Özogul, 2019). It is known that the presence of bacteria with decarboxylate activity (>7 log CFU g^{-1}) might result in BA formation (Özkütük, 2022). In the current study, bacterial growth in HDB was above 7.9 log CFU mL^{-1} and thus, foodborne pathogens and fish spoilage bacteria produced all tested BAs (Table 1). Putrescine is one BA associated with microbial food spoilage. Bacteria can produce putrescine from arginine via ornithine decarboxylase or from agmatine via agmatine deiminase (Landete *et al*., 2010). Bao *et al*. (2021) reported that *Shewanella baltica* is a common seafood spoiler that can produce putrescine from ornithine in acidic conditions. In the current study, putrescine and cadaverine formation were highest with *S. aureus* (50.64 vs. 64.51 mg L^{-1}) and *E. faecalis* (83.79 vs. 67.76 mg L^{-1}). *P. luteola* was the lower putrescine and cadaverine producer (Table 2). The presence of garlic extracts in the HDB led to lower BA accumulation by all bacteria. Similarly, Zhuang *et al*. (2019) revealed that both pomegranate peel aqueous and ethanolic extracts obstructed the proliferation of spoilage bacteria, particularly *Pseudomonas* spp. and *Aeromonas* spp. restrained the formation of BAs. Although both extracts generally showed similar suppression effects on BA production by bacteria, garlic aqueous extract did not influence putrescine production by *E. coli* but showed a higher inhibitory effect against putrescine production by *S. aureus* than ethanolic ones (Table 2). Groups treated with ethanolic garlic extracts had lesser cadaverine accumulation by *E. coli* and *P. luteola* compared to garlic aqueous extracts (Table 2). Putrescine production by *E. faecalis* in the existence of aqueous or ethanolic garlic extract was about 110-fold lower than that of the control group. Moreover, cadaverine was produced 75 and 92-fold lesser by *E. faecalis* and *S. aureus* in the existence of ethanolic extract of garlic (Table 2). Zhou *et al*. (2016) reported that in comparison with control groups, the overall BAs content in fish sausage treated with ethanolic garlic extract decreased by 27.17%.

The main polyamine in plant-derived foods is spermidine, whilst animal-derived foods had generally higher spermine content. They have an active role in the growth, maturation and regeneration of intestinal cells and exert potent antioxidant activity at physiological concentrations (Pegg, 2013). Microorganisms play an important role in the formation of spermidine and spermine in foods (Hirano *et al*., 2021). In the current study, spermidine and spermine production were the lowest by *S.* Paratyphi A (<1 mg L^{-1}) and the highest by *E. faecalis* (>25 mg L^{-1}). Garlic extracts had significant effects to suppress spermine and spermidine production by bacteria (Table 2). Tryptamine causes a toxic effect with an increase in blood pressure, and it is present in the highest amounts in sausages and meat products (Wójcik *et al*., 2021). *S. aureus* had the highest ability to produce tryptamine. Apart from *E. faecalis*, garlic extracts considerably decreased tryptamine accumulation by bacteria, mainly by *S. aureus* and *P. damseale*. *S. aureus* and *S.* Paratyphi A produced the lowest level of 2-phenylethylamine (2.91 vs. 8.27 mg L^{-1}). Among fish spoilage bacteria, *P. luteola* and *P. damselae* accumulated similar amount of 2-phenylethylamine.

Agmatine is formed from L-arginine by arginine decarboxylase and is a putrescine precursor. This pathway has been reported in *E. faecalis* ATCC11700, *Pseudomonas aeruginosa* PAO1, *Bacillus cereus* ATCC14579 and *Lentilactobacillus hilgardii* X1B (EFSA Panel on Biological Hazards (BIOHAZ), 2011). In the current study, dopamine and agmatine were mainly produced by all microorganisms tested, with more than 270 and 490 mg L^{-1} production level, respectively. Ethanolic extract of garlic did not influence dopamine production by *S. aureus*, although clear inhibition on dopamine and agmatine was observed. Serotonin formation by bacteria was below 60 mg L^{-1}. Application of garlic extracts lowered serotonin production below 7 mg L^{-1}.

Histamine is one of the BAs of most toxicity and the furthermost risk to consumer's health, which can lead various unwanted symptoms after extreme intake (Zhang *et al*., 2017). The formation of histamine in fish and seafood products is associated with the genus *Photobacterium* together with *Aeromonas hydrophila* and *Enterobacteriaceae* such as *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Morganella morganii* and *Raoultella planticola* (Gardini *et al*., 2016). Histamine production by foodborne pathogens was in the range from 0.49 mg L^{-1} for *S.* Paratyphi A to 61.99 mg L^{-1} for *S. aureus* (Fig. 1b). Fish spoilage bacteria produced histamine between 5.31 mg L^{-1} by *P. luteola*, and 15.19 mg L^{-1} by *E. faecalis* (Fig. 1a). Özogul *et al*. (2022) reported lower histamine production by foodborne pathogens (>39.29 mg L^{-1}).

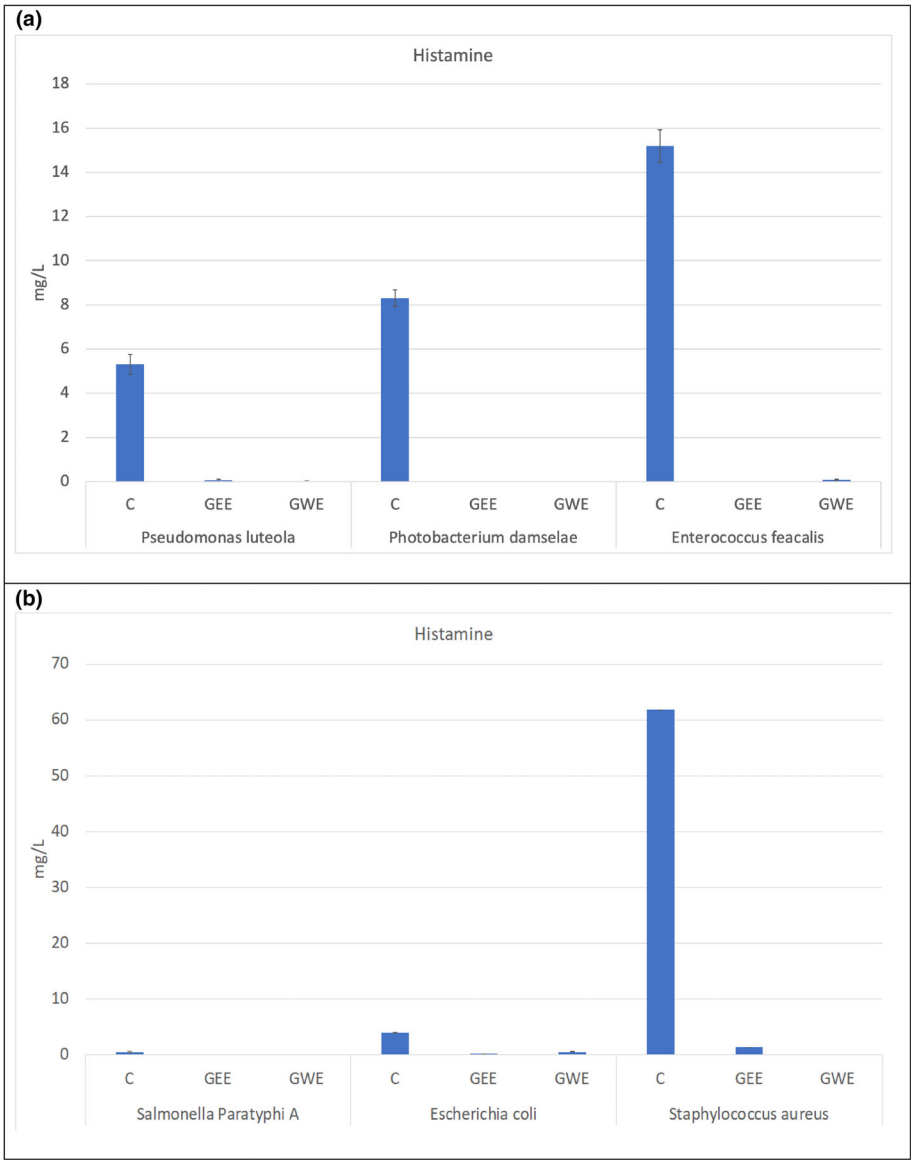

Figure 1 Histamine production by fish spoilage bacteria (a) and foodborne pathogens (b). c, Control group; GEE, garlic ethanol extract; GWE, garlic water extract.

Tyramine is known to be one of the major dietary BAs with harmful impacts, owing to high level of tyramine absorption through diet are seen as 'cheese reaction' (Kang *et al*., 2018). It was reported that tyramine displays stronger and quicker cytotoxic result than histamine (Dong *et al*., 2022). *S. aureus* accumulated tyramine at the highest level (36.45 mg L^{-1}) (Fig. 2b). Similar to our study, Özogul *et al*. (2022) found that the main tyramine-forming bacteria were *C. jejuni* and *S. aureus* accumulating 37.5 and 71.0 mg of tyramine L^{-1}, respectively. Moreover, Park *et al*. (2020) reported that the strains formed the greatest level of tyramine (301.14–315.29 μg mL^{-1}) were as *Enterococcus faecium* in a traditional Korean fermented soybean paste. Among fish spoilage bacteria, tyramine formation was the highest by *P. luteola* (15.43 mg L^{-1}) (Fig. 2a). Histamine and tyramine production by *C. albicans* were 6.77 and 26.06 mg L^{-1}, respectively (Fig. S2).

The use of garlic extract resulted in significant inhibition of histamine and tyramine production by bacteria (Figs 1 and 2) and fungus (Fig. S2). Aside from *P. damselae* and *C. albicans*, the bacterial load in the water and ethanolic extracts of garlic was statistically similar; however, variations were noted in the accumulations

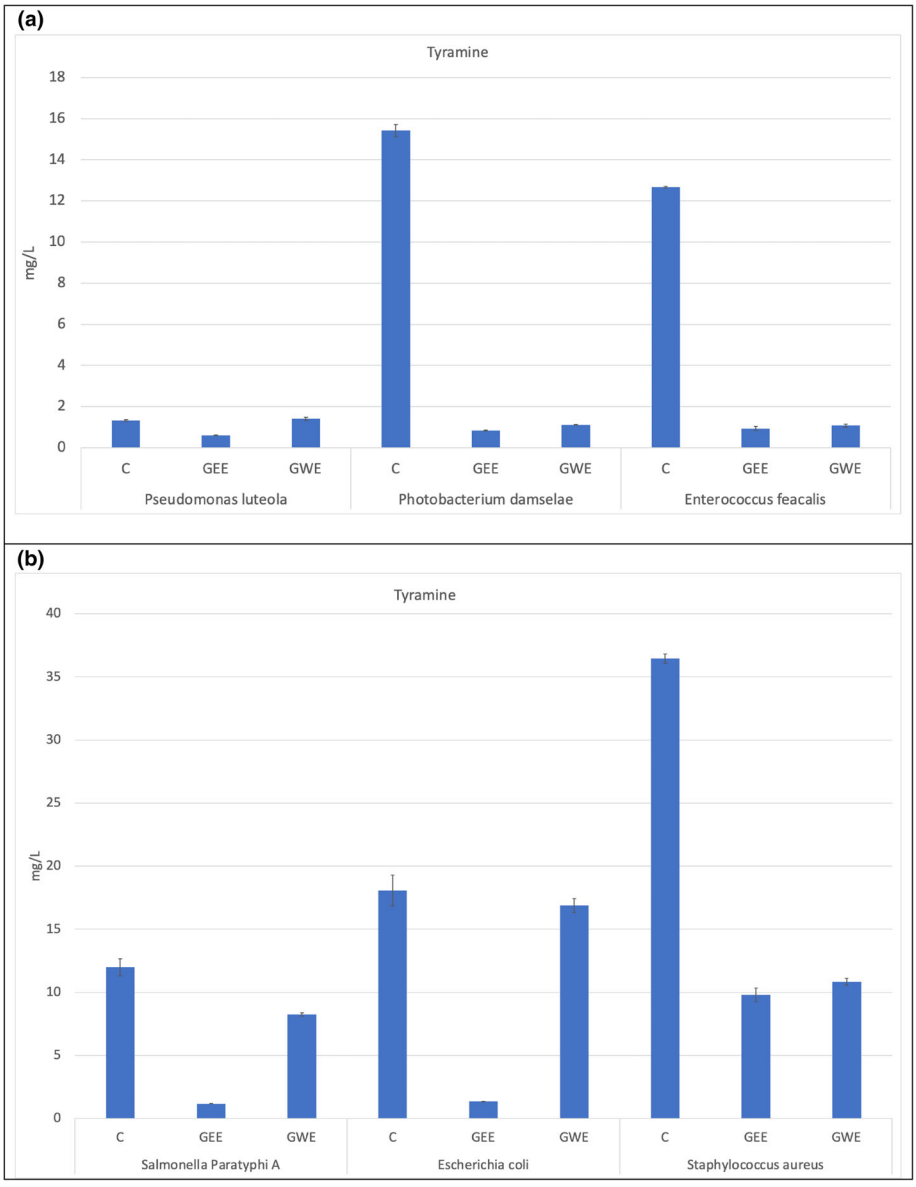

Figure 2 Tyramine production by fish spoilage bacteria (a) and foodborne pathogens (b). c, Control group; GEE, garlic ethanol extract; GWE, garlic water extract.

of their BAs. Tyramine synthesis was higher in the *C. albicans* and *P. damselae* groups treated with aqueous garlic extract than in the ethanol-treated group, despite the fact that the bacterial load at HDB was lower in the aqueous garlic extract group than in the ethanol extract group. Similarly, other studies showed a poor correlation between the production of BAs and the bacterial load (Kuley *et al.*, 2017; Burgut *et al.*, 2020; Yavuzer *et al.*, 2021; Ozogul *et al.*, 2024). Histamine production by *S. aureus* decreased to 1.34 and 0.01 mg L^{-1} in the existence of garlic ethanolic and aqueous extracts, respectively. Similar to our study, several studies reported that the histamine and other BAs formation were decreased by adding plant extracts or EOs (Şun *et al.*, 2018; Wang *et al.*, 2021; Yavuzer *et al.*, 2021; Özogul *et al.*, 2022). In addition, the presence of 5% garlic in *Myeolchi-jeot*, Korean salted and fermented anchovy decreased the histamine, putrescine, tyramine and spermidine levels by 11.7%, 11.2%, 30.9% and 17.4%, respectively (Mah *et al.*, 2009).

Conclusion

The presence of garlic ethanolic or aqueous extracts in HDB resulted noticeably in lower bacterial and fungal growth, ammonia and BAs production compared to the control due to bioactive compounds present in garlic extracts like allicin. Foodborne pathogens and fish spoilage bacteria produced all BAs tested, mainly dopamine, agmatine and tryptamine. Bacterial load did not always correlate well with BA production. Although both extracts generally showed similar effects on microbial growth inhibition and BAs production, the efficiency of the extracts in suppressing BAs also varied according to the type of BAs and foodborne microorganisms. In order to enhance safety of food, the application of garlic extracts, especially ethanolic extract on food matrixes can be used owing to their high ability to control foodborne pathogens and spoilage microorganisms and therefore lower production of BAs. Further detailed studies are needed to determine the effective dose of these garlic extracts on the inhibition of toxicologically important BAs in real food systems.

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Author contributions

Hatice Yazgan: Conceptualization; methodology; investigation; writing – original draft. **João Miguel Rocha:** Validation; resources; writing – review and editing. **Elena Bartkiene:** Validation; resources; writing – review and editing. **Esmeray Kuley:** Conceptualization; methodology; investigation; writing – original draft. **Yesim Ozogul:** Methodology; validation. **Fatih Ozogul:** Conceptualization; resources; writing – review and editing; supervision.

Conflict of interest

The authors declare no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

Ethic approval was not required for this study.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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We corrected the article one by one in line with the comments of the referees. The manuscript has been significantly improved. We had to give the necessary references to explain all the poor and unclear details. References are added below.

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The antimicrobial effects of aqueous extracts prepared by different extraction methods on important foodborne zoonotic bacteria were investigated. The bioactive components of aqueous garlic extract and aqueous garlic extract with particles were mostly sulphuric compounds. Specifically, aqueous garlic extract with particles showed better antibacterial activity against tested bacterial strains, even though both aqueous garlic extract and aqueous garlic extract with particles possessed antimicrobial capabilities against Gram-positive and Gram-negative foodborne zoonotic bacterial pathogens. This study showed that aqueous garlic extracts had substantial antibacterial effects in eliminating infections *in vitro*. The study suggested that aqueous garlic extract with particles might find application as an antimicrobial agent in the food industry.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Chromatograms of biogenic amines produced by *E. coli* in presence of garlic aqueous (a) and ethanolic (b) extracts.

Figure S2 Tyramine and histamine production by *C. albicans*.

