



Safflower and bitter melon extracts on suppression of biogenic amine formation by fish spoilage bacteria and food borne pathogens

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ABSTRACT

Safflower (*Carthamus tinctorius*) and bitter melon (*Momordica charantia* L.) ethanolic extracts for controlling growth of fish spoilage bacteria (*Acinetobacter lwoffii*, *Pseudomonas oryzae*, *Enterobacter cloacae*, *Shigella* spp., *Morganella psychrotolerans* and *Photobacterium phosphoreum*) and food-borne pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella Paratyphi A*), and their biogenic amine production were examined in anchovy infusion decarboxylase broth (AIDB). Bacterial load in AIDB was between 8.1 and 8.9 log CFU/ml. Bitter melon generally restricted growth of bacteria ($p < 0.05$). Bacterial load in the medium was not associated well with biogenic amine production. All bacteria had a high ability to form biogenic amines in AIDB. Significant discrepancies were noticed among groups in ammonia and biogenic amine production by bacteria ($p < 0.05$). *A. lwoffii* and *P. phosphoreum* were main bacteria produced the highest level of histamine (254.05 vs. 234.39 mg/L). Among food-borne pathogens, *S. aureus* was found stronger tyramine producer, with value of 736.53 mg/L. Bitter melon and safflower extract led approximately 9 and 12 fold lower histamine accumulation by *S. aureus*. The study result showed that bitter melon extract had more obvious effect, particularly for suppressing ammonia and toxicologically important biogenic amines production by bacteria, and thus could be suitable antimicrobial agent in food systems.

1. Introduction

Some members of the microorganisms named the specific spoilage organisms, are destructive microorganisms and lead to the emergence of intense off-odour and flavour associated with the spoilage of fishery products (Gram & Dalgaard, 2002; Singh et al., 2016). The microbiology of fish products is specific depending on the aquatic environment in which they are located (Ding et al., 2011). Gram-negative aerobic and facultative anaerobic rods and coliforms comprise the main spoilage microbial flora (Erkmen & Bozoglu, 2016). Microbiological spoilage of fresh and lightly preserved fish products may be due to psychrotrophic Gram-negative bacteria such as *Shewanella* spp., *Aeromonas* spp., *Pseudomonas* spp., *Photobacterium* spp., Enterobacteriaceae or *Brochothrix thermosphacta* (Gram & Dalgaard, 2002; Leroi, 2014; Løvdal, 2015).

Biogenic amines in fish have been involved as a main relevant agent of food borne illness, where intoxication caused from the digestion of foods having higher level of biogenic amines (Biji et al., 2016). There are some bacteria that decarboxylate free amino acids by means of decarboxylase enzymes in fish muscle during storage. *Scombridae* (such as

mackerel and tuna) and scombroid fish belonging to the *Scomberesocidae* families are the most common species linked with histamine fish poisoning. However, this poisoning can also be caused by non-scombroid fish species (herring, sardines and anchovies) that contain high levels of free amino acids in their muscles (Özoğul, 2001, pp. 20–36). The bacterial isolates from fish were also capable of generating various type of biogenic amine mainly dopamine, serotonin, agmatine, and tyramine. In various spoiled fish species, *Morganella*, *Hafnia*, *Vibrio*, *Aeromonas*, *Photobacterium*, *Proteus*, *Enterobacter*, *Raoultella*, *Alcaligenes*, *Flavobacterium*, *Acinetobacter*, *Shigella* and *Shewanella* were reported as amine-forming bacteria (Durak-Dados et al., 2020; Houicher et al., 2013; Kuley et al., 2017, 2019; Lakshmanan et al., 2002; Takahashi et al., 2003). In addition to fish spoilage bacteria, it has been reported that food-borne pathogens have the capacity to decarboxylate one or more amino acids and can produce various biogenic amines (Burgut, 2020; Kuley et al., 2019; Özoğul, Kacar, & Hamed, 2015; Özoğul et al., 2018).

Plants and spices, which have a wide bioactivity profile due to their unique flavors and aromas, antimicrobial and antioxidant properties,

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can be utilised as an alternative additive in the food industry (Ghidey et al., 2020; Panda et al., 2019; Sabo & Knezevic, 2019; Yavuzer et al., 2020). *Carthamus tinctorius*, which belongs to the Asteraceae family, is a plant commonly referred as safflower. Its seeds contain 30–45% oil and are used as cooking oil. It is also an economically important plant due to its medicinal values (Ahmad et al., 2020). Bitter melon (*Momordica charantia*) is an annual, thin, climbing plant that grows in the form of ivy, which is a member of the Cucurbitaceae family. Bitter melon has been used as a therapeutic for many years, especially in Asian and African countries and has various benefits in diseases such as rheumatism, stomach, intestine and diabetes, as well as skin diseases, inflammatory diseases, tumors and infections (Taylor, 2002). They are rich in phenolic compounds exerting antimicrobial and antioxidative properties (Wu & Ng, 2007; Wu & Ng, 2007).

The existence of food-borne pathogens and the production of biogenic amine mainly histamine by food-related bacteria in food make it crucial to inhibit both pathogenic and spoilage microorganisms in terms of seafood safety (Sheng & Wang, 2020). Therefore, in this study, the antimicrobial effects of safflower and bitter melon extracts on proliferation of spoilage bacteria (*Acinetobacter lwoffii*, *Pseudomonas oryzae*, *Enterobacter cloacae*, *Shigella* spp., *Morganella psychrotolerans* and *Photobacterium phosphoreum*) isolated from fish as well as food-borne pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella Paratyphi A*) and their biogenic amine production were examined in anchovy infusion decarboxylase broth (AIDB).

2. Material and method

2.1. Preparation of plant extracts

In order to obtain natural antimicrobials from the plants, 200 g of the ground plants were added in 1000 mL ethanol (Merck 1009831011, Darmstadt, Germany). They were manually shaken daily and kept in a dark environment by wrapping them with aluminum foil for 1 week. The extract obtained was filtered through filter paper. The filtrate was evaporated in the evaporator and the natural extract was obtained (Chen et al., 1992). Extracts were stored in refrigerator at 3 °C until use.

2.2. Bacterial strains

A. lwoffii, *P. oryzae*, *E. cloacae* and *Shigella* spp. were isolated from spoiled anchovy and mackerel (Kuley et al., 2019). Briefly, isolates were obtained from spoiled fish muscles packaged in vacuum and stored at 4 °C. Isolated strains were characterized by API 20E (20100) and API 20NE (2050) test strip system (BioMérieux SA, Marcy l'Etoile, France) and molecularly confirmed by PCR. *M. psychrotolerans* (DSM, 17886) and *P. phosphoreum* (DSM 15556) were also selected as spoilage and HIS forming bacteria in fish. The both strains were provided from the German Collection of Microorganisms and Cell Culture (DSMZ, Braunschweig, Germany). Among food-borne pathogens, *S. aureus* (ATCC29213) and *K. pneumoniae* (ATCC700603) were obtained from the American Type Culture Collection (Rockville, MD, USA), whilst *S. Paratyphi A* (NCTC13) was purchased from the National Collection of Type Cultures (London, UK). Nutrient broth (Merck 1.05443.0500) was used for propagation of bacterial cultures.

2.3. Antimicrobial activity of extracts

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts were analysed using Clinical and Laboratory Standards Institute (CLSI) methods (2008). Plant extracts were used with stock solution of 50 mg/mL and final concentrations of extracts were prepared as 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19 mg/mL. Each tube was assessed for bacterial growth and contrasted to the control. Positive control tubes consisted of Mueller Hilton Broth (MHB, Merck 1.10293, Darmstadt, Germany) and bacterial suspension

without extracts, whereas a tube not including MHB was evaluated as negative control. MIC was evaluated as no visible growth in a broth, while MBC was obtained by subculturing the contents of tubes of MIC exerting no growth.

2.4. Culture conditions and biogenic amine analysis

Fish infusion broth was prepared using the method of Okuzumi et al. (1981) with minor variations. Anchovy (*Engraulis encrasicolus*) was obtained from a local market (Adana, Turkey). Anchovy muscle with skin (250 g) was roughly minced and steamed with 2 volume of water (w/v) at 100 °C for 1 h. After filtration (Whatman No. 1, Sigma), they were supplemented with 1% glucose, 0.5% NaCl and 3 mg pyridoxal HCl (Sigma P9130, Steinheim, Germany). Fresh culture (0.5 mL) with 18–24 h (10^8 CFU/mL) was transferred into 9 mL of the anchovy infusion decarboxylase broth (AIDB). Safflower or bitter melon extracts at doses of 0.5% were added into the AIDB. The control was the absence of any of the extracts. The tubes were incubated at 37 °C for 72 h, except for *M. psychrotolerans* and *P. phosphoreum* groups which were kept at 28 and 20 °C for 72 h, respectively. Biogenic amine extraction and derivatisation were performed using the method of Kuley and Özogul (2011). The confirmation of biogenic amine formation was made using a rapid HPLC method (Özogul, 2004). For ammonia and trimethylamine (TMA) analysis, the same analytic method was done. For biogenic amine analysis, the mobile phase was used acetonitrile and high-performance liquid chromatography grade water. A Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) was used. The column was Spherisorb 5 Si C18 (pH-St, 250 4.6 mm, Phenomenex, Macclesfield, Cheshire, UK). HPLC conditions were performed according to method of Kuley and Özogul (2011).

2.5. Statistical analysis

SPSS 22 version software (Chicago, Illinois, USA) used for one-way variance analysis (ANOVA) and Duncan's Multiple Range Test. Calculations were done in triplicate and comparisons at a p-value of <0.05 were carried out to point out significant differences.

3. Results and discussion

Table 1 shows bacterial growth in AIDB. Bacterial growth in AIDB varied from 8.13 log CFU/ml for *E. cloacae* to 8.90 log CFU/ml for *P. oryzae*. Significant differences in bacterial loads in AIDB were observed between control and treated groups, apart from *E. cloacae* and *Shigella* spp. ($p < 0.05$). The existence of safflower extract showed no obvious difference on the growth of *A. lwoffii* and *P. phosphoreum*, whilst bitter melon extract limited growth of these bacteria ($p < 0.05$).

Table 1
Bacterial growth in anchovy infusion decarboxylase broth (log CFU/ml).

	Control	Safflower extract	Bitter melon extract
Fish spoilage bacteria			
<i>Acinetobacter lwoffii</i>	8.14 ± 0.35 ^{aa}	8.17 ± 0.08 ^a	7.99 ± 0.19 ^b
<i>Pseudomonas oryzae</i>	8.90 ± 0.06 ^a	8.28 ± 0.21 ^b	8.29 ± 0.09 ^b
<i>Enterobacter cloacae</i>	8.13 ± 0.02 ^a	7.95 ± 0.11 ^a	7.86 ± 0.61 ^a
<i>Shigella</i> spp.	8.56 ± 0.33 ^a	8.45 ± 0.11 ^a	8.19 ± 0.04 ^a
<i>Morganella psychrotolerans</i>	8.28 ± 0.03 ^a	6.32 ± 0.06 ^b	8.16 ± 0.29 ^a
<i>Photobacterium phosphoreum</i>	8.73 ± 0.23 ^a	8.49 ± 0.31 ^a	7.90 ± 0.72 ^b
Food-borne pathogens			
<i>Staphylococcus aureus</i>	8.57 ± 0.04 ^a	8.29 ± 0.00 ^b	7.55 ± 0.00 ^c
<i>Klebsiella pneumoniae</i>	8.57 ± 0.28 ^a	7.96 ± 0.00 ^b	6.48 ± 0.02 ^c
<i>Salmonella Paratyphi A</i>	8.54 ± 0.05 ^a	8.14 ± 0.04 ^b	8.56 ± 0.11 ^a

^a Mean (n = 3) ± Standard deviation. ^{a-c} Display significant differences ($p < 0.05$) between control and treated group in a column.

Antimicrobial properties of bitter melon may be due to various bioactive components of phenolic compounds, particularly gallic acid, saponins, peptides, alkaloids and vitamins (Saeed et al., 2018; Tan et al., 2016).

Unlike this study, bitter melon extract was found as highest inhibitory activity (1.1 log reduction) against *A. lwoffii* in mackerel infusion decarboxylase broth in comparison with safflower extract (Kuley et al., 2019). In the current study, safflower extract exhibited considerably lower microbial growth on *M. psychrotolerans* than control group, with 1.96 log reduction in bacterial growth. Burgut et al. (2020) indicated that broth used and extract type were significant effect on *M. psychrotolerans* load. They found that aqueous extracts of propolis had higher action to reduce bacterial load in histidine decarboxylase broth, although lesser bacterial count was in the existence of ethanolic ones in sardine infusion decarboxylase broth. Kuley et al. (2017) found addition of olive leaf extract and olive cake in AIDB showing significant inhibition on proliferation of fish spoilage bacteria such as *Proteus mirabilis* and *Pontea* spp., but ineffectiveness of olive leaf extract on most of the fish spoilage bacteria including *A. lwoffii* and *E. cloacae*.

P. oryzihabitans growth also restrained in the existence of safflower extract in AIDB. Mehrabian et al. (2000) stated that the aqueous extraction of safflower exerted antimicrobial effects on bacteria such as *Bacillus subtilis*, *Bacillus cereus* and *Bacillus mycoides*. Seok-Yeong et al. (2013) reported that safflower extract has inhibitory effects on the growth of bacteria on *S. aureus*, *Salmonella typhimurium*, *Escheria coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *Clostridium perfringens*. In the present study, 2.09 and 0.61 logarithmic decreases in the load of *K. pneumoniae* were observed in the existence of

bitter melon and safflower extract compared to the control group, respectively.

Like *K. pneumoniae*, bitter melon extract found more effective than safflower extract in growth inhibition of *S. aureus* (with 1.02 log reduction). Similarly, Coutinho et al. (2010) found ethanolic bitter melon extract inhibiting *S. aureus* growth a higher degree compared to synthetic antibiotics. Omoregbe et al. (1996) reported that ethanolic bitter melon extract was effective against *Escherichia coli*, *Salmonella Paratyphi*, *Shigella dysenteriae* and *Streptomyces griseus*. Antimicrobial activity of safflower extract was linked with their phenolic compounds such as lignan and flavonoids, with antimicrobial properties (Son et al., 2017; Yu et al., 2013).

Moreover, *S. Paratyphi A* growth did not influenced from presence of bitter melon extract, although suppression effect of safflower on the bacteria was observed. The antimicrobial activity of plant extracts might also exhibit differences depending on bacterial strains, extraction methods, kind and amount of phenolic compounds in plant extracts, harvest time, vegetative period and growing conditions of plant (Aleksic & Knezevic, 2014; Kuley et al., 2017; Savadi et al., 2020; Yalcin et al., 2017).

3.1. Ammonia and biogenic amine production by fish spoilage bacteria

Table 2 shows ammonia and biogenic amine formation by fish spoilage bacteria in AIDB. The fish spoilage bacteria appeared to produce high amounts of ammonia. Ammonia was formed more than 800 mg/L by fish spoilage bacteria. Notably lower ammonia production was

Table 2

Ammonia and biogenic amine production by fish spoilage bacteria in anchovy infusion decarboxylase broth (mg/L).

	AMN	PUT	CAD	SPD	TRP	PHEN	SPN	SER	TMA	DOP	AGM	Groups
PP	1647.65 ± 118.97 ^{a*}	404.18 ± 28.64 ^a	428.19 ± 23.73 ^a	419.34 ± 12.73 ^a	717.72 ± 24.04 ^a	654.96 ± 36.65 ^a	0.00 ± 0.00 ^c	283.79 ± 18.31 ^a	62.21 ± 2.83 ^a	1278.91 ± 100.19 ^a	289.78 ± 20.25 ^a	C
	1546.43 ± 54.43 ^a	384.92 ± 7.16 ^{ab}	118.67 ± 7.36 ^b	155.41 ± 13.47 ^b	0.00 ± 0.00 ^b	193.39 ± 12.90 ^b	104.09 ± 3.32 ^b	198.69 ± 7.40 ^b	25.83 ± 1.14 ^b	137.69 ± 10.49 ^b	60.95 ± 5.14 ^b	S
	726.78 ± 7.37 ^b	322.56 ± 26.51 ^b	415.19 ± 14.70 ^a	61.16 ± 1.66 ^c	0.00 ± 0.00 ^b	201.40 ± 19.37 ^b	132.13 ± 8.49 ^a	96.50 ± 3.37 ^c	13.32 ± 0.50 ^c	148.06 ± 9.48 ^b	36.03 ± 1.82 ^b	B
MP	1979.22 ± 99.87 ^a	193.26 ± 12.11 ^a	105.53 ± 1.58 ^c	165.54 ± 3.93 ^b	0.00 ± 0.00 ^a	849.68 ± 58.86 ^a	0.00 ± 0.00 ^c	301.72 ± 9.95 ^a	17.99 ± 1.37 ^b	877.36 ± 75.26 ^a	100.43 ± 5.29 ^a	C
	1043.90 ± 70.97 ^b	190.51 ± 6.83 ^a	176.79 ± 4.77 ^b	99.10 ± 5.26 ^c	0.00 ± 0.00 ^a	169.02 ± 3.85 ^c	84.79 ± 5.12 ^b	237.12 ± 11.21 ^b	26.74 ± 0.05 ^a	512.63 ± 30.91 ^b	48.81 ± 0.54 ^b	S
	724.73 ± 28.17 ^c	208.20 ± .56 ^a	382.34 ± 30.32 ^a	200.99 ± 3.88 ^a	0.00 ± 0.00 ^a	297.26 ± 12.29 ^b	141.43 ± 12.82 ^a	112.64 ± 2.95 ^c	11.71 ± 1.01 ^c	235.11 ± 10.17 ^c	31.46 ± 1.12 ^c	B
SH	1437.96 ± 35.98 ^a	683.54 ± 25.88 ^a	189.55 ± 9.54 ^a	119.92 ± 9.37 ^a	5.61 ± 0.28 ^a	9.24 ± 0.08 ^b	32.53 ± 1.41 ^b	78.48 ± 3.57 ^b	4.48 ± 0.12 ^c	176.78 ± 8.19 ^a	22.48 ± 0.40 ^b	C
	1330.59 ± 31.45 ^b	644.57 ± 5.96 ^a	161.69 ± 15.93 ^a	86.68 ± 1.49 ^b	0.00 ± 0.00 ^b	49.99 ± 1.40 ^a	39.83 ± 0.99 ^a	76.32 ± 2.41 ^b	12.52 ± 0.65 ^b	110.75 ± 2.55 ^c	36.06 ± 1.70 ^a	S
	1115.42 ± 25.54 ^c	756.81 ± 61.67 ^a	171.00 ± 13.97 ^a	64.93 ± 2.78 ^c	6.01 ± 1.40 ^a	10.51 ± 0.59 ^b	29.68 ± 0.96 ^b	89.93 ± 1.36 ^a	14.56 ± 0.32 ^a	150.52 ± 7.33 ^b	33.22 ± 2.55 ^a	B
EC	885.04 ± 16.73 ^b	199.96 ± 12.89 ^b	115.95 ± 8.88 ^b	113.93 ± 4.83 ^b	8.68 ± 0.62 ^b	50.92 ± 0.51 ^a	51.29 ± 2.05 ^b	108.54 ± 5.09 ^a	21.08 ± 1.59 ^b	140.63 ± 1.43 ^a	56.01 ± 1.29 ^a	C
	1029.72 ± 42.15 ^a	315.50 ± 20.22 ^a	197.16 ± 7.90 ^a	194.96 ± 17.12 ^b	58.48 ± 3.14 ^a	0.00 ± 0.00 ^c	58.83 ± 5.25 ^b	98.38 ± 7.36 ^a	54.68 ± 1.44 ^a	58.92 ± 2.80 ^b	40.14 ± 1.82 ^b	S
	832.10 ± 16.85 ^b	81.15 ± 7.58 ^c	132.53 ± 9.60 ^b	1026.45 ± 101.95 ^a	5.35 ± 0.55 ^b	38.70 ± 1.85 ^b	230.79 ± 5.78 ^a	75.28 ± 3.58 ^b	8.02 ± 0.67 ^c	140.63 ± 2.67 ^a	31.35 ± 2.32 ^c	B
PO	2770.23 ± 16.12 ^a	1345.78 ± 49.82 ^a	780.49 ± 1.86 ^a	263.34 ± 12.61 ^a	5.67 ± 0.22 ^b	48.83 ± 0.63 ^a	20.26 ± 1.81 ^b	120.77 ± 2.09 ^a	83.06 ± 2.82 ^a	174.69 ± 2.81 ^b	55.55 ± 4.48 ^a	C
	879.87 ± 42.25 ^b	732.00 ± 5.66 ^c	125.18 ± 1.15 ^c	34.88 ± 2.66 ^b	14.10 ± 1.27 ^a	24.14 ± 1.61 ^b	39.49 ± 0.72 ^a	103.42 ± 3.08 ^b	17.47 ± 0.75 ^b	229.87 ± 14.93 ^a	42.20 ± 3.11 ^b	S
	2530.24 ± 165.65 ^a	1186.98 ± 35.41 ^b	674.03 ± 18.62 ^b	8.38 ± 0.18 ^c	0.00 ± 0.00 ^c	11.18 ± 0.50 ^c	5.92 ± 0.90 ^c	88.88 ± 2.67 ^c	9.54 ± 0.02 ^c	246.98 ± 5.79 ^a	36.95 ± 2.64 ^b	B
AI	1319.87 ± 29.90 ^a	201.10 ± 0.77 ^a	126.27 ± 9.10 ^b	639.75 ± 38.01 ^a	0.00 ± 0.00 ^a	492.99 ± 39.62 ^a	205.69 ± 4.09 ^a	142.12 ± 0.51 ^b	4.53 ± 0.25 ^b	151.79 ± 7.39 ^a	36.02 ± 0.88 ^b	C
	1192.83 ± 93.30 ^a	105.19 ± 6.91 ^b	167.31 ± 8.12 ^a	423.73 ± 6.21 ^b	0.00 ± 0.00 ^a	444.85 ± 20.16 ^a	203.58 ± 9.57 ^a	215.94 ± 17.53 ^a	35.97 ± 2.78 ^a	158.08 ± 4.85 ^a	52.53 ± 4.41 ^a	S
	475.60 ± 45.52 ^b	58.83 ± 3.40 ^c	33.10 ± 0.40 ^c	156.04 ± 9.58 ^c	0.00 ± 0.00 ^a	171.02 ± 4.16 ^b	73.58 ± 6.64 ^b	30.67 ± 1.82 ^c	2.03 ± 0.49 ^b	145.73 ± 10.13 ^a	14.52 ± 1.41 ^c	B

*Mean (n = 3) ± Standard deviation. ^{a-c} Display significant differences (p < 0.05) between control and treated group in a row. PP: *Photobacterium phosphoreum*; MP: *Morganella psychrotolerans*; SH: *Shigella* spp.; EC: *Enterobacter cloacae*; PO: *Pseudomonas oryzihabitans*; AI: *Acinetobacter lwoffii*; C: Control group; S: Group treated with strawflower extract; B: Group treated with bitter melon extract. AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; TRP, tryptamine; PHEN, 2-phenylethyl amine; SPN, spermine; SER, serotonin; TMA, trimethylamine; DOP, dopamine; AGM, agmatine.

reported by bacteria isolated from spoiled sardine, anchovy and mackerel in different growth medium (Houicher et al., 2013; Kuley et al., 2017, 2019). The highest ammonia production was observed by *P. oryzihabitans* (2770.23 mg/L). Extracts used restricted ammonia formation by fish spoilage bacteria. In the existence of safflower extract in the broth, *P. oryzihabitans* produced significantly lesser ammonia (879.87 mg/L) than control group ($p < 0.05$). Apart from this bacteria, bitter melon extract showed stronger effect on suppression of ammonia production by all fish spoilage bacteria ($p < 0.05$). Özoğul et al. (2011) reported that rosemary and sage tea extracts significantly decreased ammonia formation in sardine muscle. Ammonia accumulation by fish spoilage bacteria e.g. *Photobacterium damsela*, *Pontea* spp., *Vibrio vulnificus*, *Stenotrophomonas maltophilia*, and *Pasteurella* spp. was inhibited by addition of olive leaf extract (Kuley et al., 2017).

Middlebrooks et al. (1988) have isolated *Acinetobacter lwoffii*, *Pseudomonas putrefaciens* and *Aeromonas hydrophila* with decarboxylase activity from spoiled fish. Moreover, various bacterial groups found in fish flora were reported to contribute to the formation of biogenic amines (Ahmed et al., 2012; Houicher et al., 2013; Kuley et al., 2019; Russo et al., 2010). Similarly, in the present study, the fish spoilage bacteria produced most of biogenic amines tested primarily putrescine, cadaverine, spermidine and dopamine, and significant differences were observed in the production of biogenic amines by bacteria ($p < 0.05$). Rice et al. (1976) reported that *Pseudomonas* species have amino acid decarboxylase activity, mainly in putrescine production. Putrescine and cadaverine were found in the range of 1.51–29.03 mg/kg in mahi-mahi (Bai et al., 2019). Among isolates from ice-preserved sardine and mackerel, *Kluyvera intermedia* formed histamine, putrescine and cadaverine, while *Enterobacter asburiae* strain formed solely cadaverine and putrescine (Fadhlaoui-Zid et al., 2012). In the present study, the highest putrescine and cadaverine production were seen in the *P. oryzihabitans* (1378.78 vs 780.49 mg/L) in the control group. It was observed that extract of bitter melon and safflower significantly reduced putrescine production by *P. oryzihabitans* ($p < 0.05$). The application of bitter melon extract prevented the production of putrescine by *E. cloacae* and *A. lwoffii*. The presence of extracts in the AIDB did not statistically affect the production of putrescine by *M. psychrotolerans* and *Shigella* spp. Özoğul et al. (2011) reported that the application of 1% rosemary and sage tea extract significantly reduced the accumulation of putrescine and cadaverine in sardine meat.

Putrescine and cadaverine have an essential role in increasing the toxic effect of histamine. Moreover, cadaverine provides a beneficial index in fish spoilage (Al Bulushi et al., 2009). In the present study, although the extracts applied did not show any impact on the production of cadaverine by *Shigella* spp., bitter melon extract had a suppressing effect on the cadaverine production by *A. lwoffii* ($p < 0.05$). The highest cadaverine production (780.49 mg/L) was recorded by the control *P. oryzihabitans*, whilst safflower extract significantly reduced the level of cadaverine produced by this bacterium (125.18 mg/L). Similarly, safflower extract also led lower cadaverine accumulation by *P. phosphoreum*. In addition, the extract of bitter melon resulted about 4 fold lower cadaverine production by *A. lwoffii* (33.10 mg/L) compared to control group (126.27 mg/L).

Spermidine and spermine are among the naturally occurring amines in fish muscle due to physiological and environmental factors (Veciana-Nogues et al., 1997). Krizek et al. (2004) reported that the spermine and spermidine concentrations in carp fillets wrapped in polyethylene film at chilly temperature were 0.82 and 0.9 mg/100g at the beginning and reached 0.84 and 1.11 mg/100g at the end of storage, respectively. Significant differences were observed between groups in terms of spermidine and spermine production by bacteria ($p < 0.05$). The highest spermidine and spermine production by bacteria were achieved by the control *A. lwoffii* with respective value of 639.75 and 205.69 mg/L. Bitter melon extract had an inhibitory effect on spermidine production by *A. lwoffii*, *P. oryzihabitans*, *Shigella* spp. and *P. phosphoreum* ($p < 0.05$). In addition, safflower extract significantly decreased spermidine

accumulation by *A. lwoffii* and *M. psychrotolerans* compared to the control group ($p < 0.05$). Bitter melon extract also suppressed the accumulation of spermine by *P. oryzihabitans*.

The highest tryptamine production was found by *P. phosphoreum* (717.72 mg/L), although *M. psychrotolerans* and *A. lwoffii* did not form tryptamine. Safflower and bitter melon extracts completely inhibited tryptamine production by *P. phosphoreum*. Bitter melon extract also significantly decreased tryptamine production by *P. oryzihabitans* ($p < 0.05$). 2-phenylethylamine accumulation was the highest by control *M. psychrotolerans*, with value of 849.68 mg/L. Safflower and bitter melon extracts led lower 2-phenylethylamine accumulation by *M. psychrotolerans*, *P. phosphoreum* and *P. oryzihabitans* compared to control group. In addition, bitter melon suppressed the accumulation of 2-phenylethylamine by *A. lwoffii* and *P. oryzihabitans*.

K. pneumoniae, *Staphylococcus* spp., *Pseudomonas* spp. and *Shigella* spp. have been reported to be potential histamine-producing bacteria identified in fish products (Hernandez-Herrero et al., 1999; Tsai et al., 2005). In the present study, histamine production by fish spoilage bacteria was in range from 6.64 mg/L for *Shigella* spp. to 234.39 mg/L for *P. phosphoreum* (Fig. 1). Burgut et al. (2020) found that *M. psychrotolerans* formed 87.50 and 128.92 mg/L histamine in histidine decarboxylase broth and sardine infusion decarboxylase broth, respectively, which was higher than those in this study (62.36 mg/L). In addition, *M. psychrotolerans* and *P. phosphoreum* accumulated histamine with corresponding level of 40 and 30 mg/L in mackerel infusion decarboxylase broth (Kuley et al., 2019), which was lower than values obtained in this study.

Histamine accumulation in AIDB was generally higher in control groups than treated groups ($p < 0.05$). Safflower extract resulted in about 2 and 7-fold lower histamine accumulation by *A. lwoffii* and *P. phosphoreum*, whereas in presence of bitter melon extract, approximately 3 and 4-fold lower histamine production was observed by these bacteria compared to control groups, respectively. It is known that effectiveness of extracts on biogenic amine production by fish spoilage bacteria changed depending on bacterial strains, growth medium of bacteria, type of biogenic amine, type and dose of extracts (Burgut et al., 2020; Kuley et al., 2017, 2019). Moreover, in a recent study, it was demonstrated that the presence of ethanolic and aqueous extracts of propolis in histidine decarboxylase broth noticeably inhibited histamine accumulation by *M. psychrotolerans*, although suppression action of extracts was only observed by propolis aqueous extract at concentration of 0.4% in sardine infusion decarboxylase broth (Burgut et al., 2020). In the present study, there was no obvious differences in histamine formation by *M. psychrotolerans* in the existence extracts although histamine production by other fish spoilage bacteria was restrained by addition of extracts. Important raises in histamine accumulation were noticed with olive cake for *S. maltophilia*, *Pontea* spp., and *K. pneumoniae*, whereas significant suppression effects on histamine formation by *A. lwoffii* was found by olive leaf extract and olive cake (Kuley et al., 2017).

Serotonin production by *Shigella* spp. was 78.48 mg/L, while *M. psychrotolerans* produced serotonin at level of 301.72 mg/L. Bitter melon and safflower extracts had suppressive effects on serotonin production of *M. psychrotolerans*, *P. phosphoreum* and *P. oryzihabitans* ($p < 0.05$).

Tyramine is generally formed by Gram-positive bacteria such as lactic acid bacteria and the genus *Enterococcus* (Suzzi & Gardini, 2003; Bover-Cid et al., 2014), and tyramine doses between 100 and 800 mg/kg in food are regarded as toxic for human (Ten Brink et al., 1990). Tyramine was formed by all fish spoilage bacteria in AIDB (Fig. 2). Tyramine production was the highest by *P. phosphoreum* (2020.95 mg/L) and the lowest by *P. oryzihabitans* (21.38 mg/L) (Fig. 2). *P. oryzihabitans*, *C. indologenus* and *V. vulnificus* exerted the uppermost accumulation of tyramine (187–1650 mg/L) in tyrosine decarboxylase broth (Houicher et al., 2013). Bitter melon and safflower extracts exerted significant inhibitory properties with reducing tyramine production by

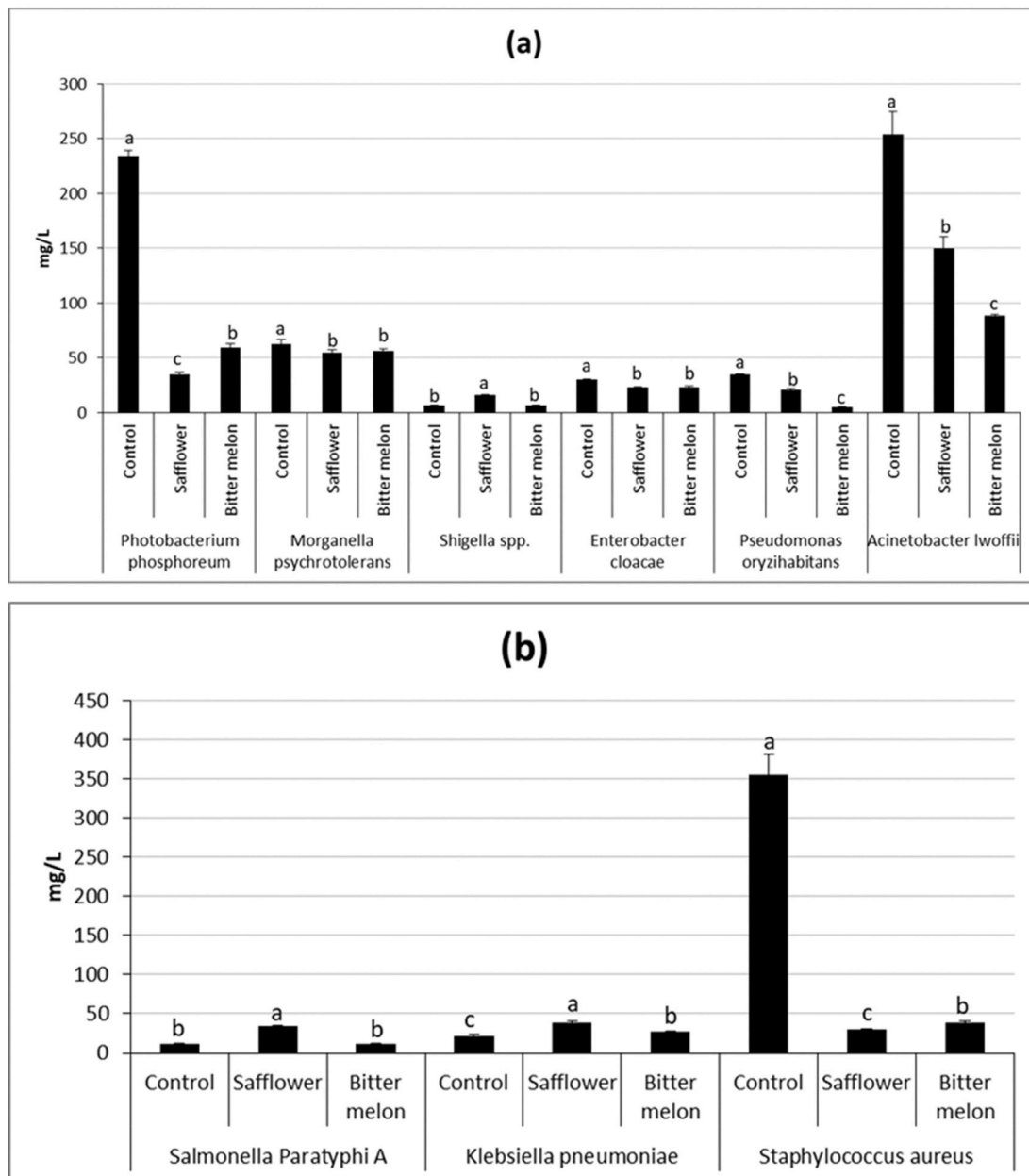


Fig. 1. Histamine production by fish spoilage (a) and food-borne pathogen bacteria (b). Data are expressed as mean value of three samples, ^{a-c} indicate significant differences ($p < 0.05$) between control and treated group.

P. phosphoreum below 43 mg/L. Suppression effects of extracts on tyramine accumulation were also observed for *E. cloacae* and *A. lwoffii*. However, the both extracts used did not affect tyramine production by *P. oryzihabitans* ($p > 0.05$), although AIDB added with extract included lesser bacterial load. This is consistent with the results of other studies confirming bacterial load in the medium being not correlated well with biogenic amine formation by bacteria (Burgut, 2020; Kuley et al., 2017).

TMA is a strong volatile amine frequently related with the characteristic “fishy” smell of spoiling seafood and its existence in spoiling fish is because of the bacterial breakdown of trimethylamine oxide (Huss, 1995). *P. phosphoreum* is known as strong TMA producer in fish. (Dalgard, 1995). TMA was the greatest accumulated amine and mostly formed by *S. maltophilia* (154 mg/L) and *Pseudomonas luteola* (121 mg/L) among fish spoilage bacteria in AIDB (Kuley et al., 2017). In the present study, among the control groups, the highest TMA-producing bacteria was *P. oryzihabitans* (83.06 mg/L). TMA production by *P. oryzihabitans* and *P. phosphoreum* was found at considerably lower

levels in the treatment groups than in the control group ($p < 0.05$). However, higher TMA content in AIDB was observed for *E. cloacae*, *A. lwoffii* and *M. psychrotolerans* in the presence of safflower extract.

Dopamine production by fish spoilage bacteria was more than 134 mg/L. Dopamine formation by *P. phosphoreum*, *M. psychrotolerans* and *Shigella* spp. was restrained in the presence of both extracts in AIDB, although existence of extracts statistically did not change dopamine production by *A. lwoffii* and *P. oryzihabitans*.

3.2. Ammonia and biogenic amine production by food-borne pathogens

Table 3 shows the production of ammonia and biogenic amine by food-borne pathogenic bacteria in AIDB. Significant differences were observed between treatment groups and control groups in terms of ammonia biogenic amine production ($p < 0.05$). Most of the food-borne pathogens produced great concentrations of ammonia. In the previous studies, much lower ammonia formation was reported for Gram-positive

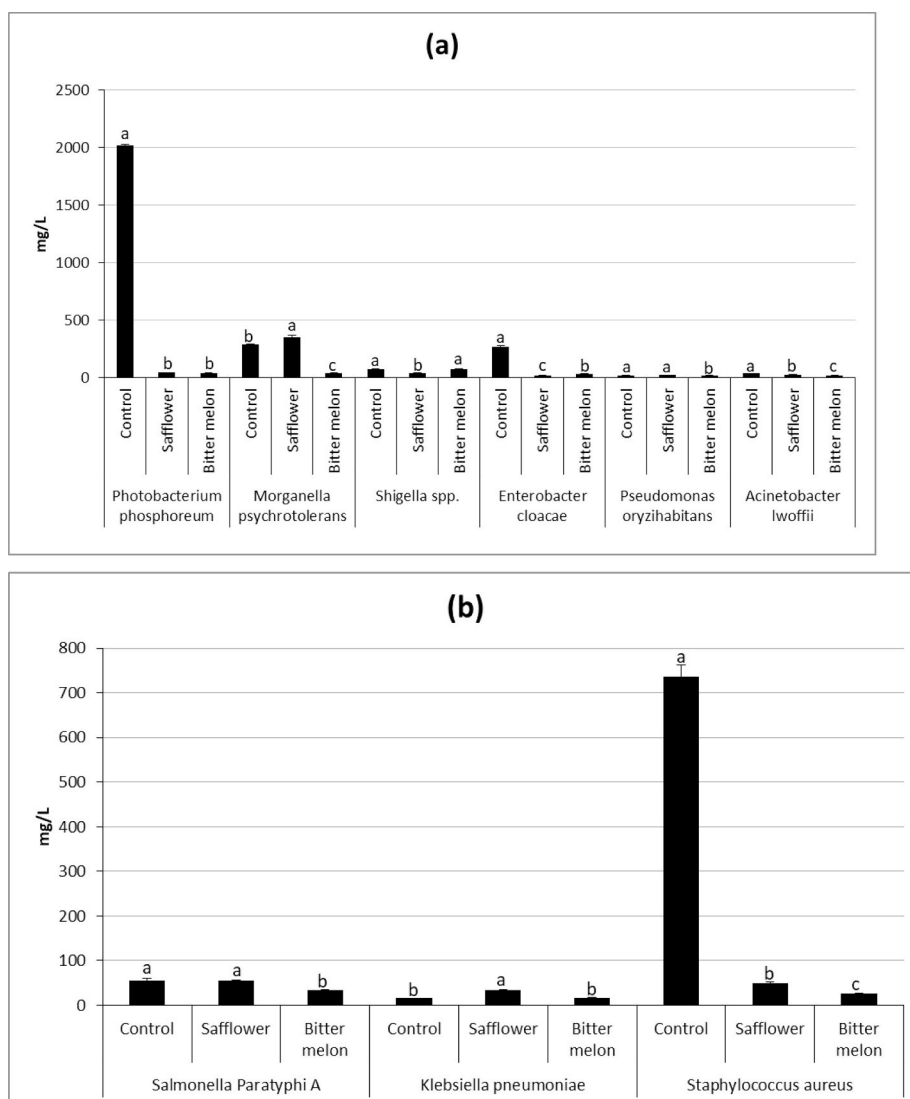


Fig. 2. Tyramine production by fish spoilage (a) and food-borne pathogen bacteria (b). Data are expressed as mean value of three samples, ^{a-c} indicate significant differences ($p < 0.05$) between control and treated group.

and negative food-borne pathogens in histidine and tyrosine decarboxylase broth (Ozogul et al., 2015; Ozogul et al., 2018; Burgut, 2020). In the present study, the highest ammonia production was occurred by *K. pneumoniae* (2155.06 mg/L). Ammonia accumulation by *K. pneumoniae* and *S. aureus* significantly decreased with the addition of bitter melon and safflower extracts ($p < 0.05$). Özogul (2004) found the ammonia production of *K. pneumoniae* as 85.55 mg/L. A similar observation was reported by Gokdogan et al. (2012) who found stimulation effect of zeolite application on ammonia production by food-borne pathogens in histidine decarboxylase broth.

Putrescine in fish tissue is mainly generated by the genus *Staphylococcus* (Wunderlichova et al., 2014). In this study, *S. aureus* produced the highest level of putrescine (288.76 mg/L) in AIDB. Kuley and Özogul (2011) reported lower putrescine level by *S. aureus* in tyrosine decarboxylase broth (74.64 mg/L). Cadaverine formed more than 75 mg/L by food-borne pathogens. Lower cadaverine and putrescine accumulation (<40 mg/L) was reported by Burgut (2020). Bitter melon extract inhibited putrescine production by *K. pneumoniae*, although safflower extract resulted in higher putrescine and cadaverine accumulation by *K. pneumoniae* and *S. Paratyphi A*. On the other hand, greatly lower putrescine and cadaverine content by *S. aureus* was found in the existence of safflower extract in AIDB ($p < 0.05$). The existence of bitter

melon statistically did not affect putrescine and cadaverine accumulation by *S. Paratyphi A*. Inhibitory effects of ethanolic propolis extracts as well as stimulatory effects of water extracts of propolis on most of food-borne pathogens tested were observed in the other study conducted by Burgut (2020).

Spermine and spermidine production of pathogens in the AIDB were above 45 and 110 mg/L. Bitter melon extract had an suppressing effect on production of spermidine by *K. pneumoniae* while safflower extract led to significantly lesser spermidine generation by *S. aureus* and *S. Paratyphi A* compared to the control group ($p < 0.05$). In addition, it is interestingly observed that spermine formation by *S. Paratyphi A* and *K. pneumoniae* was synergistically increased by the presence of safflower extract but suppressed by bitter melon extract.

Shalaby (1996) reported that histamine, tyramine and 2-phenylethylamine were the most toxic biogenic amines. *S. aureus* (355.53 mg/L) was the bacteria with the highest histamine production activity among the food-borne pathogenic bacteria in AIDB (Fig. 1). Unlike this study result, Gokdogan et al. (2012) found that *S. aureus* did not have ability to form histamine in histidine decarboxylase broth. None or very small amount (<4 mg/L) of histamine production of food-borne bacterial strains were also reported (Özogul et al., 2015; Özogul et al., 2018).

Bitter melon and safflower extract led 9 and 12-fold lower histamine

Table 3

Ammonia and biogenic amine production by food borne pathogen in anchovy infusion decarboxylase broth (mg/L).

	AMN	PUT	CAD	SPD	TRP	PHEN	SPN	SER	TMA	DOP	AGM	Groups
SP	1226.38 ±	74.27 ±	136.07 ±	158.69 ±	24.26 ±	15.93 ±	62.05 ±	139.60 ±	4.35 ±	187.64 ±	32.04 ±	C
	24.44 ^{a*}	6.51 ^b	8.26 ^b	2.50 ^b	1.42 ^a	0.31 ^b	2.20 ^b	8.12 ^b	0.07 ^b	5.60 ^c	1.42 ^c	
	1216.64 ±	212.65 ±	239.96 ±	178.57 ±	19.54 ±	131.74 ±	98.30 ±	262.55 ±	94.93 ±	298.57 ±	61.82 ±	S
KP	23.00 ^a	10.57 ^a	12.52 ^a	9.54 ^b	1.41 ^b	7.84 ^a	3.58 ^a	18.72 ^a	5.67 ^a	14.54 ^b	3.73 ^a	
	1180.67 ±	59.03 ±	133.49 ±	224.28 ±	2.28 ±	29.89 ±	47.11 ±	123.30 ±	5.91 ±	393.65 ±	48.82 ±	B
	39.64 ^a	1.31 ^b	0.42 ^b	14.80 ^a	0.39 ^c	1.33 ^b	4.13 ^c	1.30 ^b	0.58 ^b	14.86 ^a	4.02 ^b	
SA	2155.06 ±	113.22 ±	75.05 ±	110.06 ±	18.82 ±	23.39 ±	49.25 ±	69.31 ±	4.09 ±	138.79 ±	29.58 ±	C
	109.98 ^a	7.99 ^b	6.82 ^b	2.16 ^b	1.51 ^b	1.17 ^b	4.22 ^b	4.54 ^b	0.10 ^b	3.95 ^a	1.46 ^{ab}	
	1503.06 ±	163.09 ±	109.21 ±	130.80 ±	31.22 ±	107.77 ±	90.57 ±	135.47 ±	102.84 ±	113.12 ±	31.42 ±	S
SA	56.12 ^b	4.62 ^a	6.61 ^a	4.35 ^a	1.41 ^a	2.83 ^a	2.70 ^a	12.40 ^a	7.01 ^a	7.73 ^b	1.31 ^a	
	1414.47 ±	86.59 ±	74.34 ±	70.15 ±	8.17 ±	21.40 ±	36.91 ±	61.59 ±	10.69 ±	131.41 ±	26.66 ±	B
	80.09 ^b	3.09 ^c	3.57 ^b	4.29 ^c	0.27 ^c	1.52 ^b	0.16 ^c	3.74 ^b	0.55 ^b	7.50 ^{ab}	1.65 ^b	
SA	1242.24 ±	288.76 ±	130.12 ±	145.69 ±	140.95 ±	53.99 ±	145.98 ±	129.62 ±	52.18 ±	285.78 ±	44.76 ±	C
	16.65 ^a	16.21 ^a	0.42 ^a	0.96 ^a	5.72 ^a	2.35 ^b	1.98 ^a	5.18 ^a	1.92 ^a	7.70 ^a	1.93 ^a	
	1085.10 ±	80.25 ±	62.21 ±	107.88 ±	23.95 ±	88.84 ±	61.25 ±	108.06 ±	37.89 ±	226.58 ±	48.85 ±	S
SA	28.27 ^b	0.83 ^c	3.23 ^c	1.02 ^b	1.41 ^b	2.83 ^a	3.41 ^b	1.51 ^b	0.83 ^b	0.77 ^b	2.61 ^a	
	933.18 ±	206.17 ±	99.18 ±	150.30 ±	10.89 ±	35.58 ±	48.31 ±	109.94 ±	16.92 ±	240.59 ±	45.85 ±	B
	17.32 ^c	10.67 ^b	2.78 ^b	8.66 ^a	1.00 ^c	2.81 ^c	1.29 ^c	3.46 ^b	0.42 ^c	1.56 ^b	3.37 ^a	

*Mean (n = 3) ± Standard deviation. ^{a-c} Display significant differences (p < 0.05) between control and treated group in a row. SP: *Salmonella* Paratyphi A, KP: *Klebsiella pneumoniae*, SA: *Staphylococcus aureus*, C: Control group, S: Group treated with strawflower extract, B: Group treated with bitter melon extract. AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; TRP, tryptamine; PHEN, 2-phenylethyl amine; SPN, spermine; SER, serotonin; TMA, trimethylamine; DOP, dopamine, AGM, agmatine.

accumulation by *S. aureus*. However, safflower extract stimulated histamine formation by *S. Paratyphi A* and *K. pneumoniae*. As in this work, stimulation or inhibition effect of extract or antimicrobial compounds used on histamine production by food-borne pathogens were noticed in the other studies (Burgut, 2020; Gokdogan et al., 2012; Özogul et al., 2018; Özogul et al., 2015).

Serotonin production by the bacteria examined varied between 69.31 and 139.60 mg/L. Serotonin formation by *S. Paratyphi A* and *K. pneumoniae* restricted in the presence of bitter melon extract but stimulated by addition of safflower extract. Along with this, bitter melon and safflower extracts exerted a suppressing effect on serotonin production in *S. aureus*.

Among food-borne pathogens, *S. aureus* was main tyramine producer in AIDB, with value of 736.53 mg/L (Fig. 2). The tyramine production level of *S. aureus* was 469.98 mg/L in tyrosine decarboxylase broth (Özogul, Kaçar, & Kuley, 2015), but 0.38 mg/L in histidine decarboxylase broth (Özogul, Kacar, & Hamed, 2015). However, food-borne pathogens generated tyramine between 0.04 and 910 mg/L in different amino acid decarboxylase broth (Burgut, 2020; Gokdogan et al., 2012; Özogul et al., 2018). The existence of safflower and bitter melon extract in AIDB showed no obvious difference on tyramine accumulation by *S. Paratyphi A* and *K. pneumoniae*, respectively. Both extracts considerably suppressed tyramine production by *S. aureus* (p < 0.05). Similar observations were also observed in the other studies regarding the variable effects of antimicrobials on tyramine production by food-related pathogens (Burgut, 2020; Gokdogan et al., 2012; Özogul et al., 2015b, 2018).

The bacterial strains produced agmatine above 29 mg/L. The existence of extract had no statistically significant effect on agmatine formation by *S. aureus* and *K. pneumoniae*.

4. Conclusion

The study results revealed that bacteria tested, mainly fish spoilage bacteria had a high ability to accumulate most of biogenic amine including toxicologically important ones in AIDB. The study also confirmed that the number of bacteria in the medium was not directly related to the bacterial biogenic amine production. Application of bitter melon extract had more noticeable effect, particularly for suppressing ammonia, putrescine, cadaverine, histamine, tyramine, serotonin and TMA production, although the effect of the extracts varied according to bacterial strains. As a result of this study, it is suggested that ethanolic

plant extracts, especially bitter melon extract, can be used as natural antibacterial agents in foods. However, more detailed studies are required to see their real impact on food systems.

CRedit authorship contribution statement

Mebure Nüket Yavuzer: Conceptualization, Funding acquisition, Formal analysis, Writing – original draft, Conception and design of study, acquisition of data, analysis and/or interpretation of data, Drafting the manuscript, revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published. **Emre Yavuzer:** Conceptualization, Funding acquisition, Formal analysis, Writing – original draft, Conception and design of study, acquisition of data, analysis and/or interpretation of data, Drafting the manuscript, revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published. **Esmeray Kuley:** Conceptualization, Funding acquisition, Formal analysis, Writing – original draft, Conception and design of study, acquisition of data, analysis and/or interpretation of data, Drafting the manuscript, revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published.

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