International Journal of
Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.4.11

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(4):265-271 (2019)

Aqueous and ethanolic extracts of propolis for the control of tyramine production by food-borne pathogens

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Abstract

The influences of aqueous and ethanolic extracts of propolis (1%) on growth of common Gram-negative (*Salmonella* Parathyphi A, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Klebsiella pneumoniae)* and -positive *(Listeria monocytogenes, Staphylococcus aureus* and *Enterococcus faecalis*) food-borne pathogens and their biogenic amines (BAs) production were examined in tyrosine decarboxylase broth (TDB). The highest growth inhibitory activity was observed against Gram-negative *S.* Paratyphi A in the existence of ethanolic and aqueous extracts of propolis, with 2.49 and 1.9 log reduction, respectively. Ethanolic extracts of propolis were more effective than that of aqueous extract on growth inhibition of *L. monocytogenes* ($p<0.05$). Both extracts of propolis had significant effect on reducing ammonia production by bacteria ($p<0.05$). Tyramine, dopamine, agmatine and spermine were major amines formed in TDB. Tyramine production was the lowest with *S.* Paratyphi A (1.94 mg/L) and highest with *E. faecalis* (254.93 mg/L). The existence of ethanolic propolis extracts in TDB led to significantly fewer tyramine production by Gram-positive *S. aureus, L. monocytogenes* and *E. faecalis,* and Gram-negative *C. jejuni* ($p<0.05$). Histamine produced lower than 1.3 mg/L by all food-borne pathogens. Ethanolic extracts of propolis generally led to lower histamine production by bacteria. The influence of propolis on BAs production varied according to type of extracts, specific BAs and bacterial strains. However, the aqueous of propolis generally showed a synergistic effect on most of BAs mainly tyramine production by bacteria. Thus, the use of propolis ethanolic extracts appeared to be more suitable than aqueous extract to control tyramine production in foods.

Keywords: Propolis, Tyramine, Food-borne pathogens, Food safety

Introduction

Food safety is a major public health concern worldwide (Liu et al., 2019). Food-borne illnesses are often related with pathogens, toxins and chemicals, and are a global public health problem for a variety of causes. New risks constantly occur while others are inhibited (Camino-Feltes et al., 2017). Pathogenic bacteria have a capacity to form BAs via amino acid decarboxylation action. Some bacteria species including *Bacillus, Citrobacter, Klebsiella, Escherichia, Proteus, Pseudomonas* and *Photobacterium* may decarboxylate one or more amino acids (Silla-Santos, 1996, Karovičová and Kohajdová, 2005).

Digestion of food having excessive levels of BAs are occupied in several toxicological symptoms which resulted in various kinds of foodborne illness (headaches, low blood pressure, heart palpitations, edema, vomiting, and diarrhea) (Maintz and Novak, 2007). Therefore, the existence of BAs can influence both the feature and the safety of foods (Gram and Dalgaard, 2002). BAs have been found in many foods including fish, meat, cheese, vegetables and wines (Lorenzo et al., 2007). Tyramine is known as the most commonly accumulated BAs in cheese (Fernandez et al., 2007). Tyramine can cause physiological reactions such as peripheral vasoconstriction, improved cardiac output, elevated respiration, increased blood glucose, and release of norepinephrine (Shalaby, 1996). In view of the fact that the detection of the “cheese reaction” hyperten-

Cite this article as:
Burgut, A. (2019). Aqueous and ethanolic extracts of propolis for the control of tyramine production by food-borne pathogens. Int. J. Agric. Environ. Food Sci., 3(4), 265-271
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.11
Received: 01 September 2019 **Accepted:** 17 December 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 265-271
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

sive crisis caused by tyramine intake among individuals on monoamine oxidase inhibitor (MAOI) drugs, several studies have focused on the tyramine content in foods (Marcobal et al. 2012). Control of BAs is crucial to ensure the safety of fermented foods (Li et al., 2018). BAs production can be restricted by preventing bacterial proliferation or the decarboxylase action of bacteria (Wendakoon and Sakaguchi 1995).

Propolis is a natural constituent accumulated by bees from native flora (Majiene et al., 2007). Propolis have natural combinations of several secondary metabolites that exert a variety of bioactivity e.g. antibacterial, antiulcer, antioxidant, and anti-viral activities (De Figueiredo et al., 2015). Since propolis is safe for humans if extensive dose is not taken (Satoshi et al., 2005), it possesses a wide range of uses such as preservatives in food products (fruits, juice, soft drinks, fish and meat products) and also in veterinary pharmaceutical applications (Casquite et al. 2016). Antimicrobial activity of propolis is due to their primary ingredients of flavonoids, phenolic compounds diterpenic acids and aromatic acids (Afrouzan et al., 2018). Propolis extracts exhibited the highest antimicrobial activity towards the Gram-positive food-borne pathogen bacteria e.g. *Bacillus cereus* and *S. aureus* (Nedji and Loucif-Ayad, 2014). The propolis exerted a noticeable antibacterial activity against the Gram-positive strain (*L. monocytogenes*) and restricted action against Gram-negative *Salmonella* Enteritidis depending on different propolis dose (Temiz et al., 2011). Propolis extracts had an inhibitory effect towards *S. aureus* isolated from instant soups, although their antimicrobial effects varied depending on their geographical regions (Apaydın and Gümüş, 2018).

Although many studies have been done about the antibacterial properties of propolis, there are limited studies regarding its impact on tyramine and other BAs accumulation by bacteria. Thus, the aim of the study was to examine the impact of aqueous and ethanolic extracts of propolis on tyramine and other BAs produced by common Gram-negative and positive food-borne pathogens.

Material and Method

Food-borne pathogens

Enterococcus faecalis ATCC29212, *Staphylococcus aureus* ATCC29213, *Klebsiella pneumoniae* ATCC700603, *Campylobacter jejuni* ATCC 33560 and *Listeria monocytogenes* ATCC19112 were purchased from the American Type Culture Collection (Rockville, MD, USA). *Salmonella* Parathyphi A NCTC13 and *Yersinia enterocolitica* NCTC 11175 were provided from the National Collection of Type Cultures (London, UK, Özogul et al., 2011).

Preparation of propolis extracts

Propolis from *Apis mellifera* was obtained using a commercial plastic trap in August 2018 (Adana, Turkey). Crude propolis was ground into powder and extracted with ethanol (70%) or water (100%). They placed in daily shakable containers for 48 h. Solutions of propolis were prepared aseptically and protected from light. They were stored in a dark place at 4 ºC until analysis.

Culture Conditions and Bas Analysis

The production of BAs from all food-borne pathogens in this work was monitored using tyrosine decarboxylase broth (TDB) suggested by Klausen and Huss (1987). The extraction process and derivatisation of BAs were performed in accordance with the method of Kuley and Ozogul (2011). The mobile phase contained acetonitrile (Sigma 439134, Steinheim, Germany) and grade water for the amine analyses. A high-performance liquid chromatography (HPLC, Shimadzu, Kyoto, Japan) was used to detect BAs. ODS Hypersil (5µ, 250x6 mm, Phenomenex, UK) was used as column.

Monitoring bacterial growth in TDB

Estimation of total viable counts in TDB was made in triplicate. Plate count agar (Fluka 70152; Steinheim, Switzerland) was used to growth of bacteria. Spread plates with appropriate dilution of 0.1 ml were incubated for 2 days at 30˚C.

Statistical Analysis

Mean and standard deviation of three replicates were measured. The significance of differences ($p<0.05$) was determined using Oneway ANOVA with SPSS 15.0 version (SPSS Inc., USA).

Result and Discussion

Bacterial growth in TDB

Bacterial load in TDB in the existence or absence of propolis extracts was shown in Table 1. Bacterial loads in control groups were in range from 8.41 log cfu/ml for *S.* Paratyphi A to 8.95 log cfu/ml for *Y. enterocolitica*. Significant differences in bacterial load apart from *S. aureus* were observed between control and propolis treated groups ($p<0.05$). By contrast, propolis extracts exhibited different extents of inhibitory effects against *S. aureus,* depending on concentration, collecting area and time (Lu et al., 2005; Apaydın and Gümüş, 2018). Presence of water or ethanolic propolis extracts in TDB resulted in lower bacterial growth ($p<0.05$), although extracts statistically did not affect growth of *S. aureus*. The highest inhibitory effects were observed against Gram-negative *S.* Paratyphi A (>1.9 log reduction), *Y. enterocolitica* (>1.2 log reduction) and *C. jejuni* growth (>1 log reduction). Propolis acted against both Gram-positive and Gram-negative bacteria.

The antimicrobial action of propolis is related to its natural ingredients and is different in individual countries (Przybyłek and Karpiński, 2019). However, the propolis was also reported to have better action against Gram-positive bacteria than Gram-negative (Kim and Chung, 2011; Pobiega et al., 2019). Among Gram-positive bacteria, the highest growth inhibition of propolis extracts was found against *E. faecalis*, with about 0.7 log reduction. The effects of ethanolic and aqueous propolis extracts on bacterial growth were statistically similar except for *L. monocytogenes*. However, Al-Ani et al. (2018) found that aqueous extract of propolis exerted poor bactericidal action against Gram-negative bacteria. Similarly, ethanolic propolis extracts were more effective than that of water extract on growth inhibition of *L. monocytogenes* in TDB ($p<0.05$). Ethanol extract of propolis exhibited strong antilisterial activity (Pobiega et al., 2019; Temiz et al., 2011). The antibacterial action of propolis is as a consequence of the direct action on the microorganism and encouragement of the immune sys-

tem causing in initiation of natural defences of the organism (Sforcin and Bankova, 2011; Przybyłek and Karpiński, 2019).

Biogenic amine production by bacteria

Figure 1 shows tyramine accumulation by food-borne pathogens. Tyramine production was the lowest with *S.* Paratyphi A (1.94 mg/L) and highest with *E. faecalis* (254.93 mg/L). *Enterococcus* spp. are important tyramine producer in fermented foods and able to yield TYR more than 520 mg/L (Connil et al., 2002; Özogul and Özogul, 2007; de Palencia et al., 2011). Tyramine accumulation was the weakest (6.42 mg/L) with *K. pneumoniae* among food-borne pathogens tested (Özogul et al., 2015). In the present study, *K. pneumoniae* accumulated tyramine at a moderate level (29.53 mg/L).

Presence of propolis ethanolic extracts in TDB led to significantly fewer tyramine production by Gram-positive *L. monocytogenes, E. faecalis, S. aureus* and Gram-negative *C. jejuni* ($p<0.05$). However, aqueous extracts of propolis caused considerably higher tyramine formation by all of food-borne pathogens, mainly Gram negative-bacteria. The highest stimulating effect of aqueous propolis extracts was found for *S.* Paratyphi A, with 146-fold higher tyramine production, which was not consistent with result of bacterial load in TDB. Tyramine production by *K. pneumonia* and *Y. enterocolitica* were also 11 and 13 fold higher with propolis aqueous extracts. The presence of 6 mg tyramine in one or two usual servings of food is thought to be sufficient to cause a mild adverse event while 10–25 mg will produce a severe adverse event in those using MAOI drugs (Da Prada et al., 1988). A limit of 200–800 mg in one or two usual servings has been proposed for tyramine in foods (Da Prada et al., 1988; Marcobal et al., 2012). Food borne-pathogens produced tyramine between 4.93 (*Y. enterocolitica*) and 37.64 mg/L (*L. monocytogenes*) in the presence of ethanolic propolis extract.

Table 1. Bacterial growth in tyrosine decarboxylase broth with or without propolis extracts (log cfu/mL)

	Control	Ethanolic extracts of propolis	Water extracts of propolis
Gram-positive bacteria			
L. monocytogenes	8.69±0.01a	8.51±0.02c	8.62±0.01b
E. faecalis	8.56±0.01a	7.88±0.10b	7.77±0.01b
S. aureus	8.55±0.05a	8.51±0.00a	8.50±0.00a
Gram-negative bacteria			
S. Parathyphi A	8.41±0.22a	6.51±0.16b	5.92±0.21b
K. pneumoniae	8.58±0.00a	7.83±0.08b	7.70±0.09b
Y. enterocolitica	8.95±0.08a	7.68±0.03b	7.72±0.06b
C. jejuni	8.90±0.05a	7.68±0.07b	7.85±0.11b

*Data are stated as mean value of three samples, Mean value±Standard deviation.
[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a column.

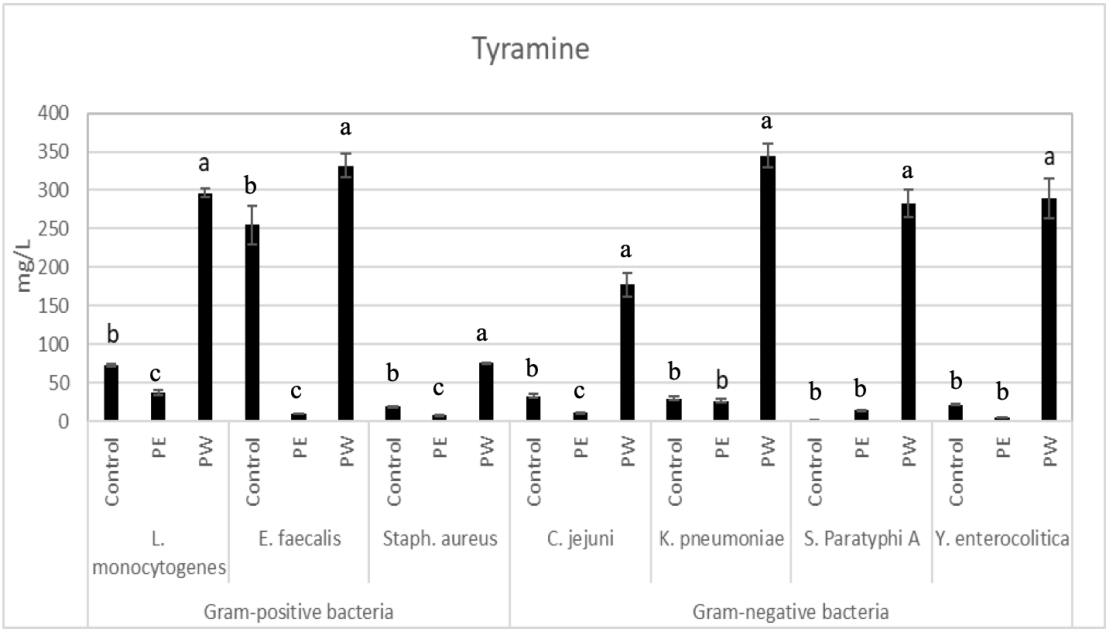

Figure 1. Tyramine accumulation by food borne pathogens in the absence or existence of propolis extracts. PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. [a–c] Show statistically significant differences ($P < 0.05$) between control and treated group

Table 2 and 3 illustrate ammonia and BAs production by Gram-positive and -negative food-borne pathogen in the absence or presence of propolis extracts, respectively. Ammonia production was in range from 167.63 mg/L by *C. jejuni* to 469.57 mg/L by *K. pneumoniae*. The highest ammonia accumulation was reported for *K. pneumoniae* and *S. aureus* (470-525 mg/L) in TDB (Kuley and Özogul, 2011), which was consistent with current results. Both extracts of propolis were significantly effective on reducing ammonia production by bacteria ($p<0.05$). Ethanolic extracts of propolis was more effective on reducing ammonia production by *E. faecalis* (2 fold-lower) and *S. aureus* (3-fold lower) than that of aqueous extracts ($p<0.05$), although both extracts of propolis showed statistically similar inhibitory effect on ammonia production by most of the bacteria.

Food-borne bacteria produced all amine tested apart from tryptamine. Tyramine, dopamine, agmatine and spermine were main amines produced in TDB, which was in agreement with result of Özogul et al. (2015). Putrescine production was the highest with *Y. enterocolitica* (35.75 mg/L) and *C. jejuni* (32.50 mg/L), whilst *C. jejuni* (40.32 mg/L) and *L. monocytogenes* were main cadaverine producer. *S. aureus* and *S.* Paratyphi A produced considerably higher concentrations of putrescine than other food-borne pathogens (De las Rivas et al., 2006). In the current study, *S. aureus* and *S.* Paratyphi A produced putrescine at the level of 15.29 and 2.70 mg/L, respectively. Putrescine and cadaverine production by *L. monocytogenes* and *C. jejuni* was significantly inhibited by both propolis extracts. Moreover, presence of ethanolic extracts generally led to considerably lower putrescine and cadaverine production, although aqueous extracts of propolis mostly induced higher putrescine and cadaverine accumulation in TDB broth. Spermidine production by bacteria was above 4 mg/L and generally suppressed by both extracts. Spermine was produced at the highest level by *L. monocytogenes* and *E. faecalis* (about 60 mg/L). Although spermine production by *L. monocytogenes* and *Y. enterocolitica* considerably suppressed by presence of aqueous or ethanolic extracts of propolis, aqueous extracts of propolis resulted in higher spermine production by Gram-positive *S. aureus* and Gram-negative *C. jejuni* and *K. pneumoniae.*

Histamine is known as the causative agent of histamine intoxication and causes diarrhea, headache, rhinoconjunctival symptoms and other reactions in immunocompromised patients (Maintz and Novak, 2007; Comas-Basté et al., 2019). The US Food and Drug Administration (1995) recommended an upper limit of histamine to 5 mg/100 g (50 ppm) in fish (Al-Bulushi et al., 2009), whilst the European Commission (Commission Regulation EC No. 1441/2007, 2007) has suggested that the mean content of histamine in fish should not be above 10 mg/100g. Histamine produced lower than 1.3 mg/L by all food-borne pathogens. Ethanolic extracts of propolis was generally led to lower histamine production by bacteria. However, inhibitory effect of aqueous extract of propolis on histamine formation was just detected for *Y. enterocolitica* and *C. jejuni*, whilst it stimulated histamine production by *E. faecalis*, *S. aureus* and *K. pneumoniae*. The highest histamine production (3.24 mg/L) was found for *E. faecalis* in the existence of aqueous extracts of propolis. Serotonin production by bacteria was <6 mg/L in TDB. Serotonin accumulation by the most of bacteria was also inhibited by ethanolic propolis extract. Suppression effects of aqueous extract on serotonin production were observed for *L. monocytogenes*, *S. aureus* and *C. jejuni*.

TMA production by bacteria was between 1.25 and 50.34 mg/L by *S.* Paratyphi A and *K. pneumoniae,* respectively. Apart from *K. pneumoniae* and *Y. enterocolitica*, stimulatory effect of propolis extracts on TMA production was found. *K. pneumoniae* and *S.* Paratyphi A had good ability to produce dopamine in TDB, with corresponding value of 554.84 and 523.52 mg/L, whilst Gram-positive bacteria produced dopamine below 145 mg/L.

Propolis ethanolic extract had a significant effect on suppression of dopamine production by Gram-negative bacteria mainly *C. jejuni*, but ineffective against Gram-positive bacteria apart from *L. monocytogenes*. However, aqueous extract of propolis increased dopamine production of all Gram-positive bacteria tested, whilst it did not affect dopamine formation by *C. jejuni* and *K. pneumoniae*. Agmatine accumulation was the uppermost by *C. jejuni* and *K. pneumoniae*, with corresponding value of 95.50 and 90.81 mg/L. Both propolis extracts resulted in lower agmatine production by these bacteria ($p<0.05$), whereas there were no substantial differences in agmatine production by these bacteria between control and aqueous extracts groups. However, agmatine production by Gram-positive bacteria increased with aqueous extracts of propolis ($p<0.05$). The antimicrobial mechanisms of propolis are multiple and complex, being determined by the synergistic effects of phenolic compounds and other biologically active components (Hazem et al., 2017). Increase in most of BAs in the presence of aqueous extracts of propolis may be due to the fact that the basic biologically active constituents of propolis are hardly soluble in water (Kubiliene et al., 2015). Other reasons for the increase in BAs may be due to the presence of other synergistic conditions, such as changes in water activity and pH. Therefore, more detailed studies are needed to determine this effect.

Conclusions

Propolis extracts showed good antimicrobial activity against food-borne pathogens apart from *S. aureus* in TDB. The highest growth inhibitory activity of propolis extracts was observed for Gram-negative *S.* Paratyphi A, *C. jejuni* and *Y. enterocolitica*. Bacterial growth did not generally associate well with BAs production. The influence of propolis on BAs formation varied according to the type of extracts, specific BAs and bacterial strains, although ammonia production by bacteria was suppressed in the existence of propolis extract. The study results revealed that aqueous extract of propolis showed synergistic effects on the most of BAs production by bacteria mainly tyramine. In conclusion, it has been suggested that the use of the ethanolic propolis extract in food products may be more suitable than the aqueous extract of propolis.

Table 2a. Ammonia and BAs accumulation by Gram-positive food borne pathogen in the absence or presence of propolis extracts (mg/L).

	AMN	PUT	CAD	SPD	PHEN	SPN
LM	206.42±8.17a 123.08±7.59b 129.44±5.23b	3.99±0.10a 0.50±0.00c 0.87±0.02b	33.27±1.07a 5.17±0.08c 19.60±0.80b	7.53±0.04a 0.00±0.00b 0.00±0.00b	2.73±0.12b 0.55±0.07c 24.28±0.95a	62.21±4.15a 24.68±1.16c 45.99±3.19b
EF	213.70±3.80a 89.13±2.90c 115.92±4.99b	3.45±0.28b 0.00±0.00c 12.42±0.12a	7.38±0.86b 6.25±0.08b 37.42±0.44a	21.25±0.43a 13.95±0.15b 0.00±0.00c	8.62±0.63b 0.92±0.03c 32.47±0.58a	61.23±5.07a 53.91±4.85ab 44.50±0.91c
SA	274.58±11.76a 88.92±5.35c 135.25±13.35b	15.29±1.31b 7.45±0.12c 26.47±0.36a	10.15±0.03b 2.42±0.08c 17.52±1.26a	4.16±0.13a 2.01±0.00b 3.86±0.21a	0.93±0.03c 2.23±0.05b 5.38±0.07a	4.82±0.14b 5.19±0.44b 27.07±0.06a

*Data are expressed as mean value of three samples, Mean value±Standard deviation.

[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a row.

LM: *Listeria monocytogenes*, EF: *Enterococcus faecalis*, SA: *Staphylococcus aureus*, C: control group without propolis extract addition, PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; PHEN, 2-phenylethyl amine; SPN, spermine

Table 2b. Ammonia and BAs accumulation by Gram-positive food borne pathogen in the absence or presence of propolis extracts (mg/L).

	HIS	SER	TMA	DOP	AGM	Groups
LM	1.29±0.04a 0.28±0.00b 1.28±0.03a	4.06±0.16a 1.85±0.04c 3.38±0.05b	1.88±0.02c 3.44±0.06b 5.64±0.84a	70.50±3.69c 128.82±6.92b 317.28±14.66a	39.79±2.75c 60.44±0.64b 76.34±7.14a	C PE PW
EF	0.89±0.05b 0.42±0.01c 3.24±0.06a	1.56±0.04b 1.42±0.08b 3.72±0.20a	8.28±0.77c 21.99±0.16b 27.76±1.60a	62.35±1.77b 64.17±3.77b 155.24±1.94a	25.15±1.13c 54.33±1.94b 64.54±1.31a	C PE PW
SA	0.38±0.00b 0.13±0.01b 2.43±0.23a	3.25±0.13a 1.08±0.01c 2.42±0.14b	3.06±0.16c 3.75±0.02b 13.20±0.27a	144.87±7.37b 129.87±7.88b 459.91±29.80a	15.79±0.86b 16.74±0.77b 64.39±2.91a	C PE PW

*Data are expressed as mean value of three samples, Mean value±Standard deviation.

[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a row.

LM: *Listeria monocytogenes*, EF: *Enterococcus faecalis*, SA: *Staphylococcus aureus*, C: control group without propolis extract addition, PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. HIS: histamine, SER, serotonin; TMA, trimethylamine; DOP, dopamine, AGM, agmatine

Table 3a. Ammonia and BAs accumulation by Gram-negative food borne pathogen in the absence or presence of propolis extracts (mg/L)

	AMN	PUT	CAD	SPD	PHEN	SPN
YE	272.12±21.91a 118.28±8.15b 82.75±3.28b	35.75±0.07b 1.91±0.13c 60.20±2.78a	17.05±0.10a 15.21±1.50a 14.20±1.35a	19.63±0.80a 8.86±0.46b 0.00±0.00c	10.21±0.23b 0.00±0.00c 11.45±0.48a	55.85±1.43a 27.70±0.02c 36.53±0.15b
CJ	167.63±11.25a 97.37±4.66b 98.08±6.87b	32.50±2.76a 0.00±0.00c 25.97±1.37b	40.32±1.89a 7.39±0.17c 33.73±2.36b	10.27±0.29a 0.00±0.00c 1.26±0.01b	0.72±0.05b 0.54±0.06b 1.11±0.10a	35.35±2.31b 35.04±0.27b 67.37±2.65a
KP	469.57±24.54a 94.62±6.89b 107.91±1.24b	1.36±0.14b 0.35±0.03b 61.94±5.38a	4.85±0.49b 3.29±0.02b 35.43±2.26a	31.23±2.13a 0.00±0.00b 2.16±0.13b	0.39±0.01c 0.90±0.13b 2.48±0.16a	32.36±2.46b 30.52±2.45b 48.01±3.92a
SP	268.48±23.57a 102.63±7.72b 143.94±12.71b	2.70±0.18b 0.69±0.02c 26.71±0.33a	24.57±1.70a 13.69±0.15c 19.18±1.80b	18.39±1.07a 17.55±0.07a 4.57±0.00b	0.78±0.00b 0.00±0.00b 16.82±1.29a	53.30±2.78a 31.89±1.27b 50.91±3.90a

*Data are expressed as mean value of three samples, Mean value±Standard deviation.

[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a row.

YE: *Yersinia enterocolitica*, CJ: *Campylobacter jejuni*, KP: *Klebsiella pneumoniae*, SP: *Salmonella* Paratyphi A, C: control group without propolis extract addition, PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; PHEN, 2-phenylethyl amine; SPN, spermine

Table 3b. Ammonia and BAs accumulation by Gram-negative food borne pathogen in the absence or presence of propolis extracts (mg/L)

	HIS	SER	TMA	DOP	AGM	Groups
YE	0.99±0.01a 0.63±0.05c 0.78±0.04b	2.66±0.27b 1.10±0.08c 3.93±0.32a	12.27±0.55b 3.84±0.22c 16.77±1.05a	263.42±15.39b 125.20±7.35c 308.47±16.99a	74.88±1.52b 85.09±5.02ab 86.22±2.43a	C PE PW
CJ	1.18±0.03a 0.47±0.01c 0.86±±0.07b	5.79±0.28a 0.73±0.03b 1.02±0.04b	5.39±0.19b 24.97±0.01a 6.12±0.50b	393.80±14.95a 0.00±0.00c 270.91±3.14b	95.50±8.19a 48.31±3.24b 85.92±0.73a	C PE PW
KP	0.38±0.00b 0.42±0.02b 0.84±0.06a	2.48±0.02b 1.10±0.00c 3.71±0.46a	50.34±0.81a 13.40±1.27c 35.32±0.36b	554.84±37.36a 205.63±7.97b 630.83±29.43a	90.81±8.81a 64.23±3.84b 93.57±2.62a	C PE PW
SP	0.70±0.03a 0.31±0.01b 0.77±0.02a	2.24±0.16b 0.77±0.08c 4.95±0.29a	1.25±0.00c 3.15±0.14b 15.25±0.26a	523.52±13.94a 296.97±2.15b 521.92±3.47a	25.57±1.00b 36.07±0.37b 131.67±6.35a	C PE PW

*Data are expressed as mean value of three samples, Mean value±Standard deviation.

[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a row.

YE: *Yersinia enterocolitica*, CJ: *Campylobacter jejuni*, KP: *Klebsiella pneumoniae*, SP: *Salmonella* Paratyphi A, C: control group without propolis extract addition, PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. HIS: histamine, SER, serotonin; TMA, trimethylamine; DOP, dopamine, AGM, agmatine

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