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 *vivo* 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°C) 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 Sedick 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 (Ashan 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 10⁶ CFU/ml and it inoculated to sausage mixture by sterile pipette and mixed well to achieve final count about 10⁷ 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.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean meat</td>
<td>69.50</td>
<td>Water (as ice)</td>
<td>13.00</td>
</tr>
<tr>
<td>Fat tissue</td>
<td>8.00</td>
<td>Spices mixture *</td>
<td>0.86</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.00</td>
<td>Curing agents **</td>
<td>0.44</td>
</tr>
<tr>
<td>Starch</td>
<td>4.10</td>
<td>Garlic</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Onion</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Spices mixture</strong></td>
<td>10.0</td>
<td><strong>Curing agents</strong></td>
<td>2.18</td>
</tr>
<tr>
<td>General spices</td>
<td>11.5</td>
<td>Sodium nitrite</td>
<td></td>
</tr>
<tr>
<td>Red pepper</td>
<td>45.0</td>
<td>Sodium ascorbate</td>
<td>15.16</td>
</tr>
<tr>
<td>Black pepper</td>
<td>15.0</td>
<td>Sodium glutamate</td>
<td>36.74</td>
</tr>
<tr>
<td>Coriander</td>
<td>2.5</td>
<td>Sodium pyrophosphate</td>
<td>45.92</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fennel</td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mustard</td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Preserving Efficiency of Sausage Inoculated With Listeria Monocytogenes by Ginger Extract

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, shaken 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:

TBA (mg malonaldehyde/Kg sample) = 7.8 X OD538

Total protein estimation, Kjeldahl method was performed according to Latimer (2016) using Kjeltac system 2020 digestor, Tecator Inc., Herndon, VA, USA. Firstly, 1.0 g sausage sample was hydrolyzed by adding 15 mL of concentrated H2SO4 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), Cephadine (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>
<thead>
<tr>
<th>Lab. code</th>
<th>Antibiotics</th>
<th>Concentration per disc</th>
<th>Group</th>
<th>L. monocytogenes ATCC7644 Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9</td>
<td>Cloxacillin</td>
<td>5 mg</td>
<td>Penicillin</td>
<td>0</td>
</tr>
<tr>
<td>AX</td>
<td>Amoxicillin</td>
<td>25 mcg</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>P</td>
<td>Penicillin</td>
<td>10U</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>ZOX</td>
<td>Cefitoxime</td>
<td>30 mg</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>CL</td>
<td>Cephalexin</td>
<td>30 mcg</td>
<td>Cephalosporin</td>
<td>0</td>
</tr>
<tr>
<td>CE</td>
<td>Cephadrine</td>
<td>30 mcg</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>ATM</td>
<td>Aztreonam</td>
<td>10 mg</td>
<td>Beta-lactam</td>
<td>29</td>
</tr>
<tr>
<td>FOX</td>
<td>Cefoxitin</td>
<td>30 mcg</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>AK</td>
<td>Amikacin</td>
<td>30 mg</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>CN</td>
<td>Gentamycin</td>
<td>10 mcg</td>
<td>Aminoglycoside</td>
<td>20</td>
</tr>
<tr>
<td>TOB</td>
<td>Tobramycin</td>
<td>10 mcg</td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

Table 2. Sensitivity of L. monocytogenes ATCC7644 to different antibiotics.
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, erthyromycin, 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 ciprofluaxcin, tetracycline, erthyromycin 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 monoterpenes which cause membrane disruption and facilitated its solubility (Sivakumar and Bautista-Banos, 2014; Hadjilouka et al., 2015).
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).

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 OD600 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.](image)

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

<table>
<thead>
<tr>
<th>Ginger Concentrations (mgL−1)</th>
<th>OD600</th>
<th>Reduction rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2500</td>
<td>0.621</td>
<td>63.7</td>
</tr>
<tr>
<td>5000</td>
<td>0.085</td>
<td>95.0</td>
</tr>
<tr>
<td>10000</td>
<td>0.071</td>
<td>95.6</td>
</tr>
<tr>
<td>20000</td>
<td>0.066</td>
<td>96.1</td>
</tr>
<tr>
<td>50000</td>
<td>0.044</td>
<td>97.4</td>
</tr>
<tr>
<td>100000</td>
<td>0.024</td>
<td>98.6</td>
</tr>
<tr>
<td>200000</td>
<td>0.007</td>
<td>99.6</td>
</tr>
<tr>
<td><em>Control (without ginger)</em></td>
<td>1.712</td>
<td>--</td>
</tr>
</tbody>
</table>

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 (CFU/mL) 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 \textit{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-shagalois 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 \textit{L. monocytogenes} during storage period 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 4-5°C 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 3°C and 12°C of sausage.

Periodical changes of \textit{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 \textit{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, \textit{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*.**

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

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

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.

**References**


**Bacteriological Analytical Manual (BAM) (2002).** Published by FDA (Foods Program Compendium of Analytical Laboratory Methods).


Gill, A.O. and Holley, R.A. (2006). Disruption of *Escherichia coli*, *Listeria monocytogenes* and...


Latimer, G.W. (2016). Official methods of analysis of AOAC international. AOAC international, Gaithersburg, MD, USA.


Naik, M., Fomba, B., Jaykumar, E. and Bhat, J. (2010). Antimicrobial activity of lemongrass (Cymbopogon citratus) oil against some selected
Hamoda, Mayar, E. et al.

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كفاءة حفظ السجق الملقح ببكتريا Listeria monocytogenes

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تعتبر زيادة فترة الحفظ للمنتج تحديًا صعبًا يواجه مصنعي المنتجات الحيوية خاصة المنتجات ذات فترة الحفظ القصيرة مثل السجق. لذلك، فإن استخدام عوامل حفظ الطبيعية أثناء التصنيع يمكن أن يكون له تأثير صحي واقتصادي جيد.

لذلك تم تصميم تجربتي الأولى أجريت لدراسة حساسية بكتريا Listeria monocytogenes ATCC7644 مقاومة لخمسة مضادات حيوية تنتمي إلى أربع مجموعات هي البنسلين، والسالفانوسورين، والمانتشيكل، والتركسيلين.

بعد ذلك، تم دراسة تأثير الزيوت والمستخلصات المائية والإيثانولية والميثانولية لثلاثة نباتات طبية هي الزنجبيل وحشيشة الليمون والزعتر كمواد فعالة لثبيط نشاط بكتريا L. monocytogenes المقاومة للمضادات الحيوية لاختيار 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 والتقسيم عليه بمستخلصات L. monocytogenes بمعدلات 1/10 MIC و 1 MIC. تم تحضير فئتين من كل المعالجة بمعدلات قشرة نباتية تم تخزينها لمدة 21 يومًا. وتم تحليل النتائج من خلال عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية المختلفة. تم تسجيل عدد البكتريا الهوائية وعدد البكتريا في التحليلات الميكروبية مختلفة.

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