DOI:10.22059/IJVM.2023.362975.1005428 Iranian Journal of Veterinary Medicine Original Article

Online ISSN: 2252-0554

The Effect of Some Nano Plant Extract on Bacteria Producing Biogenic Amines Isolated From Minced Meat

Amany Omar Selim[1], Marwa Magdy Abdel Salam[2],Rasha Nagiub Abdallah Hassan [2],Gehan Elsaeid Mustafa[3] , Zeinab AbdELrahman Mahdy[1]

1-Bacteriology Department,Benha Provincial Laboratory, Animal Health Research Institue,Agriculture Research Center(ARC), Cairo,Egypt..

2- Foof Hygiene Deptartment, Benha Provincial Laboratory, Animal Health Research Institute, Agriculture Research Center,Benha, Egypt

3- Biochemistry Deptartment, Benha Provincial Laboratory, Animal Health Research Institute, Agriculture Research Center,Benha, Egypt

Abstract

Background: Biogenic amines are the end products of bacterial decarboxylation of amino acids which occur as a result of bacterial contamination. Those may cause a series of problems for human health such as allergic reactions, itching, breathing difficulties, fever and hypertension.

Objective: This study aimed to isolate different bacteria that can produce decarboxylase enzymes and trail to control it by using Garlic, Onion and Ginger nano-emulsions .

Methods: Isolation and identification of some bacteria producing decarboxylase enzymes from minced meat, Preparation of Garlic, Ginger and Onion nano-emulsions (60%) and investigating their cytotoxicity by Sulforhodamine B (SRB) assay. Then, the antibacterial effect of the prepared nano-emulsions against the isolated bacteria was explored by determination of their MICs and measuring biogenic amines levels by HPLC.

Results: The most common bacteria isolated from samples were Salmonella species "*Salmonella* typhimurium*1*,4{5},12: i:1.2 and *S. arizonae*", *E. coli* "serotype O44:K74 and O125:K70", *Klebseilla pneumonia, Enterobacter spp, S. aureus, Aeromonas hydrophilia, Proteus marbalis, Pasteurella multocida* and *Lactobacillus* species. The biogenic amines detected on positive samples were Putrescine, Cadaverine, Spermidine, Spermine, Putrescine, B-phenyl ethyl amine, Histamine and tyramine. The sizes of the ginger oil nanoemulsion 60% , garlic oil nanoemulsion 60% and onion oil nanoemulsion 60% were (222.6 ± 2.22 nm, 420.7 ± 36.95 nm and 202.9 ± 2.1 nm) respectively, indicating that they were safe and stable. The antibacterial effect of the used nano emulsions showed that *Salmonella spp*, *E. coli* and *S. aureus* were the most sensitive strains while *Klebsiella pneumonia* and *Enterobacter* spp. were the most resistant ones. The level

of the detected biogenic amines were reduced greatly after addition the oil nanoemulsions 7.5% to examined samples.

Conclusion: Using of plant extract as ginger, garlic and onion nanoemulsions oils as antibacterial agents and for reduction of biogenic amines was more effective.

Key words: Biogenic amines, minced meat, HPLC, natural Nano emulsions, and bacteria producing biogenic amines.

Introduction

Biogenic amines (BAs) are low molecular weight compounds with biological activity, produced as a result of the decarboxylation of amino acids or amination and transamination of aldehydes and ketones during the metabolic processes in living cells (**Jaguey-Hernández et al., 2021).** It has multifunctional roles as physiological substances used for neurotransmission, regulation of growth and blood pressure, and other important roles in the intestinal immune system **(Erdag et al., 2019).** However, when they increased over the acceptable level, it leads to an adverse effect on nervous, respiratory, and cardiovascular systems and/or allergic reactions (**Visciano et al., 2020**). It may be polar or semi-polar compounds with an aliphatic (putrescine, cadaverine, spermine, and spermidine), aromatic (tyramine, phenylethylamine), or heterocyclic (histamine, pyrrolidine) structure (**Papageorgiou et al., 2018).**

These low-molecular-weight elements are formed mainly by enzymatic decarboxylation of different amino acids present in meat through microbial enzyme activity during storage (**Zhang et al., 2019**). Several groups of microorganisms were reported to produce decarboxylase enzymes like Enterobacteriaceae, Micrococcaceae, and Pseudomonadaceae (**Balamatsia et al., 2006**). Biogenic amines are produced by the action of enzymes on free amino

acids in meat during storage **(Ruiz-Jiménez and Luque de Castro, 2006**). The amount and proportion of these compounds reflect the quality and safety of the raw materials and the processing methods. Therefore, biogenic amines can be used as indicators of the hygienic conditions of meat products for instance: putrescine and cadaverine combination act as an index of acceptability in fresh meat and their quantities increase during microbial spoilage even during chilled storage (**Triki et al., 2018; Algahtani et al., 2020**).

Meat safety has been recently at the forefront of societal considerations. Also there is increased necessity to prevent or even reduce the frequency and concentration of traditional and developing foodborne pathogens, **Brashears and Chaves,(2017**). So, many manufacturing techniques were developed to decrease or even prevent biogenic amines formation through decrease the microbial growth and decarboxylase activity One of them is addition of natural preservatives and coatings (**Saleh et al., 2017; Eldaly et al., 2018; Mahmoud, 2019).**

Natural products, such as essential oils (EOs) represent complex mixtures of aromatic and volatile liquids frequently distilled from plant, and it has distinctive flavors, antioxidation, and antibacterial effects (**Khan et al., 2019**). Garlic, Onion, and Ginger were the most used ingredients as a flavor enhancement in meat. Garlic has a wide spectrum of actions, not only antibacterial, antifungal, and antiprotozoal, but also it has beneficial effects on the cardiovascular and immune systems (**Saad et al., 2019).** Garlic significantly reduce the contents of putrescine, cadaverine, histamine, tyramine, and spermidine ($p < .05$) **(Mah et al., 2009).** Onion extract has been considered a natural preservative with antifungal and antibacterial effects against a wide variety of Gram-negative and Gram-positive bacteria **(Kabrah et al., 2016).** So, those help in the

inhibition of the biogenic amine formation by their antibacterial activity. Also, ginger contains a higher amount of amine oxidases which help in reducing biogenic amine formation by inhibiting the growth of bacteria **(Yeunyongsuwan and Kongkiattikajorn, 2005; Lu et al., 2015**).

This study aimed to explore the antibacterial effect of different nano plant extracts (garlic, onion, and ginger) against some bacteria producing biogenic amines and measuring the concentration of biogenic amines before and after treatment with these nano-emulsions.

Material and Methods

Sample collection

A total of 210 fresh minced meat samples collected from butcher shops at Al Qalyubia Governorate, Egypt. Weight 100 gram for each sample and placed it in sterile plastic bags then put it into icebox and transported as soon as possible to the laboratory.

This research is excluded from ethical limitations, because the animals were not touched directly by the authors.

Bacteriological examination

Decarboxylase activity

Samples were prepared according to **(APHA, 2001).** Then one ml from each prepared sample was inoculated into nutrient broth and incubated at 37ºC for 24 hrs. A loopful from incubated nutrient broth was streaked over lysine iron agar in order to determine the ability of bacteria to

form biogenic amines due to its decarboxylase and deaminase activity. The agar was incubated at 37ºC for 24 hrs.

Bacteriological isolation and identification (Paul *et al.*, 2009 and Markey *et al.*, 2013).

According to the results of lysine agar, the suspected bacteria were inoculated on MacConkey agar, XLD agar, 10% sheep blood agar, Baired parker agar and MRSA agar. Colonies were examined for their morphology, pigmentation and hemolytic ability. Then biochemical tests were performed. Finally, subculture the isolated strains into brain heart broth with 30% glycerin and kept in -18 ºC for preservation and until further tests were done.

Serological identification of the isolated *E. coli* and *Salmonella* species

This done by using the slide agglutination test technique **(Markey *et al.*, 2013**). Serotyping of *E. coli* isolates was performed using rapid diagnostic *E. coli* antisera sets (Anti-Coli, Sifin-Germany) obtained from the Animal Health Research Institute, Dokki, Egypt. While for *Salmonella*, Anti-Salmonella I (A-E+Vi) and anti-salmonella phase H_1 and H_2 (SIFIN) obtained from the Animal Health Research Institute, Dokki, Egypt, were used. The serotyping of *Salmonella* was done according to the Kauffman-White scheme (**Grimont & Weill 2007).**

Preparation, characterization and cytotoxicity assay of Garlic, Ginger and Onion nano-emulsions

Garlic, Ginger and Onion nano-emulsions (60%) were prepared in Nanomaterials Research and Synthesis Unit in Animal Health Research Institute, Dokki, Egypt. according to Rao and McClements (2011) Nano-emulsion oil parepared by adding 60 ml of each **Garlic, Ginger and Onion oil-emulsions** to 10 ml of tween 80 and 30ml distilled deionized water which were mixed for half in heamogeneous blender 1500watt and then add distilled deionized water slowly

to the mixed oil phase. The droplet size, surface charge (zeta potential), size distribution (polydispersity indexes [PDI]), and electrical conductivity of the nanoemulsions was measured by Zetasizer Malvern Instrument (Malvern, UK). At fixed angle of 173° at 25° C. Samples were analyzed in triplicate.

Cytotoxicity assay

Sulforhodamine B (SRB) assay was done to investigate the cytotoxicity of prepared nano-emulsions **(Skehan et al., 1990).** Different concentrations of nano-emulsion (0.006, 0.06, 0.6, 6, and 60 %) were tested against rat heart/ myocardium cell line, obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). The Cells were maintained in DMEM media supplemented streptomycin (100 mg/mL), penicillin (100 units/mL), and 10% heat-inactivated fetal bovine serum and incubated in a humidified atmosphere containing 5% CO2 at 37°C.

Antibacterial effects of (Ginger, Garlic and Onion) nano-emulsions

This was done by using microdilution method (MIC) according to **Kowalska-Krochmal and Dudek-Wicher (2021**). In 96 well-plates., 50 ul of peptone water broth was dispensed into each well of the column1.Then 50 ul of the garlic nano-emulsion was added in column "1". Double serial dilutions were performed using a multichannel pipette for transferring and mixing garlic nano-emulsion from column 1-6 in order to obtain different concentrations of the nano emulsion (60, 30, 15, 7.5, 3.75 and 1.875 %). Finally, 50 ul of each isolated bacteria inoculum (5×10^8 cfu\ ml) was inoculated in one row. Negative control well contained pepton water only, while positive control well was inoculated with the microbe in pepton water. The

plate was incubated at 37ºC for 24hrs. After incubation, A loopful from each concentration was inoculated on nutrient agar to determine MIC, which known as the lowest concentration that showed no bacterial growth. MIC for Onion and Ginger nano emulsion was determined as previously described with Garlic nano emulsion.

Biogenic amines determination.

Sampling

Different biogenic amines (Treptamine, B-phenyl ethyl amine, Putrescine, Cadaverine, Histamine, Serotonin, Tyramine, Spermidine and Spermine) were detected in six selected samples; represented as (two samples were lysine positive, two samples were lysine positive with production of H_2S, one sample produced red lysine with H_2S and the last one was lysine negative). They were subjected to four treatment before measuring biogenic amines by High performance liquid chromatography (HPLC), including:

1st group: free from any addition.

2nd group: treated with 7.5% of Garlic nano-emulsions to samples.

3rd group: treated with 7.5% of Onion nano-emulsions to samples.

4th group: treated with 7.5% of Ginger nano-emulsions to samples.

Extraction and Formation of dansylamines

Treptamine,B-phenyl ethyl amine, Putrescine, Cadaverine, Histamine, Serotonin, Tyramine, Spermidine and Spermine were extracted and determined according to Mietz and Karmas (1977), Ayesh (2012), sultan and Marrez 2014 with some modifications

Reagents

a) Dansyl chloride solution: 500mg of dansyl chloride (5- {Dimethylamino} naphtalene -1-sulfonyl chloride) were dissolved in 100 ml acetone.

b) Standard solutions: Stock standard solutions of the tested amines: 25mg of each standard were dissolved in 25ml distilled water individually.

Extraction

Twenty five grams of homogenised sample were blended with 125 ml of 5% TCA for 3 min using a warning blender. Filtration was achieved using filter paper Watman No.(1). Ten millilitres of the extracts was transferred into a culture tube with 4g NaCl and 1 ml of 50 % NaOH then shacked and extracted three times by 5 ml n-butanol / chloroform (1: 1 v/v) stoppered and shacked vigorously for 3.0 min. Centrifugation for 5.0 min. at 3000 rpm and the upper layer was transferred to 50 ml separating funnel using disposable Pasteur pipette. To the combined organic extracts (upper layer), 15 ml of n-heptane was added and extracted three times with 1.0 ml portions of 0.2N HC1, the HCl layers were collected in a glass stoppered tube. Solution was evaporated just to dryness using water bath at 95°C with aid of a gentle current of air.

Formation of dansylamines:

One hundred µl of each stock standard solution were transferred to a vial 50 ml and dried. About 0.5 ml of saturated $NaHCO_3$ solution was added to the residue of the sample extract (or the standard). Stoppered and carefully mixed to prevent loss-due to spattering. Carefully, 1.0-ml dansyl chloride solution was added and mixed-thoroughly using vortex mixer. The reaction mixture was incubated at 55°C for 45 min**.** About 10 ml of distilled water was added to the reaction mixture, stoppered and shacked vigorously using vortex mixer. The extraction of dansylated biogenic amines was carried out using three times of 5.0 mI portions of diethylether, stoppered, shacked carefully for 1.0 min and the ether layers were collected in culture tube using disposable Pasteur pipette. The combined ether extracts were carefully evaporated at 35°C in dry bath with aid of current air. The obtained dry film was dissolved in 1ml methanol, then 10 µl was injected in HPLC.

Apparatus

-High performance liquid chromatography (HPLC) used for dansylamines determination was an Agilent 1260 affinity system (Germany) equipped with auto sampler, pump, UV detector set at 254 nm wavelength. Agilent Poroshell 120 EC-C18 4um (4.6 mm × 150 mm) column was used for biogenic amines separation. Data were integrated and recorded using Chromeleon Software program.

Statical analysis

Statistical software Minitab17. The significance level for statistical analyses was $P \leq 0.05$.

Results

Decarboxylase activity of samples and their bacterial isolation.

The results of lysine iron agar inoculation differ according to types of bacteria present in minced meat samples and its ability to make decarboxylation, or deamination and formation of hydrogen sulphide. **Table (1)** represented bacterial species isolated and their decarboxylase activity. The results showed that (31.9%) of samples gave lysine positive. Their bacteriological analysis revealed isolation of (*E. coli, Klebseilla pneumonia, Enterobacter spp and S.aureus)*, while (27.6%) of samples yielded lysine positive with production of H_2S and the following bacteria was isolated (*Salmonella spp, Aeromonas hydrophila*). Moreover, (21.4%) of samples made deamination to lysine (represent by red color of indicator) with production of H_2S the bacteria isolated was (*Proteus marbalis* and with red color only for *Pasteurella multocida)*, Also, the negative results were detected by (19.04%) of samples and *Lactobacillus* species were isolated from them.

Table (1) Isolated bacterial species and their decarboxylase activity

Decarboxylase activity	Number of samples	%*	Isolated bacteria

Lysine positive	67	31.9%	***E. coli*** ***Klebseilla pneumonia*** ***Enterobacter spp*** ***S. aureus***
Lysine positive with H_2S production	58	27.6%	***Salmonella spp*** ***Aeromonas hydrophila***
Red lysine with H_2S	45	21.4%	***Proteus marbalis*** ***Pasteurella multocida***
Lysine negative	40	19.04%	***lactobacillus spp***

*percentage related to total number of samples (n = 210)

The results of *Salmonella* and *E. coli* strains serotyping

Salmonella strains were related to *Salmonella* typhimurium 1,4{5},12:i:1.2 and *S. arizonae*. While *E. coli strains* belonged to O44:K74 and O125:K70.

Characterization of Oil nano-emulsions (Ginger, Garlic and Onion).

The nano-emulsion was characterized by TEM nano-emulsion size, with a narrow size distribution indicating greater homogeneity in nanodroplet size (the homogeneous of

nanoparticles, measured by PDI, the smaller the PDI the more homogeneous nanoparticles) and zeta potential indicates moderate stable suspensions, as in table (2)

Table (2) Characterization of Oil nano-emulsions (Ginger, Garlic and Onion).

	ginger oil nano-emulsion 60%	**garlic oil nano-emulsion 60%**	**onion oil nano-emulsion 60%**
Particle size	222.6 ± 2.22 nm	420.7 ± 36.95 nm	202.9 ± 2.1 nm
PDI	0.338 ± 0.012	0.432 ± 0.023	0.28 ± 0.016
Zeta potential	-14.4 ± 0.75 mv	-25.1 ± 0.2 mv	-15.8 ± 0.35 mv

The viability % of the rat cells (H_9C_2) using SRB assay using different concentrations of Ginger oil, Garlic oil and Onion oil nano-emulsions (60, 6, 0.6, 0.06 and 0.006 %) after three days post inoculation showed the following results recorded in **Table (3).** In which the IC50 > 60% for Onion, Garlic and Ginger oil nano-emulsions as shown in (**Figure 1, 2, 3**)

Table (3) Viability of rat cells during using different concentrations of nano-emulsions.

Concentration	ginger oil nano-emulsion	garlic oil nano-emulsion	onion oil nano-emulsion
60	82.7832	52.9538	91.9771
6	98.4174	98.9923	97.0489
0.6	100.328	99.4962	99.8429
0.06	100.203	100.088	99.7756
0.006	99.8304	100.441	99.7083
Ic50	>60%	>60%	>60%

A B

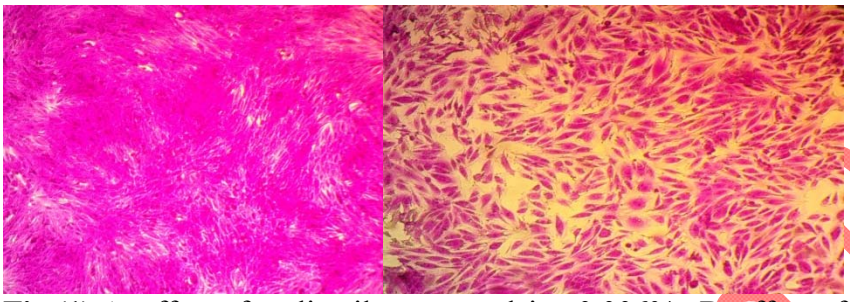

Fig (1) A: effect of garlic oil nano-emulsion 0.006% B: effect of garlic oil nano-emulsion 60%

A B

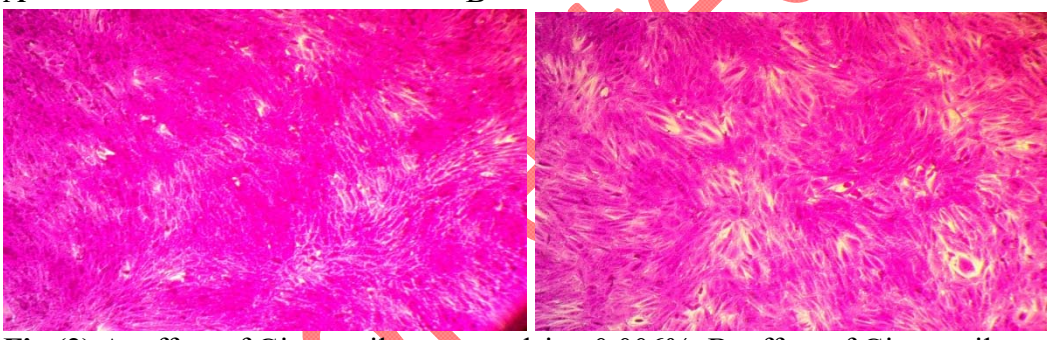

Fig (2) A: effect of Ginger oil nano-emulsion 0.006% B: effect of Ginger oil nano-emulsion 60%

A B

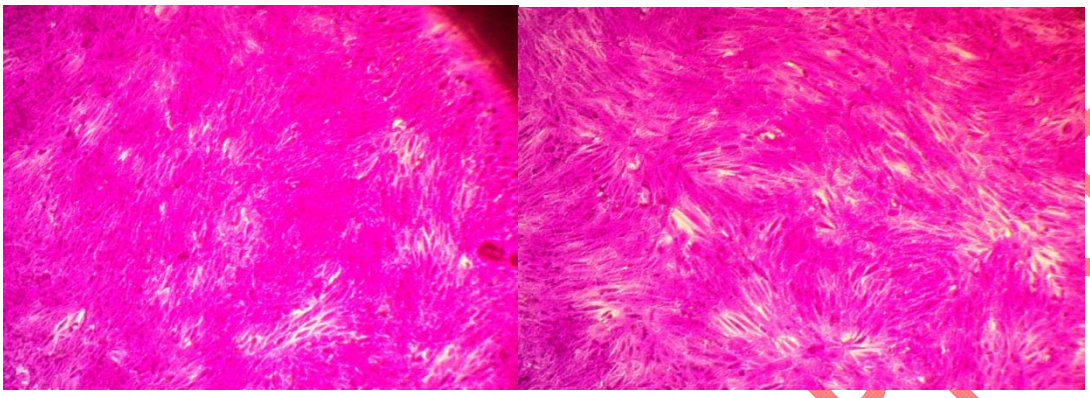

Fig (3) A: effect of Onion oil nano-emulsion 0.006% B: effect of Onion oil nano-emulsion 60%

Antibacterial activity of Garlic, Ginger and Onion Nano-emulsions (In-Vitro MIC)

The antimicrobial activity and microdilution susceptibility test of nano-emulsions used was determined using the MIC value as the lowest concentration of nano-emulsion which caused inhibition of bacterial growth. The results tabulated in **Table (4)** explained that the **Garlic** nano-emulsion was greatly affected in *E. coli* and *Salmonella* at conc. 3.75%, while inhibited growth of *Klebsiella pneumonia, Aeromonas hydrophila, Enterobacter species, Proteus marbiles* and *S. aureus* at conc. 7.5%, and for lactobacillus species at concentration 15%. Whereas **Onion** nano-emulsion hinders growth of *E. coli*, *Salmonella, Proteus marbiles* and *S. aureus* at conc. 7.5%, followed by inhibition to *Aeromonas hydrophila and Lactobacillus* at conc. 15%, and for *Klebsiella pneumonia* and *Enterobacter* species at conc. 30%. Moreover, **Ginger** nano-emulsion reduced growth of *E. Coli*, *Salmonella, Proteus marbiles* and *S. aureus* and *Lactobacillus* at

conc. 7.5%, while for *Aeromonas hydrophila* inhibition occurs at conc. 30%, and at 60% for *Klebsiella pneumonia and Enterobacter species.*

This indicated that the Garlic nano-emulsion has a greatly antibacterial effect over a wide range of bacteria than Onion and Ginger nano-emulsions.

Table (4) In- Vitro MIC of Garlic, Ginger and Onion Nano-emulsions.

Isolates	MIC of garlic	MIC of onion	MIC of ginger
E. coli	**3.75%**	**7. 5%**	**7.5%**
Salmonella spp	**3.75%**	**7. 5%**	**7.5%**
Klebsiella pneumonia	**7. 5%**	**30%**	**60%**
Aeromonas hydrophila	**7. 5%**	**15%**	**30%**
Enterobacter SPP	**7. 5%**	**30%**	**60%**

Proteus marbalis	**7. 5%**	**7. 5%**	**7.5%**
S. aureus	**7.5%**	**7.5%**	**7.5%**
Lactobacillus spp	**15%**	**15%**	**7.5%**

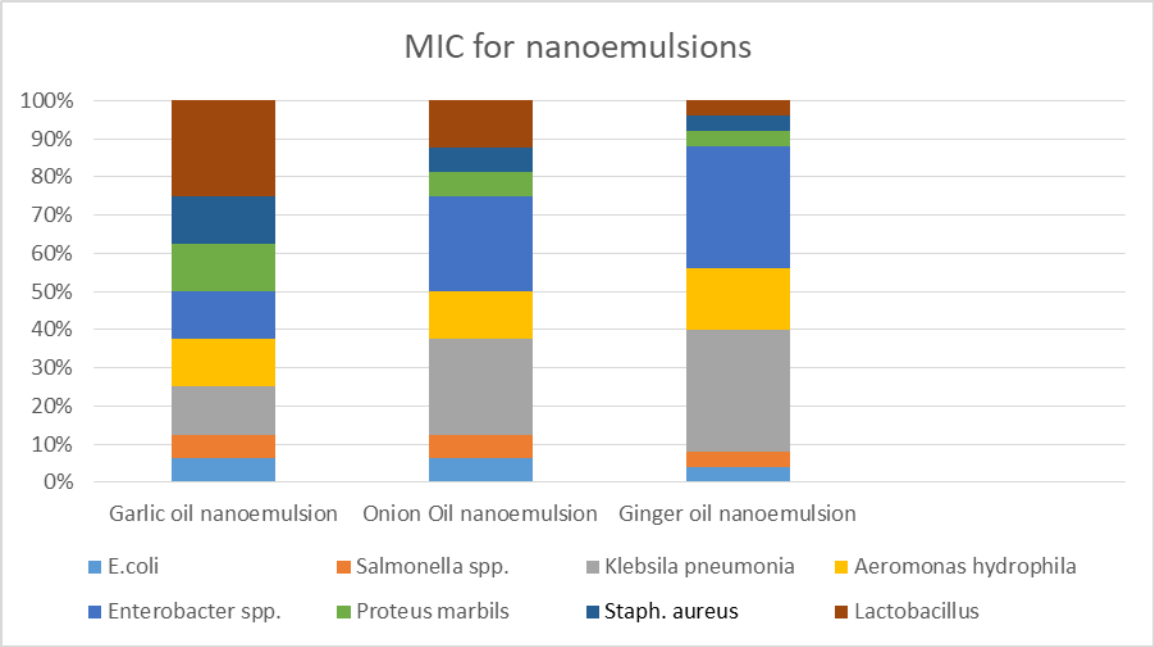

Fig (4) In- Vitro MIC of Garlic, Ginger and Onion nano-emulsions.

Biogenic amines detection by HPLC

According to the results reported in Table (5,6,7,8) the level of **Putrescine** varied from 8.30, 17.87, 11.19, 5.66, 4.35, and 2.08 mg\kg in the first untreated group to be 5.82, 12.53, 6.07, 3.97, 3.05, and 1.46 mg\kg for second group treated with garlic nano-emulsion. While varied to be 4.77, 10.26, 6.43, 3.25, 2.51, and 1.21mg\kg for third group treated with Onion nano-

emulsion. For ginger nano-emulsion treated group it was 7.86, 16.92, 10.60, 5.36, 4.12, and 1.97 mg\kg.

Moreover, the level of **Cadaverine** differed from 38.59, 28.70, 26.50, 20.60, 0.87, and 3.60 mg\kg in the first untreated group to be 2.66, 1.98, 1.72, 1.42, 0.06, and 0.25 mg\kg for second group treated with garlic nano-emulsion. Whilst the third group treated with Onion nano-emulsion had 1.52, 1.12, 1.03, 0.8, 0.03, and 0.14 mg\kg. In ginger nano-emulsion treated group, the level of **Cadaverine was** 1.29, 0.96, 0.89, 0.69, 0.03, and 0.12 mg\kg

Furthermore, the level of **Spermidine** differed from 5.22, 1.36, 15.22, 8.33, 3.91, and 7.76 mg\kg in the first untreated group to be 0.58, 0.15, 1.38, 0.93, 0.44, and 0.87 mg\kg for second group treated with garlic nano-emulsion. While varied to be 0.84, 0.22, 2.45, 1.34, 0.63, and 1.25 mg\kg for third group treated with Onion nano-emulsion. But it was 1.002, 0.26, 2.92, 1.6, 0.75, and 1.49 mg\kg for ginger nano-emulsion treated group.

Likewise, the level of **Spermine** differed from 18.33, 4.17, 5.32, 5.18, 24.22, and 5.48 mg\kg in the first untreated group to be 3.22, 0.73, 0.91, 0.91, 4.25, and 0.96 mg\kg for second group treated with garlic nano-emulsion. Although varied to be 2.93, 0.68, 0.87, 0.85, 3.97, and 0.90 mg\kg for third group treated with Onion nano-emulsion, and 4.71, 1.07, 1.37, 1.33, 6.22 and 1.41 mg\kg for ginger nano-emulsion treated group.

But the level of **Histamine and tyramine** was not detected in all treated groups. It varied from 2.31, 1.63, 1.51, 0.76, 2.05, and 0.72 mg\kg for histamine, and 2.15, 0.82, 1.35, 1.51, ND, and 1.19 mg\kg for tyramine in the first group.

Table (5) Biogenic amines in first group (untreated group).

Sample code	Treptamine mg/kg	B-phenyl ethyl amine mg/kg	Putrescine mg/kg	Cadaverine mg/kg	Histamine mg/kg	Serotonin mg/kg	Tyramine mg/kg	Spermidine mg/kg	Spermine mg/kg
1	0.56	0.18	8.30	38.59	2.31	ND	2.15	5.22	18.33
2	ND	0.28	17.87	28.70	1.63	ND	0.82	1.36	4.17
3	ND	ND	11.19	26.50	1.51	ND	1.35	15.22	5.32
4	ND	ND	5.66	20.60	0.76	ND	1.51	8.33	5.18
5	ND	ND	4.35	0.87	2.05	ND	ND	3.91	24.22
6	ND	ND	2.08	3.60	0.72	ND	1.19	7.76	5.48

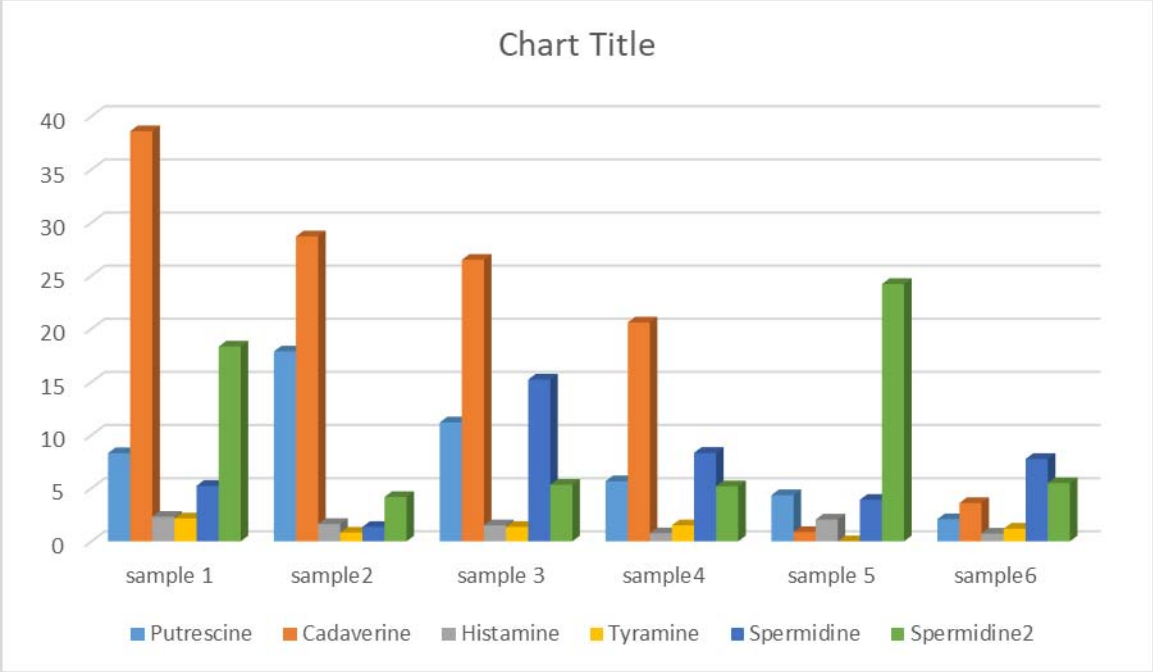

Fig (5) Biogenic amines in first group (untreated group).

Table (6) Biogenic amines in the second group (treated with 7.5% Garlic oil nano-emulsion).

Sample code	Treptamine mg/kg	B-phenyl ethyl amine mg/kg	Putrescine mg/kg	Cadaverine mg/kg	Histamine mg/kg	Serotonin mg/kg	Tyramine mg/kg	Spermidine mg/kg	Spermine mg/kg
1	ND	ND	5.82	2.66	ND	ND	ND	0.58	3.22
2	ND	ND	12.53	1.98	ND	ND	ND	0.15	0.73
3	ND	ND	6.07	1.72	ND	ND	ND	1.38	0.91
4	ND	ND	3.97	1.42	ND	ND	ND	0.93	0.91
5	ND	ND	3.05	0.06	ND	ND	ND	0.44	4.25
6	ND	ND	1.46	0.25	ND	ND	ND	0.87	0.96

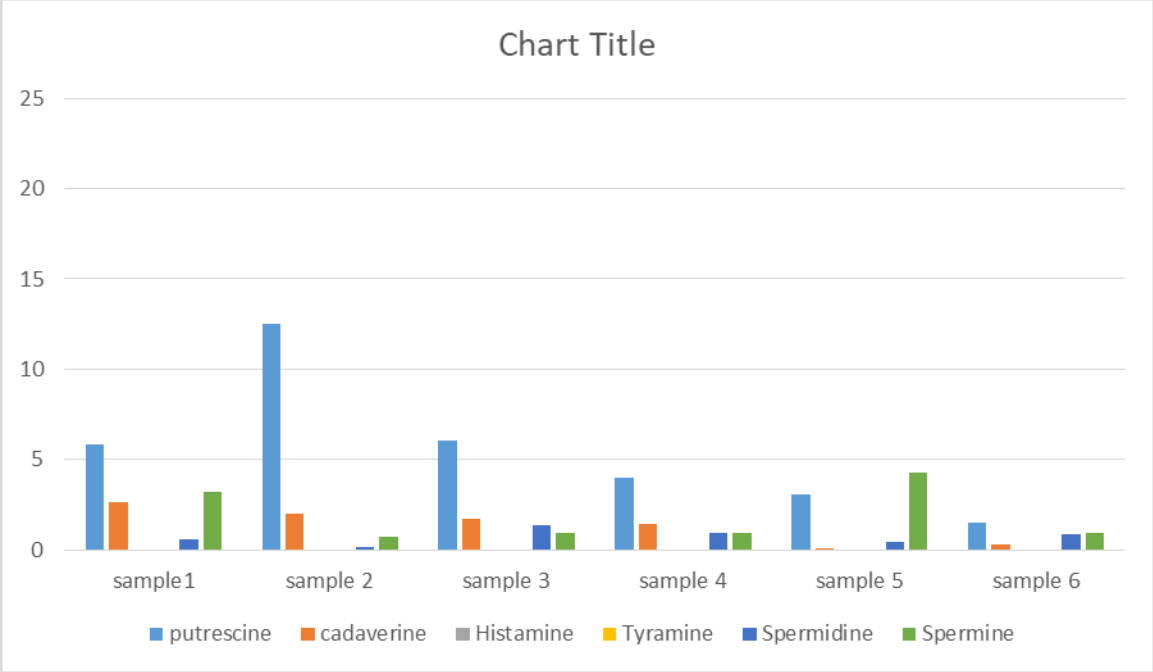

Fig (6) Biogenic amines in the second group (treated with 7.5% Garlic oil nano-emulsion).

Table (7) Biogenic amines in the third group (treated with 7.5% Onion oil nano-emulsion).

Sample code	Treptamine mg/kg	B-phenyl ethyl amine mg/kg	Putrescine mg/kg	Cadaverine mg/kg	Histamine mg/kg	Serotonin mg/kg	Tyramine mg/kg	Spermidine mg/kg	Spermine mg/kg
1	ND	ND	4.77	1.52	ND	ND	ND	0.84	2.93

2	ND	ND	10.26	1.12	ND	ND	ND	0.22	0.68
3	ND	ND	6.43	1.03	ND	ND	ND	2.45	0.87
4	ND	ND	3.25	0.8	ND	ND	ND	1.34	0.85
5	ND	ND	2.51	0.03	ND	ND	ND	0.63	3.97
6	ND	ND	1.21	0.14	ND	ND	ND	1.25	0.90

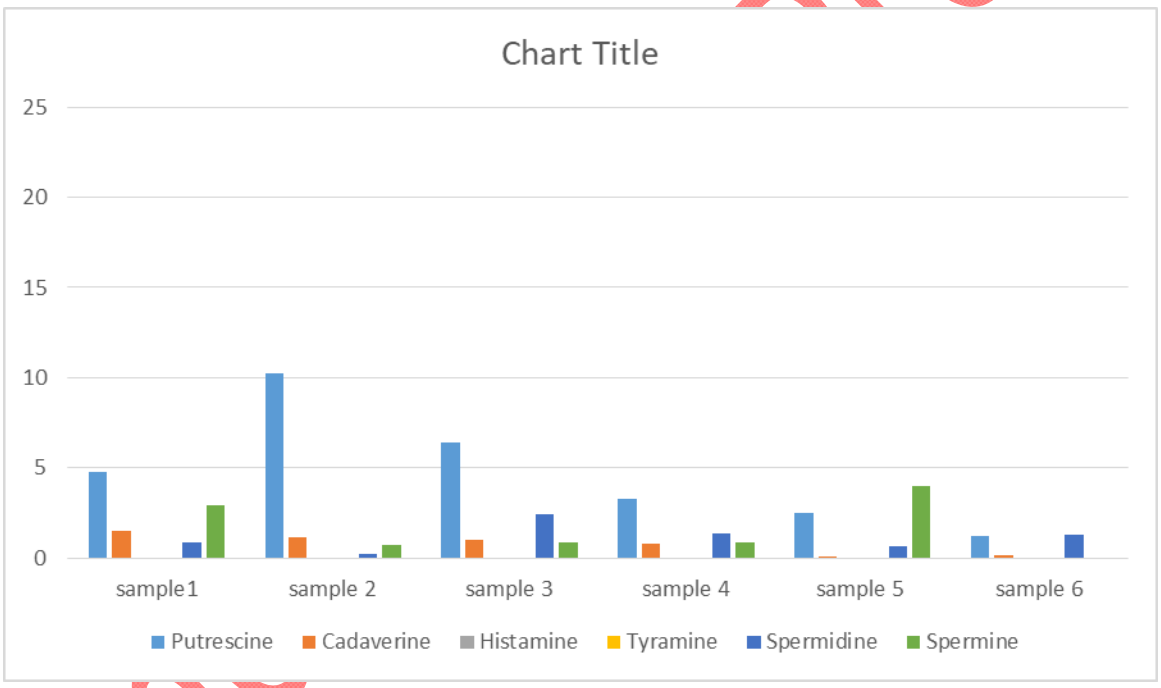

Fig (7) Biogenic amines in the third group (treated with 7.5% Onion oil nano-emulsion).

Table (8) Biogenic amines in the fourth group (treated with 7.5% Ginger oil nano-emulsion).

Sample code	Treptamine mg/kg	B-phenyl ethyl amine mg/kg	Putrescine mg/kg	Cadaverine mg/kg	Histamine mg/kg	Serotonin mg/kg	Tyramine mg/kg	Spermidine mg/kg	Spermine mg/kg
1	ND	ND	7.86	1.29	ND	ND	ND	1.002	4.71
2	ND	ND	16.92	0.96	ND	ND	ND	0.26	1.07
3	ND	ND	10.60	0.89	ND	ND	ND	2.92	1.37
4	ND	ND	5.36	0.69	ND	ND	ND	1.6	1.33
5	ND	ND	4.12	0.03	ND	ND	ND	0.75	6.22
6	ND	ND	1.97	0.12	ND	ND	ND	1.49	1.41

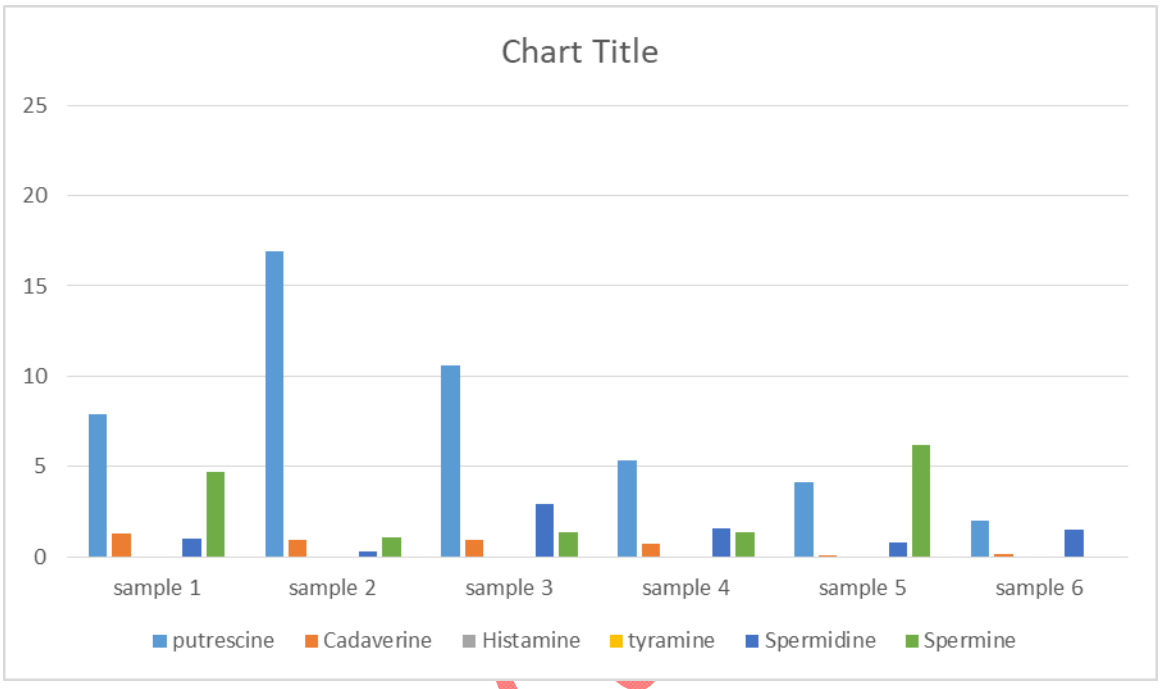

Fig (8) Biogenic amines in the fourth group (treated with 7.5% Ginger oil nano-emulsion).

Discussion

Presence of Biogenic amines in food act as indicator for bacterial decarboxylation of amino acids and their types and amount depend on the presence of different bacteria in foods (**Ruiz-Capillas et al., 2007**). For examples, species of many genera such as *Bacillus*,

Citrobacter, Clostridium, Klebsiella, Escherichia, Proteus, Pseudomonas, Salmonella, Shigella, Photobacterium and the *lactic acid bacteria "Lactobacillus, Pediococcus and Streptococcus"* are capable of decarboxylating one or more amino acid (**Ekici & Omer, 2020**). This can be detected by using simple method as using media contain pH indicator as Bromcresol Purple to determine the ability of microorganism to form biogenic amines and to differentiate between bacteria (**Kalhotka et al., 2012**). In the present study, lysine iron agar was used to isolated bacteria producing decarboxylase enzyme. Many bacteria were isolated as (*E. coli, Klebseilla pneumonia, Enterobacter spp, S. aureus, Salmonella spp, Aeromonas hydrophila, Proteus marbalis, Pasteurella multocida and* Lactobacillus spp), as tabulated in **Table (1).** This came in accordance with that mentioned by **Jairath et al., (2015**) who reported that decarboxylase activity in meat products is attributed mainly to Enterobacteriaceae, Pseudomonadaceae, Micrococcaceae and lactic bacteria. **Li et al., (2020**) reported that several bacteria can produce biogenic amines like *Enterobacteriaceae and pseudomonas*, some strains belonging to the genera *Staphylococcus* and *Bacillus*, and LAB are isolated from meat and meat products. In addition, **Pircher et al., (2007**) detected the presence of different biogenic amines (cadaverine, histamine, putrescine and tyramine) in raw meat and fermented sausages and isolated bacteria were Enterobacteriaceae and Lactobacillus species. While **Bermúdez et al., (2012)** isolated a group of gram-positive bacteria as (lactic acid bacteria (LAB), *Staphylococcus* and *Bacillus*) from cheese and traditional sausage and found that they were formed biogenic amines.

The importance of obtained safe food was increased globally and using plant-based products as additives for both raw and processed meat products have been investigated widely in order to avoid the development of aminogenic contaminant bacteria and in turn, to reduce

biogenic amines content as well (**Lu et al., 2015**). So in this study, the antibacterial effect on ginger, Garlic and Onion nano-emulsions was determined with conc. 60% against different aminogenic producing bacteria. These nanoemulsions characterization recorded that the mean diameter were (222.6 ± 2.22 nm, 420.7 ± 36.95 nm, and 202.9 ± 2.1 nm respectively) for (ginger oil 60%, Garlic oil 60% and Onion oil nano-emulsions 60% respectively) and their zeta potential were ((-14.4 ± 0.75 mv, -25.1 ± 0.2 mv, -15.8 ± 0.35 mv) respectively **(Table 2).** This came nearly to that reported by **Hassan and Mujtaba (2019)** for garlic oil nano-emulsion and with **Ningsih et al., (2020)** for ginger oil nano-emulsion.

PDI value is a parameter for determining the size distribution of droplets. Generally, a small PDI value indicates a narrow size distribution, while a value higher than 0.7 represents a broad size distribution (**Gul et al., 2018**). The narrow size distribution indicates greater homogeneity in nanodroplet size (the smaller the PDI the more homogeneous nanoparticles). While Zeta potential represents the electrical charge of the particles and characterizes the colloidal system's behavior, which is vital for the stability of nano-emulsion (**Pabast et al., 2018**). The transformation of crude essential oils to nanoforms helps in increase their distribution and their antibacterial activity as previously reported by **Ma et al., (2016) and Carpenter and Saharan (2017**). Also, it was supposed that essential oil in nano-emulsions had an improved physicochemical stability and dispersibility in food matrices, leading to easier access to bacteria and consequently higher antibacterial activity (**Donsì & Ferrari, 2016**). Cytotoxicity of the used nano-emulsions was tested against the rat cells (H_9C_2) using SRB assay and found that they were safe to the cell until 60% concentration. Also, they have antibacterial effect on isolates until 7.5% concentration and reduction the biogenic amines. Also, the ginger oil nano emulsion has

repaired effect on cell at concentration 0.06% and 0.6% while the garlic oil nano emulsion at concentration 0.006% and 0.06% as recorded in Table (3), Fig (1,2 &3). Many authors recorded the effect of ginger nano emulsion as anti-inflammatory and repairing the cells as **Zhang et al., (2016), Sung et. al., (2019) and Al-Badawi et. al., (2022).**

The antibacterial activity and minimum inhibitory concentration of the used nano-emulsions (ginger oil 60%, Garlic oil 60% and Onion oil nano-emulsions 60%) were recorded in **Table (4),** in which the MIC of garlic oil nano-emulsions mainly occur at 7.5% for most examined bacteria , this differ with **Liu et al., (2022**) who reported that the MIC of garlic oil nano-emulsion was 1.25% against MRSA, and with **Hassan and Mujtaba, (2019**) and **Hassan et al., (2020)** who determined that Garlic oil nano-emulsion have greater effect toward Gram-positive bacteria more than Gram- negative ones.. And this came in accordance with **Zheng et al., (2013)** who found that garlic nano emulsion showed strong antibacterial activity against *S. aureus* at higher concentration.

The MIC for Onion oil nano-emulsion in most bacteria appeared to be 7.5% while it may increase for 30% for other bacteria. The antibacterial effect of the Onion was previously reported by **Kabrah et al., (2016)** who determined that Onion extract is effective in vitro against many bacterial species including *Bacillus subtilis, Salmonella, and E. coli*. Similarly, this inhibiting effect was also noted on *Staphylococcus aureus* and results showed a complete inhibition of all strains tested at a concentration of 6.5 mg/ml. It's noted that the partial size of nano-emulsion is pivotal in determining the antimicrobial ability of agents where reduced particle size of nano-emulsion, thus leading to increased exposure to microbial membrane and

enhanced antibacterial activity **Liu et al., (2022**). So, the antibacterial effect of onion extract was enhanced by its transformation to nano form. In addition, the Ginger oil nano-emulsion has the same concept; their conservation to nanoparticles enhanced their effect. The MIC of ginger oil nano-emulsion mainly appears at conc. 7.5% while it may increase to 60% in the case of *K. pneumonia* and Enterobacter spp. This came in accordance with **Thakur et al., (2013)** who reported that the ethanolic ginger extract showed more potent against *E.coli*, and moderately inhibited the *P. aeruginosa, K. pneumonia*. The ginger extract contains many different bioactive compounds with antimicrobial activities that appear to be more sensitive to gram-positive bacteria than the gram-negative ones (**Gurumayum, 2015**).

The results tabulated in **Table (5), Fig (5)** determined the level of biogenic amines presented in six samples (two samples were lysine positive, two samples were lysine positive with production of H_2S, one sample produced red lysine with H_2S and the last one was lysine negative). The level of putrescine, cadaverine, tyramine and Histamine were higher among the six samples, this mainly occur due to bacterial contamination of the samples or bad storage condition as recorded by **Doeun et al., (2017**). And this came in agreement with **Stadnik and Dolatowski (2010)** who mentioned that tyramine, cadaverine, putrescine and histamine were the dominant biogenic amines in meat and meat products. Cadaverine represented the greatest amine present in meat due to presence of precursor lysine in high amount in meat (**Vinci and Antonelli, 2002**).

Meat represents a good source for biogenic amines production, this occurred due to presence of great amount of protein that act as a start point for bacterial decarboxylation and

subsequently biogenic amines formation (**Schirone et al., 2022**). The presence of one or more biogenic amines in meat samples act as indicators of freshness, quality, and spoilage in meat and meat products (**Triki et al., 2018**). The ratio between Spermine and Spermidine evaluates the quality of raw meat (**Jastrzebska et al., 2015**). While the sum of Cadaverine and Putrescine act as index for microbial decayed and level of Histamine and Tyramine begin to elevate after several days of spoilage, there is no standards or guidelines are reported for presence of histamine in meat (**Schirone et al., 2022**).

The biogenic amine index (BAI) consists of the total of putrescine, cadaverine, histamine, and tyramine and according to **Hernandez-Jover et al., (1997) who** mentioned that the BAI value of less than 5 mg\kg represents fresh meat and of good quality, while between 5 and 20 mg\kg it is still acceptable with some signs of deterioration. But, between 20 and 50 mg\kg and above 50 mg\kg the meat is of low quality and spoiled.

The results tabulated in **Table (6), Fig (6)** determined the level of biogenic amines in minced meat samples after treatment with 7.5% from **Garlic oil nano-emulsion** and as previously seen the level of biogenic amines were decreased to low level and histamine and tyramine disappeared completely, this means effective treatment of samples with **Garlic oil nano-emulsion.** This came in accordance with **Zhou et al., (2016)** that reported that Garlic extract mainly reduced biogenic amine producing bacteria and found that the level of histamine and spermidine in the samples handled with garlic extract was reduced significantly than that of the control ones. Also, it assured the previous study of **Mah et al., (2009**) who reported that addition of 5% garlic during ripening of food reduced the biogenic amine level (putrescine,

cadaverine, histamine, tyramine, and spermidine) significantly by 8.7%. The results recorded in **Table (7), Fig (7)** detected the level of biogenic amines in minced meat samples after treatment with 7.5% with **Onion oil nano-emulsion,** In which the level of biogenic amine markedly decrease in the treated samples than the untreated ones**,** Similarly results detected by **Majcherczyk and Surówka (2019),** that addition of onion caused a reduction in the total biogenic-amine content when compared with the control sample without an additive.

While the results in Table (8), Fig (8) declared the level of biogenic amines in minced meat samples after addition of 7.5% **Ginger oil nano-emulsion,** and as previously described with other additives the level of biogenic amines decreased markedly with this treatment**.** This came in accordance with **Kongkiattikajorn, (2015)** who found that the addition of ginger extract led to a reduction in total biogenic amines concentration by 64.7% in samples added with ginger extract, as compared to control samples. Also **Lu et al., (2015**) reported a marked reduction in biogenic amines by using plant extract like (cinnamon, clove, and ginger). This occurred by inhibition the growth of biogenic amine producing bacteria. Many authors reported the effect of Garlic, Ginger and Onion, but there is no previous research in the effect of their nano-emulsions and the level of biogenic amines formation in food, so this work aimed to focus on this item.

Conclusion

Using nano-emulsions of Garlic, Ginger and Onion nano-emulsions leaded to significant reduction in the formation of undesired biogenic amines in minced meat and control the bacterial growth in it.

Declarations

a. Ethics approval and consent to participate.
Ethical approval for animal research was not required as live animals were not used in this study as the samples were taken after slaughter at the abattoir.
Samples were collected from minced meat as routine commercial food and fiber.
b. Contest for publication:
Not applicable

c. Availability of data and materials
The datasets generated and/or analyzed during the current study are available in figure file and table file.
d. Competing interest:
The authors declared that they have no competing interests.
e. Funding:
This research did not receive any funding.
f. Authors&; contributions
Amany O. Selim[1], Marwa M.M. Abdel Salam[2], Rasha N.A. Hassan[2], Gehan E.A. Mustafa [3]and Zeinab A.M. Mahdy[1]: Studied design, shared laboratory examination and data analysis, Amany O. Selim and Zeinab A.M. Mahdy[1] prepared final draft of manuscript. All authors had read and approved the final manuscript.

g. Acknowledgments:
The authors are grateful to the stuff of animal health research institute Benha branch for their help

References

Al-Badawi, H. M., Waly, E. N., Eid, M.E. & Soliman, N.A. (2022). Histopathological Impact of Ginger Loaded Nanoparticle Versus Ginger Extract as A Novel Therapy of Experimentally Induced Acute Ulcerative Colitis. E.J.H., 2(45) ,442-456.

Al-gahtani, F. D., Morshdy, A. E., Hussein, M. A., Abouelkheir, E. S., Adeboye, A., Valentine, A. & Elabbasy, M. T. (2020). Biogenic Amines and Aflatoxins in Some Imported Meat Products: Incidence, Occurrence, and Public Health Impacts. Journal of Food Quality, 2020, 8718179. https://doi.org/10.1155/2020/8718179

APHA "American Public Health Association" (2001). Compendium of Methods for the Microbiological examination of Foods. 4th Ed. F.P. Downes and K. Ito (editors), APHA. Washington D.C., USA**.**

Ayesh, A.M., Ibraheim, M.N., El-Hakim, A.E. & Mostafa, E.A.H. (2012). Exploring the contamination level by biogenic amines in fish samples collected from markets in Thuel-Saudi Arabia. African Journal of Microbiology Research, 6 (6), 1158-1164.

Balamatsia, C. C., Paleologos, E. K., Kontominas, M. G. & Savvaidis, I. N. (2006). Correlation between microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored aerobically or under modified atmosphere packaging at 4 °C: Possible role of biogenic amines as spoilage indicators. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 89(1), 9–17. https://doi.org/10.1007/s10482-005-9003-4

Bermúdez, R., Lorenzo, J.M., Fonseca, S., Franco, I. & Carballo, J. (2012). Strains of Staphylococcus and Bacillus isolated from traditional sausages as producers of biogenic amines Frontiers **in Microbiology.** Food Microbiology, 3, 151 **-157.**

Brashears, M. M. & Chaves, B. D. (2017). The diversity of beef safety: A global reason to strengthen our current systems. Meat Science, 132, 59–71. https://doi.org/https://doi.org/10.1016/j.meatsci.2017.03.015

Carpenter, J. &Saharan, V.K. (2017). Ultrasonic assisted formation and stability of mustard oil in water nanoemulsion: Effect of process parameters and their optimization, Ultrason. Sonochem, 35, 422–430, https://doi.org/10.1016/j. ultsonch.2016.10.021.

Doeun, D., Davaatseren, M. & Chung M.S. (2017). Biogenic amines in foods. Food Sci. Biotechnol, 26, 1463–1474.

Donsì, F. & Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food, J. Biotechnol, 233, 106–120. https://doi.org/10.1016/j. jbiotec.2016.07.005

Ekici, K. & Omer, A.K. (2020). Biogenic amines formation and their importance in fermented foods. BIO Web Conf, 2020, 17, 00232.

Eldaly, E. A., Mahmoud, A. F. A. & Abobakr, H. M. (2018). Preservative effect of chitosan coating on shelf life and sensory properties of chicken fillets during chilled storage. Journal of Nutrition and Food Security, 3(3), 139–148.

Erdag, D., Merhan, O. & Yildiz, B. (2019). Biochemical and pharmacological properties of biogenic amines. In Biogenic Amines; Proestos, C., Ed.; IntechOpen: London, UK, 2019, 1–14.

Grimont, P.A.D. & Weill, F.X. (2007). Antigenic formulae of the Salmonella serovars. 9 edn. Paris, France: WHO Collaborating Centre for Reference and Research on Salmonella, 2007.

Gul, O., Saricaoglu, F.T., Besir, A., Atalar, I. & Yazici, F. (2018). Effect of ultrasound treatment on the properties of nano-emulsion films obtained from hazelnut meal protein and clove essential oil. Ultrason. Sonochem, 41, 466–474. https://doi.org/ 10.1016/j.ultsonch.2017.10.011.

Gurumayum, S. (2015). Invitro Antimicrobial Activity And Preliminary Phytochemical Screening Of Methanol, Chloroform And Hot Water Extracts Of Ginger (Zingiber Officinale). Asian Journal of Pharmaceutical and Clinical Research, (8), 176-180.

Hassan, I.A.M.K., Shany, S.A.S. & Salam, S.H.S. (2020). Virulence and resistance determinants in *Pseudomonas aeruginosa* isolated from pericarditis in diseased broiler chickens in Egypt. J. Adv. Vet. Anim. Res., 7(3), 452-463. DOI: 10.5455/javar.2020.g441

Hassan, K.A.M. & Mujtaba, M.D.A. (2019). Antibacterial efficacy of garlic oil nano-emulsion. AIMS Agriculture and Food, 4(1), 194–205. DOI: 10.3934/agrfood.2019.1.194

Hernández-Jover, T., Izquierdo-Pulido, M., Veciana-Nogués, M.T. & Vidal-Carou, M.C. (1997). Biogenic amine sources in cooked cured shoulder pork. J. Agric. Food Chem., 44(10), 3097-3101. http://dx.doi.org/10.1021/jf960250s

Jaguey-Hernández, Y., Aguilar-Arteaga, K., Ojeda-Ramirez, D., Añorve-Morga, J., González-Olivares, L.G. & Castañeda-Ovando, A. (2021). Biogenic amines levels in food processing: Efforts for their control.in foodstuffs. Food Res. Int., 2021, 144, 110341.

Jairath, G., Singh, P.K., Dabur, R.S., Rani, M. & Chaudhari, M. (2015). Biogenic amines in meat and meat products and its public health significance: a review. J. Food Sci. Technol., 52(11), 6835–6846.

Jastrzebska, A., Kowalska, S. & Szłyk, E. (2015). Studies of levels of biogenic amines in meat samples in relation to the content of additives. Food Addit. Contam., (33), 27–40.

Kabrah, A.M., Faidah, H.S., Ashshi, A.M. & Turkistani, S.A. (2016). Antibacterial Effect of Onion. Sch. J. App. Med. Sci., 4(11D), 4128-4133. http://saspublisher.com/sjams/

Kalhotka, L., Manga, I., Přichystalová, J., Hůlová, M., Vyletělová, M. & Šustová, K. (2012). Decarboxylase activity test of the genus Enterococcus isolated from goat milk and cheese. ACTAVET. BRNO., (81), 145–151; doi:10.2754/avb201281020145

Khan, A., Nadeem, M., Bhutto, M. A., Yu, F., Xie, X., El-Hamshary, H., ElFaham, A., Ibrahim, U. A. & Mo, X. (2019). Physico-chemical and biological evaluation of PLCL/SF nanofibers loaded with oregano essential oil. Pharmaceutics, 11(8), 386. https://doi.org/10.3390/ pharmaceutics11080386

Kongkiattikajorn, J. (2015). Effect of Ginger Extract to Inhibit Biogenic Amines Accumulation during Nham Fermentation. J. Food Chem Nanotechnol., 1(1): 15-19.

Kowalska-Krochmal, B. & Dudek-Wicher, R. (2021). The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation and Clinical Relevance. Pathogens. 4, 10(2), 165. doi: 10.3390/pathogens10020165. PMID: 33557078; PMCID: PMC7913839.

Li, Y., Yu, Z., Zhu, Y. & Cao, Z. (2020). Selection of nitrite-degrading and biogenic amine-degrading strains and its involved genes. Food Qual. Saf. 4, 225–235.

Liu, M., Pan, Y., Feng, M., Guo, W., Fan, X., Feng, L., Huang, J. & Cao, Y. (2022). Garlic essential oil in water nanoemulsion prepared by high-power ultrasound: Properties, stability and its antibacterial mechanism against MRSA isolated from pork. Ultrasonics Sonochemistry, 90, 106201. https://www.elsevier.com/locate/ultson

Lu, S., Ji, H., Wang, Q., Li, B., Li, K., Xu, C. & Jiang, C. (2015). The effects of starter cultures and plant extracts on the biogenic amine accumulation in traditional Chinese smoked horsemeat sausages. Food Control, 50, 869–875. https://doi.org/10.1016/j.foodcont.2014.08.015

Ma, Q., Davidson, P.M., Critzer, F. & Zhong, Q. (2016). Antimicrobial activities of lauric arginate and cinnamon oil combination against foodborne pathogens: Improvement by ethylenediaminetetraacetate and possible mechanisms, LWT, (72), 9–18. https://doi.org/10.1016/j.lwt.2016.04.021.

Mah, J.-H., Kim, Y. J. & Wang, H.-J. (2009). Inhibitory effects of garlic and other spices on biogenic amine production in Myeolchi-jeot, Korean salted and fermented anchovy product. Food Control, 20(5), 449–454. https://doi.org/10.1016/j.foodcont.2008.07.006.

Mahmoud, A. (2019). Effect of Lettuce, Marjoram and Cumin Essential Oils on the Quality and Shelf Life of Minced Meat during Refrigerated Storage. Zagazig Veterinary Journal, 47(3), 288–297. https://doi.org/10.21608/zvjz.2019.13680.1047

Majcherczyk, J. & Surówka, K. (2019). Effects of onion or caraway on the formation of biogenic amines during sauerkraut fermentation and refrigerated storage. Food Chem, 298, 125083. http://dx.doi.org/10.1016/j.foodchem.2019.125083 PMID: 31261001

Markey, B.K., Leonard, F.C., Archambault, M., Cullinane, A. & Maguire, D. (2013). Clinical Veterinary Microbiology. Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto**.**

Mietz, J.L. & Karmas, E. (1978). Polyamine and histamine content of rock fish, salamon, lobster and shrimp as an indicator of decomposition. J. AOAC, 61 (1), 139-145.

Ningsih, I.Y., Faradisa, H., Cahyani, M.D. , Rosyidi, V.A. & Hidayat, M.A. (2020). The formulation of ginger oil nanoemulsions of three varieties of ginger (Zingiber officinale Rosc.) as natural antioxidant. J Res Pharm., 24(6), 914-924. https://doi.org/10.35333/jrp.2020.251

Pabast, M., Shariatifar, N., Beikzadeh, S. & Jahed, G. (2018). Effects of chitosan coatings incorporating with free or nano-encapsulated Satureja plant essential oil on quality characteristics of lamb meat. Food Control, (91), 185–192. https://doi.org/ 10.1016/j.foodcont.2018.03.047.

Papageorgiou, M., Lambropoulou, D., Morrison, C., Kłodzińska, E., Namieśnik, J. & Płotka-Wasylka, J. (2018). Literature update of analytical methods for biogenic amines determination in food and beverages. Trends Analyt Chem., (98), 128-42. http://dx.doi.org/10.1016/j.trac.2017.11.001.

Paul, D.V., George, M.G., Dorothy, J., No-el, R.K., Wolfgang, L., Fred, A.R., Karl-Heinz, S. & William, B.W. (2009). "Bergey's Manual of Systematic Bacteriolo-gy". 2ndEd., Springer, Dordrecht, Heidel-berg, London, New York.

Pircher, A., Bauer, F. & Paulsen, P. (2007). Formation of cadaverine, histamine, putrescine and tyramine by bacteria isolated from meat, fermented sausages and cheeses. *Eur.FoodRes.Technol.,* 226, 225–231.

Rao, J., & McClements, D. J. (2011). Formation of flavor oil microemulsion, nanoemulsions and emulsion influence of composition and preparation method . J. of Agri. and food chem., 59(9), 5026-5035.

Ruiz-Capillas, C., Jiménez Colmenero, F., Carrascosa, A.V. & Muñoz, R. (2007). Biogenic amine production in Spanish dry-cured "chorizo" sausage treated with high-pressure

and kept in chilled storage. Meat Science, 77 (3), 365-371, ISSN 0309-1740, https://doi.org/10.1016/j.meatsci.2007.03.027.

Ruiz-Jiménez, J. & Luque de Castro, M.D. (2006). Pervaporation as interface between solid samples and capillary electrophoresis. Determination of biogenic amines in food. J Chromatogr A, 1110(1-2), 245- 53.

Saad, S.M., Shaltout, F.A., Abou Elroos, N.A. & El-nahas, S.B. (2019). Antimicrobial Effect of Some Essential Oils on Some Pathogenic Bacteria in Minced Meat. J Food Sci Nutr Res., 2 (1), 012-020.

Saleh, E.A., Morshdy, A. E. M., Hafez, A. E., Hussein, M. A., Elewa, E. S. & Mahmoud, A. F. A. (2017). Effect of pomegranate peel powder on the hygienic quality of beef sausage. Journal of Microbiology, Biotechnology and Food Sciences, 6(6). https://doi.org/10.15414/jmbfs.2017.6.6.1300-1304

Schirone, M., Esposito, L., D'Onofrio, F., Visciano, P., Martuscelli, M., Mastrocola, D. & Paparella, A. (2022). Biogenic Amines in Meat and Meat Products: A Review of the Science and Future Perspectives. Foods, 11, 788. https://doi.org/10.3390/ foods11060788

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J. & Vistica, D., et.al. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening .J. Natl Cancer Inst, 82(13),1107-1112.

Stadnik, J., & Dolatowski, Z.J. (2010). Biogenic amines in meat and fermented meat products. Acta Sci. Pol., Technol. Aliment., 9(3), 251-263.

Sultan, Y.Y & Marrez, D.A. (2014). Control of histamine formation by *Morganilla morganii* in synthetic media and mackerel fish using blue green alga, *Spirulina platensis*. Alexandria Journal of Food Science and Technology, 11, 1-10.

Sung, J., Yang, C., Viennois, E., Zhang, M. & Merlin, D. (2019). Isolation, Purification, and Characterization of Ginger-derived Nanoparticles (GDNPs) from Ginger, Rhizome of *Zingiber officinale*. Bio Protoc., 5, 9(19), e3390. doi: 10.21769/BioProtoc.3390. PMID: 31737748; PMCID: PMC6857645.

Thakur, R., Yadav, K. & Khadka, K.B. (2013). Study of antioxidant, antibacterial and anti-inflammatory activity of cinnamon (Cinamomum tamala), ginger (Zingiber officinale) and turmeric (Curcuma longa). American Journal of Life Sciences, 1(6), 273-277. http://www.sciencepublishinggroup.com/j/ajls

Triki, M., Herrero, A., Jiménez-Colmenero, F. & Ruiz-Capillas, C. (2018). Quality assessment of fresh meat from several species based on free amino acid and biogenic amine contents during chilled storage. Foods, 7(9). https://doi.org/10.3390/foods7090132

Vinci, G. & Antonelli, M.L. (2002). Biogenic amines: quality index of freshness in red and white meat. Food Control, 13, 519–524.

Visciano, P., Schirone, M. & Paparella, A. (2020). An overview of histamine and other biogenic amines in fish and fish products. Foods, 9, 1795.

Yeunyongsuwan, K. & Kongkiattikajorn, J. (2005). Study of amine oxidases from cereal seedlings and local plants. In Proceedings of 43rd Kasetsart University Annual Conference: Veterinary Medicine, Science, Thailand.

Zhang, M., Viennois, E., Prasad, M., Zhang, Y., Wang, L., Zhang, Z., Han, M.K., Xiao, B., Xu, C., Srinivasan, S. & Merlin, D. (2016). Edible ginger-derived nanoparticles: A novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. Biomaterials, 101, 321-340. doi: 10.1016/j.biomaterials.2016.06.018.

Zhang, Y. j., Zhang, Y., Zhou, Y., Li, G. H., Yang, W. Z. & Feng, X. S. (2019). A review of pretreatment and analytical methods of biogenic amines in food and biological samples since 2010. Journal of Chromatography A, 1605, 360361. https://doi.org/10.1016/j.chroma.2019.07.015.

Zheng, H.M., Li, H.B. & Wang, D.W., et al. (2013). Preparation methods for monodispersed garlic oil microspheres in water using the microemulsion technique and their potential as antimicrobials. J Food Science, 78.

Zhou, X., Qiu, M., Zhao, D., Lu, F. & Ding, Y. (2016). Inhibitory Effects of Spices on Biogenic Amine Accumulation during Fish Sauce Fermentation. Journal of Food Science, 81(4), M913-M920. doi: 10.1111/1750-3841.13255.