Annals of Agric. Sci., Moshtohor
Vol. 59(1) (2021), 111 – 122

ISSN 1110-0419
https://assjm.journals.ekb.eg

Preserving Efficiency of Sausage Inoculated With *Listeria Monocytogenes* by Ginger Extract

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Abstract

Increasing the shelf life of the product is considered a challenge facing meat product manufacturers, especially products with a short shelf life such as sausage. Therefore, the use of natural agents during processing could have important good health and economic feedback. In this concern, two experiments were designed, the first one was achieved *in vitro* to study the antibiotics sensitivity of *Listeria monocytogenes* ATCC7644 for sixteen different antibiotics. *L. monocytogenes* was resistant to five antibiotics belonging to four groups of antibiotics penicillin, cephalosporin, macrolide, and tetracycline. After that, the effect of oils and aqueous, ethanolic and methanolic extracts of three medicinal plants (ginger, lemongrass, thyme) as effective agents for these bacteria to select the more potent as antibacterial agent against *L. monocytogenes* bacteria in the processed sausage. The results showed that the ethanolic extract of ginger is the most effective in inhibiting the tested bacteria compared to the other extracts .Also, the lowest inhibitory concentration (MIC) of the extract was 5000 mgL^{-1}. In the next experiment, fresh beef sausage was prepared and inoculated with of *L. monocytogenes* then, treated with MIC of ginger ethanolic extract compared to chemical preservatives (sodium benzoate, sodium nitrate, potassium sorbate, sodium propionate) and kept for 21 days to estimate the periodical changes in the different microbial groups. Total count of aerobic bacteria and coliforms besides the survived *L. monocytogenes* cells were recorded the obtained data found that all numbers gradually decreased to reach the minimum after 21 days of preservation, In contrast with yeasts and fungi, which increased their number to reach the maximum after 21 days. Moreover, after 21 days the corruptions indicators (pH, thiobarbituric acid (TBA), protein content) in sausage inoculated with *L. monocytogenes* were estimated. The results showed that there were no significant differences in the pH values between all treatments, moreover, treating sausage with ethanolic ginger extract reduced the TBA and protein content compared to the other treatments.

Keywords: plant extracts; antimicrobial activity; MIC; *L. monocytogenes*; sausage.

Introduction

Among Gram-positive bacteria, *Listeria* spp. considered an important genus in public health field, it includes 10 species in which *L. monocytogenes* is the most important one **(Bertsch *et al.*, 2014)**. *L. monocytogenes* can grow in different environments depending on under investigation strain. Some strains can grow in high concentrations of salt, wide range of temperatures (1- 45^5C) and pH (4.6- 9.6), besides it can survive on the food surfaces by forming biofilms **(Castellano *et al.*, 2008)**. *L. monocytogenes* can be transmitted to humans during the consumption of impure foods, especially perishable foods such as meat and meat products like sausage **(Gandhi and Chikindas, 2007)**. Although it is very rare to occur, listeriosis is a human disease can be fatal and cause critical health problems affected around 600 million people and cause dying of about 420,000 people around the world each year as a result of contaminated foods consumption **(Altuntas *et al.*, 2012; Park *et al.*, 2015)**. Antibiotics is the primary choice of chemical therapy for foodborne pathogens like *L. monocytogenes* infection, the repeated use cause accumulation of its residues and increasing number of antibiotic-resistant strains which cause many health and environmental problems **(Oliver *et al.*, 2011; Nazir *et al.*, 2017)**. The World Health Organization (WHO) has listed the processed meat as carcinogenic products, mainly due to the chemicals added to these products, thus increasing consumer awareness about the dangers of these chemicals and the tendency to replace with natural preservatives has become an urgent necessity **(Pereira *et al.*, 2019)**. Among the most important alternatives to these chemicals, plant extracts have been approved **(Pisoschi *et al.*, 2018)**. Ginger extracts have been reported to inhibit growth of *Listeria monocytogenes* **(Natta *et al.*, 2008)** and its antimicrobial activity was recorded at 2000 $mgml^{-1}$ **(EL Sediek *et al.*, 2012).** The unique flavors of ginger have been derived from its oils (volatile and/or non-volatile) besides some of nippy compounds like gingerol, shagaol, and zingiberene which lead to widely use in food industries **(Ravindran and Babu, 2004)**.

Fresh sausage is a meat product without thermal treatment thus it susceptible to spoilage by bacteria, which makes it not suitable for human consumption due to the occurrence of some undesirable sensory changes in appearance, texture, odor, and flavor **(Archer, 2002)**. This bacterial contamination may be resulted from the raw materials, cooking utensils, preparation venue, besides the personal hygiene of manufacturers **(Rane, 2011)**. The corruption factors of meat products included lipids oxidation, changes in protein content and pH values **(Wen, 2013)**.

For this purpose, two experiments were achieved, the first one to evaluate the antimicrobial activities of extracts and oils of three plants (ginger, thyme, lemongrass) against food-borne antibiotics-resistant pathogen *Listeria monocytogenes* under laboratory conditions. Then, the most effective and efficient one was selected as natural preservative agent to beef sausage infected with *L. monocytogenes* besides followed up its shelf-life during storage period.

Materials and methods

Pathogenic bacterium

Gram-positive bacterium *Listeria monocytogenes* ATCC7644 was obtained from Dairy Microbiological Laboratory, National Research Center, Dokki, Giza, Egypt. Five colonies of the pathogenic bacterium 24 h old grown on Mueller-Hinton agar were transferred aseptically to 100 ml test tube containing 50 ml of Tryptone soy broth medium and incubated at 37±0.2˚C for 24 h then kept at 4˚C for further.

Antibiotics sensitivity test

The standard Kirby-Bauer disk diffusion method was used in this experiment to determine the antimicrobial sensitivity profiles of the tested bacterial strain for sixteen antibiotics belonging to different groups that obtained from Oxoid, UK as described by **(Bauer *et al*., 1966).**

Medical plants

Three medical plants, ginger (*Zingiber officinale*), thyme *(Thymus vulgaris*) and lemongrass (*Cymbopogon citratus*) were obtained from Ornamental Farm, Faculty of Agriculture, Benha University, Egypt and used for aqueous, methanolic and ethanolic extraction besides their oils. Preparation of extracts and oils were achieved in Food Industries and Nutrition Division National Research Center, Dokki, Giza, Egypt.

The aqueous extract was prepared by soaking 1.0 g of fine powder in 200 mL of hot distilled water and then freeze dried **(Ijeh *et al.*, 2005)**. The methanolic extract was done by methanol (80%) at 60◦C in water bath for 3 h, filtered and evaporated in vacuum at 60◦C to extract non-volatiles (phenolics and flavonoids) compounds **(Rajeswari *et al.*, 2012)**. For ethanolic extract, 50 g of dried plant powder was added to 250 ml concentrated ethanol and kept in a shaker for 72 h. Extraction is repeated till the clear colorless solvent is obtained, the solvent's residue was evaporated and stored at 0-4˚C in an airtight container **(Asha *et al.*, 2017).**

Determination of antimicrobial activity

The effect of plant extracts and essential oils under study (thyme, lemongrass, ginger) on the growth of *L. monocytogenes* was studied by determination of the inhibition zone and minimum inhibitory concentration **(Sleigh and Timburg, 1981)**.

Preparation of experimental sausage samples

Preparation of the pathogenic inoculum

L. monocytogenes was activated in nutrient broth at 37˚C for 18-24 h till cell count recorded about 10^9 CFU/ml and it inoculated to sausage mixture by sterile pipette and mixed well to achieve final count about 10^4 CFU/g **(Uyttendaele *et al.*, 2001)**.

Preparation of sausage mixture

The recipes used in sausage preparation as well as spices mixture and curing agents are shown in Table (1). Minced beef meat was purchased from a local market and enriched with fat tissues and spices. Curing agents were dissolved in portion of water then mixed with sausage components. The remaining water (as ice) and other recipes were then added. Afterwards, the mixture was grounded to get a homogeneous and then treated by different treatments **(Lingnert and Lundgren, 1980).**

Table 1. Recipes used in the preparation of experimental sausage samples and constitution of spices mixture and curing agents.

Ingredients	%	Ingredients	%
Lean meat	69.50	Water (as ice)	13.00
Fat tissue	8.00	Spices mixture *	0.86
Sodium chloride	3.00	Curing agents **	0.44
Starch	4.10	Garlic	0.60
		Onion	0.50
Spices mixture *	**%**	**Curing agents ****	**%**
General spices	10.0	Sodium nitrite	2.18
Red pepper	11.5	Sodium ascorbate	15.16
Black pepper	45.0	Sodium glutamate	36.74
Coriander	15.0	Sodium pyrophosphate	45.92
Nutmeg	2.5		
Clove	4.0		
Fennel	6.0		
Mustard	6.0		

Treatments

- **Controls**
  1) Sausage mixture only (prepared as above).
  2) Sausage mixture + *L. monocytogenes*.
  3) Sausage mixture + curing agents.
  4) Sausage mixture + curing agents + *L. monocytogenes*.
- **Ginger ethanol extraction treatments**
  1) Sausage mixture + Ginger ethanol extract
  2) Sausage mixture + *L. monocytogenes*+ Ginger ethanol extract
  3) Sausage mixture + curing agents + Ginger ethanol extract
  4) Sausage mixture + curing agents + *L. monocytogenes* + Ginger ethanol extract

Assessments

Microbial counts

All microbial counts were calculated as colony forming units per g of sample according to **(BAM, 2002)**. The mesophilic aerobic bacteria were counted using plate agar count after 48±2 h incubation at 35±1°C. Coliform group was determined using solid medium method onto plates of violet red bile agar medium **(Difco, 1984),** plates were incubated for 24 h at 35°C, purple colonies were counted as coliforms. Enumeration of yeasts and molds were carried out using potato dextrose agar medium **(Oxoid, 1990).** Plates were incubated at 22-25°C for 3-5 days. *L. monocytogenes* was detected using *Listeria* selective enrichment medium at 30°C for 7 day. Then, plates containing selective oxford agar with *Listeria* supplement was streaked from enrichment flasks and incubated at 35°C for 48 h, typical colonies of *L. monocytogenes* will form black zones around the colonies.

Chemical analysis

pH estimation, 10 g of sausage were blended with 90 ml distilled water for 30 sec in blender then, the pH value was measured using digital pH meter model (WHEATON100) **(Zaika *et al.*, 1976)**.

Thiobarbituric acid test, mixing 10 g of sausage with 47.5 ml distilled water for 2 min and pH was adjusted to 1.5 by 2.5 ml of 4 *M* HCl, then added an antifoaming agent and glass beads and heated to collect about 50 ml of extract. After that, 5 ml of collected extract was transferred into a glass-stoppered tube, 5 ml of TBA reagent (0.2883 g TBA reagent in 100 ml of 90% glacial acetic acid) was added, stoppered, shacked and heated in boiling water for 45 min. Blank was prepared using 5 ml water with 5 ml reagent. Then, the tubes were cooled for 10 min and measured spectrophotometrically at 538 nm **(Pearson *et al.*, 1981)**. TBA was calculated as the following equation:

$$\text{TBA (mg malonaldehyde/Kg sample)} = 7.8 \text{ X } OD_{538}$$

Total protein estimation, Kjeldahl method was performed according to **Latimer (2016)** using Kjeltec system 2020 digestor, Tecator Inc., Herndon, VA, USA. Firstly, 1.0 g sausage sample was hydrolyzed by adding 15 mL of concentrated H_2SO_4 containing two copper catalyst tablets in a heat block and kept at 420°C for 2 h then let to cool and distilled water was added to the hydrolysates before neutralization and titration. Total protein was calculated by multiplying total nitrogen with conversion factor of 6.25 **(Kjeldahl, 1883).**

Results and discussion

Sensitivity of *Listeria monocytogenes* to antibiotics

Antibiotics sensitivity of *L. monocytogenes* ATCC7644 was tested by disc diffusion assay against sixteen antibiotics. Disc diffusion assay resulted in *L. monocytogenes* ATCC7644 was resistant to five antibiotics namely Cloxacillin (C9), Erythromycin (E15), Cephradine (CE), Doxycycline (DO) and Cephalexin (CL) (Table 2; Figure 1). Also, results showed that *L. monocytogenes* was susceptible to eleven antibiotics among them Ofloxacin (OFX) was the best one with 34 mm followed by Imipenem (IPM), Aztreonam (ATM), Ceftizoxime and Amoxicillin + clavulanic acid with 32, 29 and 28 mm, respectively (Table 2 and Figure 1).

Table 2. Sensitivity of *L. monocytogenes* ATCC7644 to different antibiotics.

Lab. code	Antibiotics	Concentration per disc	Group	*L. monocytogenes* ATCC7644 Inhibition zone (mm)
C9	Cloxacillin	5 mg		0
AX	Amoxicillin	25 mcg	Penicillin	22
P	Penicillin	10U		12
ZOX	Ceftizoxime	30 mg		28
CL	Cephalexin	30 mcg	Cephalosporin	0
CE	Cephradine	30 mcg		0
ATM	Aztreonam	10 mg	Beta-lactam	29
FOX	Cefoxitin	30 mcg		9
AK	Amikacin	30 mg		21
CN	Gentamycin	10 mcg	Aminoglycoside	20
TOB	Tobramycin	10 mcg		20

OFX	Ofloxacin	5 mg	Fluoroquinolone	34
E15	Erythromycin	15 mg	Macrolide	0
DO	Doxycycline	30 mcg	Tetracycline	0
IPM	Imipenem	10 mcg	Carbapenem	32
AMC	Amoxicillin + Clavulanic acid	20/10 mcg	Penicillin + Beta-lactam	26

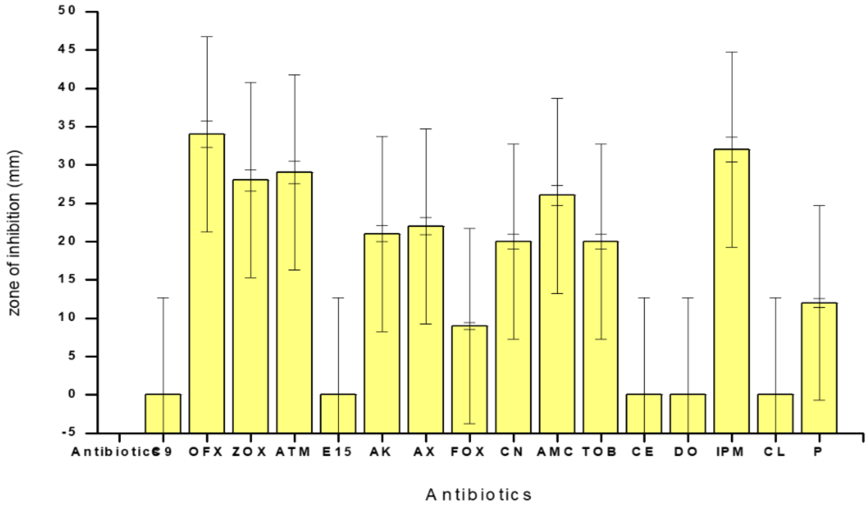

Figure 1. Sensitivity of *L. monocytogenes* ATCC7644 to different antibiotics

Although many researchers approved penicillin alone or in combination with gentamicin as the effective therapy for listeriosis besides other antibiotics like vancomycin, erythromycin, tetracycline and chloramphenicol **(Pesavento *et al.,* 2010; Altuntas *et al.,* 2012)**, our results proved that *L. monocytogenes* ATCC7644 was resistant to most of these antibiotics (Table 2; Figure 1). On the other hand, many researchers reported that *L. monocytogenes* was resistant to penicillin, vancomycin, gentamycin, cefotaxime, cefepime, oxacillin, and licosamides while sensitive to ciprofluxacin, tetracycline, erythromycin and ampicillin **(Rafieian-Kopaei *et al.,* 2016; Olaimate *et al.,* 2018)** which confirm our results. So, the failure of antibiotic therapy for listeriosis might be due to the variance between strains in their response to different antibiotics **(Chiamaka *et al.,* 2019)**.

Effect of plant oils and extracts on *Listeria monocytogenes*

Regarding the effect of ginger, lemongrass and thyme oils on *L. monocytogenes*, Figure (2) indicated that the inhibition percentage of all oils was gradually increased from 10% reached to its maximum at 50% and decreased thereafter to give lower percentage at 100%. Trend of results was true with lemongrass and thyme oils and may be due to that the concentrated oils gave only dehydrated effect and can't kill the bacterial cells. Furthermore, **Gutierrez *et al.* (2009)** proved that the thyme oil contains more than 60 ingredients, such as carvacrol, thymol, p-cymene and g-terpinene. Among them, carvacrol and thymol have been found to possess antimicrobial activity against *L. monocytogenes* **(Gill and Holley, 2006)**. On the other hand, ginger oil inhibited *L. monocytogenes* at 50% only.

Among all tested oils, lemongrass was the best one because it was able to inhibit *L. monocytogenes* at different concentrations, this might be attributed to that lemongrass oil contains antioxidants and aromatic compounds as citral, myrcene, geraniol, and geranyl acetate which responsible for its antimicrobial activity against wide range of microorganisms such as molds and yeasts, Gram- negative and positive bacteria **(Naik *et al.,* 2010)**. **Bonada de Silva *et al.* (2008)** found that these components can affect the bacterial cell growth by diffusion of monoterpene which cause membrane disruption and facilitated its solubility **(Sivakumar and Bautista-Banos, 2014; Hadjilouka *et al.,* 2015)**.

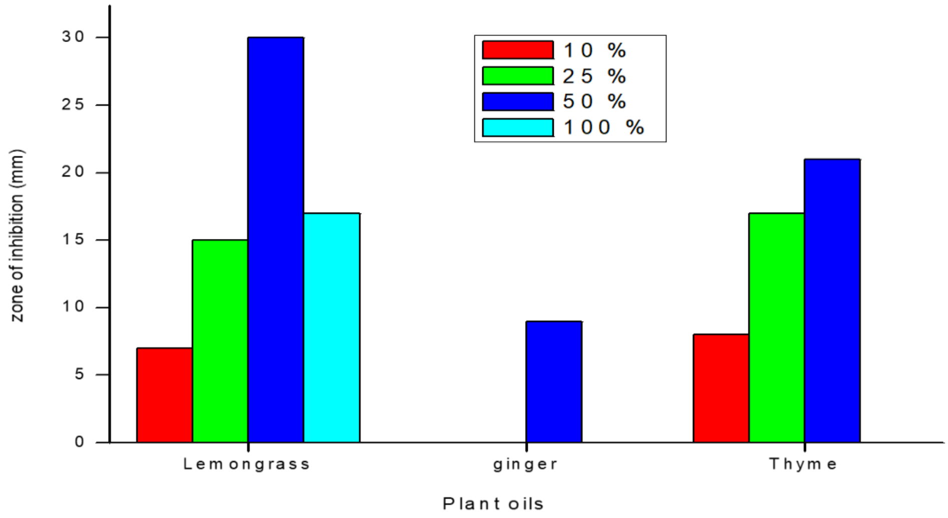

Figure 2. Effect of plant oils on *L. monocytogenes*

Concerning the effect of plant extracts on *L. monocytogenes*, data presented in Figure (3) indicated that the aqueous extracts of all used plants revealed no inhibition zones. **Indu *et al.* (2006)** recorded that the aqueous extract of ginger was incapable of being antibacterial agent to *L. monocytogenes* at any concentration (10-100%). In this point we can speculated that the dried leaves contained essential oils, acids, tannins, alkaloids, steroids which their activities depend upon their solubility in various solvents, so this explain why ethanolic and methanolic extracts showed activity while aqueous extracts not **(Al-Daihan *et al.*, 2013)**. It means that the extraction method and used solvent influenced the antibacterial properties of plants.

Also, the ethanolic extract of ginger was able to inhibit *L. monocytogenes* growth at different concentrations with inhibition zones ranged from 6.0 to 23 mm (Figure 3). Moreover, inhibition zones (22 and 23 mm) were recorded when *L. monocytogenes* treated with the ethanolic extract of lemongrass at 50 and 100%, respectively. Whereas the ethanolic extract of thyme inhibited *L. monocytogenes* when applied at 100% only. This might be due to that it contains flavonoids and tannins which responsible for this activity **(Revathi *et al.*, 2012)**.

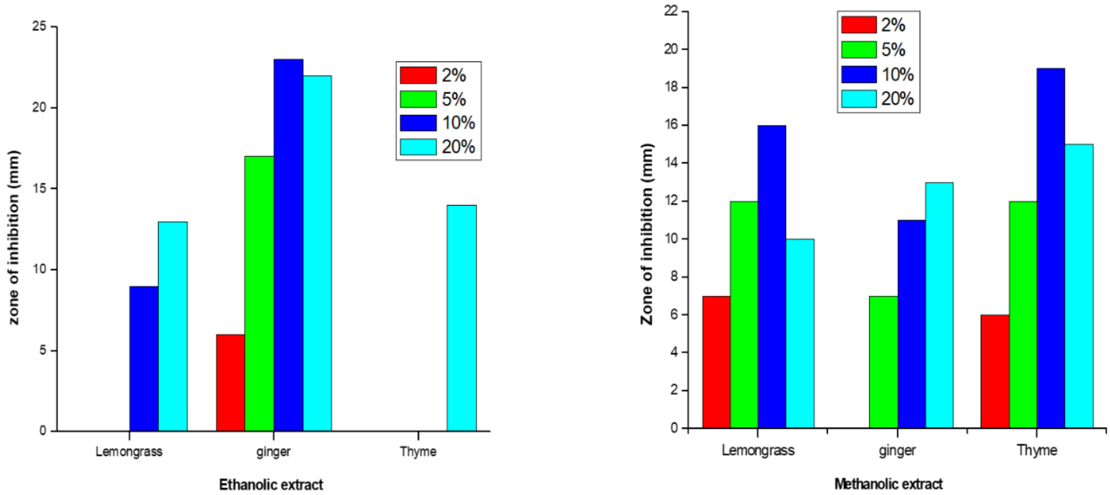

Figure 3. Effect of plant extracts on *L. monocytogenes*, **(A)** Ethanolic extract; (**B)** Methanolic extract

With regard to the methanolic extracts, data recorded in Figure 3 showed that all various concentrations of ginger, lemongrass and thyme were able to inhibit *L. monocytogenes* at different values ranged from 6 to 19 mm except ginger at 10% recorded no inhibition zone, the highest inhibition zone was recorded at 50% thyme. Additionally, the methanolic extract of lemongrass had the ability to inhibit *L. monocytogenes* at different concentrations and this may be due to its phytochemical components like flavonoids, alkaloids, and tannins which considered as antimicrobial agents **(Gopinath *et al.*, 2013)**.

Minimum inhibitory of concentration

According to results of the previous experiment, ginger ethanolic extract was the most efficient agent against *L. monocytogenes* as recommended by **Paul *et al.* (2015)** who reported that among the most popular plants used as natural preservative, ginger is well known to have antioxidant activity and effective antimicrobial agents. Reduction of *L. monocytogenes* growth compared to control was recorded under treatment by different concentrations of ginger extract. This trend of results was true cross all hours. Additionally, the growth rate was sharply reduced after 12 h after inoculation when ginger extract was added at 200000 mgL^{-1} and this effect was indicated by the reduction in OD_{600} value (Figure 4). This result was logic because ginger had a bacteriostatic effect which directly proportional to the concentration as confirmed by **Deepa and Vrinda (2015)**.

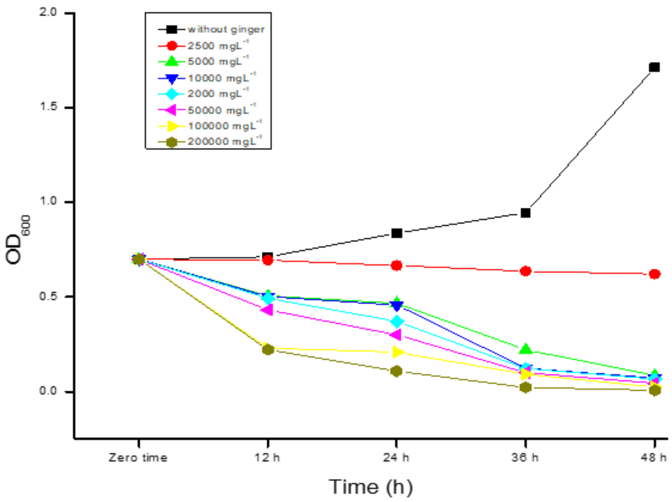

Figure 4. Hourly changes of *L. monocytogenes* growth under different concentrations of ginger ethanolic extract.

The minimum inhibitory concentration (MIC) of ginger ethanolic extract on *L. monocytogenes* was showed in Table (3), the MIC was identified as ginger extract required for inhibition of 95% of *L. monocytogenes* growth. The ginger extract at different concentrations from 200000 to 5000 mgL^{-1} had good antibacterial action against *L. monocytogenes* with reduction rate ≥ 95%, whereas at 2500 mgL^{-1} had a moderate antibacterial action with 63.7% reduction in growth rate.

Table 3. Minimum inhibitory concentration of ginger ethanolic extract against *L. monocytogenes*

Ginger Concentrations (mgL^{-1})	OD_{600}	Reduction rate (%)
2500	0.621	63.7
5000	0.085	95.0
10000	0.071	95.6
20000	0.066	96.1
50000	0.044	97.4
100000	0.024	98.6
200000	0.007	99.6
*Control (without ginger)	1.712	--

Compared to results by **Deepa and Vrinda (2015)**, our results recorded high concentration of ginger extract which required to inhibit 95% of *L. monocytogenes* growth although many researchers reported that Gram-positive bacteria were more sensitive than Gram-negative bacteria **(Onyeagba *et al.*, 2004)**. These results were controlled by many factors like the difference among *L. monocytogenes* strains and the diversity of ginger used. Finally, as presented in this study the MIC of ginger ethanolic extract against *L. monocytogenes* was 5000 mgL^{-1} and this concentration was used in the next experiment for sausage preservation.

Sausage experiment

Effect of ginger ethanolic extract on periodical changes of microbial counts (CFUmL^{-1}) in sausage infected with *L. monocytogenes*

According to results of the previous experiments, ginger ethanolic extract applied at as a natural preservative agent for sausage at 5000 mgL^{-1} as resulted from MIC experiment. The total microbial count of sausage as affected by various treatments of ginger ethanolic extract of *L. monocytogenes* are presented in Figure (5), results indicated that, total bacterial count of sausage treated with ginger ethanolic extract were decreased with decreasing period of storage up to 21 days (Figure 5 A). These results might be attributed to the presence of gingerols besides more than 50 components as discussed by **Abdalla and Abdallah (2018).** Also, the extraction method of ginger affects its components as reported by **Ali *et al.* (2008)** that the concentrations of gingerols in dry ginger are reduced than of fresh ginger, but the concentrations of 6-shagaols increases. This supports our results that we extracted dried ginger.

Concerning coliform group counts, data presented in Figure (5 B) showed the effect of ginger ethanolic extract on *L. monocytogenes* during storage of experimental sausage. The obtained results indicated that, a fair decrease was noticed in coliform group counts of all sausage treatments with increasing of storage period. The highest decrement rate was shown in sausage +C+L+G, where coliform group completely disappeared at 15 days for sausage +C+L+G and Sausage +C+ G. This result might be attributed to the presence of some antimicrobial compounds in ginger extract like α-pinene, borneol, camphene and linalool **(Nychas and Skandamis, 2003)**.

Yeasts and molds count of experimental sausage treated with ginger ethanolic extract during 21 days of cold storage at 4-5ºC were determined and data were presented in Figure (5 C). The initial yeasts and molds in a straight line till 7 days, then increasing gradually till 15 days and Significantly increases until the day 21. The same trend of results was obtained by **Samelis and Metaxopoulos (1998)** who reported that yeast counts increasing during manufacturing and storage in air at 3ºC and 12ºC of sausage.

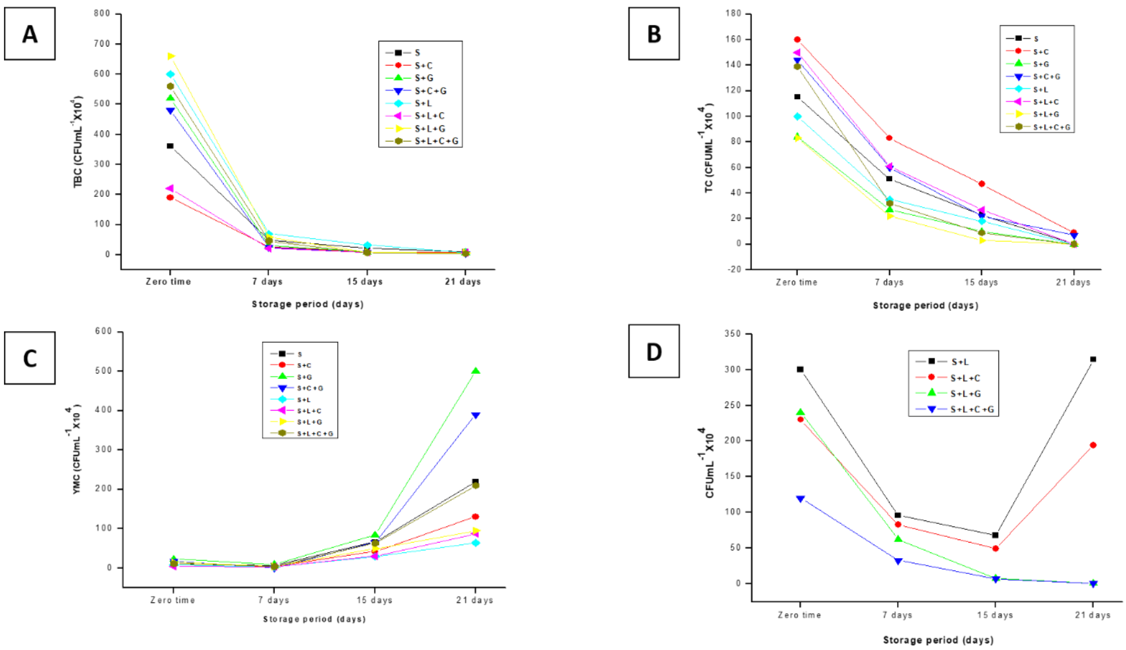

Figure 5. Effect of ginger ethanolic extract on periodical changes of microbial counts (CFU/mL) in sausage infected with *L. monocytogenes*: **A)** total bacterial count; **B)** coliform; **C)** yeasts and molds; **D)** survived *L. monocytogenes*.

S: sausage; C: chemical preservatives; G: ginger ethanolic extract; L: *Listeria monocytogenes*

Also, **Hanušová *et al.* (2009)** proved that molds could cause spoilage of sausage and grow rapidly on its surface, so the surface should be treated with various preservatives either synthetic or natural to prevent the growth of molds. Another recommendation by **Ture *et al.* (2008)** is to apply antifungal agents especially rosemary extract.

Periodical changes of *L. monocytogenes* count in sausage treated with ginger ethanolic extract for 21 days were showed in (Figure 5 D). Results indicated that the presence of ginger alone or in combination with chemical preservative caused gradually decrease in *L. monocytogenes* count from zero time to reach its lowest count after 21 days of incubation. These results were logic and due to the presence of ginger components such as zingerone, gingirdiol, zingibrene, and particularly gingerol and shagol, sesquiterpenes such as farnesene, corcomin and beta-Bisabolene which affected its antimicrobial activities **(Giriraju and Yunus, 2013; Azadpour *et al.*, 2016)**. On contrast, *L. monocytogenes* count in sausage infected with pathogenic bacteria but without any preservatives besides sausage treated with chemical preservatives decreased sharply after seven days and then increased dramatically after 15 days.

Foods prepared from meat contained high quantity of lipids and proteins, lipids play an important role due to their autoxidation that leads to several undesirable compounds formation **(Paul *et al.*, 2015)**. These compounds, in cooperation with surface-contaminating microorganisms are responsible for food spoilage, which ultimately leads to the occurrence of many human diseases **(Mielnik *et al.*, 2008)**. Chemical oxidants used to decrease lipid oxidation were found to be carcinogenic, which led to growing interest of natural antioxidants as we were worked on **(Valko *et al.*, 2007)**. Amines that formed in foods as a result of protein spoilage during storage or enzymatic or thermal processing are considered a public health concern due to physiological and toxic effects **(Vinci and Antonelli, 2002)**. Lipid oxidation, protein putrefaction and microbial growth in sausages can controlled by either synthetic or natural food preservatives **(Estevez and Cava, 2006)**.

The natural preservatives like plant extracts have acquired great interest due to their phenolic content, suggesting that antioxidant action is similar to that of synthetic phenolic antioxidants **(Paul *et al.*, 2015)**. Besides, the meat products containing natural antioxidants became preferable by the consumers **(Valko *et al.*, 2007)**, which led us to select new natural antioxidants as effective alternatives.

Corruptions indicators in sausage infected with *L. monocytogenes*

The pH values of prepared sausage were ranged from 6.80 to 7.23 for all treatments besides no differences were recorded in pH between sausage treated with different types of agents, except for the sausage infected with *L. monocytogenes* only (Table 4). This proved that no fermentation was done during sausage preparation. The highest pH value was recorded in sausage infected with *L. monocytogenes* and this may be due to the production of alkaline secondary metabolites which buffering pH value. While the lowest value was recorded in sausage without any treatment and this indicate that some of native microorganisms may be grown and cause decrease in pH. Additionally, the recorded neutral pH in treatments with either chemical or ginger extract indicated that these agents prevent growth of native contaminants. These results were in congruence with those obtained with **Alirezalu *et al.* (2018)** that the highest pH value was recorded at the end of storage period. On contrary, a decrease in the pH value of beef pies treated with olive leaf extract was recorded from 5.7 to 5.5 during the 12-day storage period compared to the control **(Hayes *et al.*, 2010).**

Regarding thiobarbituric acid (TBA) content in sausage treated with various agents, data in Table (4) showed that higher TBA value was recorded in sausage without any additions and this may be due to that no preservation agent was added to stop lipid oxidation in meat. Furthermore, sausage infected with *L. monocytogenes* only has the highest TBA content (1.095 $mgKg^{-1}$) compared to treatments with chemical or ginger extract. This trend of results was in harmony with those obtained by **Ghonaimy (2004)** who recorded higher TBA (1.274 $mgKg^{-1}$) in sausage infected with *L. monocytogenes* compared with sausage without any additions (1.134 $mgKg^{-1}$) after 15 days of storage. The four treatments of sausage with ginger extract with/without *L. monocytogenes* or chemicals recorded lower values of TBA compared to other treatments. This may be due to that plant extracts are rich in healthier components, antimicrobial activity and antioxidant capacity and have particular importance in improving the sausage quality **(Alirezalu *et al.*, 2018)**.

Table 4. Corruptions' indicators in processed sausage infected with *L. monocytogenes*.

Treatments	pH	TBA (mg/Kg)	Protein (%)
Sausage	6.80	0.990	17.3
Sausage + *L*	7.23	1.095	17.2
Sausage + C	6.93	0.819	17.0
Sausage + C + *L*	6.89	0.858	16.9
Sausage + G	6.93	0.727	16.3
Sausage + *L* + G	6.89	0.761	16.0
Sausage + C + G	6.81	0.628	15.9
Sausage + C+ *L* + G	6.97	0.733	15.7

TBA: Thiobarbituric acid *L*: *L. monocytogenes*
C: chemical preservatives G: gingers ethanolic extract

Concerning change in protein content due to various treatments, data in Table (4) indicated that the addition of ginger extract did not result in differences in protein content. The highest values were recorded in sausage without any addition followed by sausage infected with *L. monocytogenes* then sausage treated with chemical preservatives only. Moreover, data also proved that the presence of ginger extract with or without any other additions decrease protein content compared to other treatments. These results were consistent with those obtained by **Alirezalu *et al.* (2018)** who reported that the treatment of sausage with olive extract didn't affect protein content compared to control without any additions and recorded 13.72 to 13.96 % in control and olive extract treatment, respectively.

Conclusion

It was concluded that the extracts of medical plants especially the ethanolic extracts like ginger produced high bacteriostatic effect against antibiotic-resistant *Listeria monocytogenes in vitro.* Hence, the addition of ginger ethanolic extract as natural preservative to improve quality and increase shelf-life of sausage. Finally, ginger extract approved as a sufficient protective agent against lipid oxidation in prepared sausage besides its antimicrobial activity.

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كفاءة حفظ السجق الملقح ببكتريا *Listeria monocytogenes* بواسطة مستخلص الزنجبيل

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تعتبر زيادة فترة الحفظ للمنتج تحديًا صعباً يواجه مصنعي منتجات اللحوم خاصة المنتجات ذات فترة الحفظ القصيرة مثل السجق. لذلك، فإن إستخدام عوامل الحفظ الطبيعية أثناء التصنيع يمكن أن يكون له نتائج صحية وإقتصادية جيدة.

لذلك تم تصميم تجربتين الأولى أجريت لدراسة حساسية بكتريا *Listeria monocytogenes* ATCC7644 لستة عشر مضاد حيوي، ولقد وجد أن بكتريا *L. monocytogenes* مقاومة لخمسة مضادات حيوية تنتمي إلى أربع مجموعات هى البنسلين، والسيفالوسبورين، والماكروليد، والتتراسيكلين.

بعد ذلك، تم دراسة تأثير الزيوت والمستخلصات المائية والإيثانولية والميثانولية لثلاثة نباتات طبية هى الزنجبيل وحشيشة الليمون والزعتر كعوامل فعالة لتثبيط نشاط بكتريا *L. monocytogenes* المقاومة للمضادات الحيوية لإختيار أكثرها فعالية وتطبيقها كعامل مضاد لهذه البكتريا في السجق المصنع.

ولقد أظهرت النتائج أن المستخلص الإيثانولي للزنجبيل هو الأكثر فاعلية في تثبيط البكتريا المختبرة مقارنة بالمستخلصات الأخرى وأن أقل تركيز مثبط منه (MIC) هو 5000 مجم/لتر.

وفي التجربة الثانية، تم تحضير السجق الطازج وتلقيحه بـ *L. monocytogenes* ثم معاملته بـ MIC للمستخلص الإيثانولي للزنجبيل مقارنة بالمواد الحافظة الكيميائية (بنزوات الصوديوم, نترات الصوديوم، سوربات البوتاسيوم، بروبيونات الصوديوم) وحفظه لمدة 21 يومًا ومتابعة التغيرات الدورية في المجاميع الميكروبية المختلفة. تم تسجيل العدد الكلي للبكتريا الهوائية وبكتريا القولون بالإضافة إلى بكتيريا *L. monocytogenes* ووجد أن جميع الأعداد تناقصت تدريجيًا من بداية التجربة لتصل إلى الحد الأدنى بعد 21 يومًا من الحفظ ،على عكس ذلك مع الخمائر والفطريات والتي زاد تعدادها من بداية التجربة ليصل إلى الحد الأقصى بعد 21 يومًا. علاوة على ذلك ، بعد 21 يومًا تم تقدير مؤشرات الفساد (الأس الهيدروجيني، حمض الثيوباربيتوريك (TBA)، المحتوى البروتينى) في السجق الملقح ببكتريا *L. monocytogenes*. ولقد أوضحت النتائج أنه لا يوجد فروق معنوية في قيم الأس الهيدروجيني بين جميع المعاملات ولكن وجد أن معاملة السجق بمستخلص الزنجبيل الإيثانولي قلل من محتوى TBA والبروتين مقارنة بالمعاملات الأخرى.

الكلمات الدالة: المستخلصات النباتية ، النشاط التضادى ، أقل تركيز مثبط ، *L. monocytogenes* ، السجق