Microbiological Quality of Ground Pork during Storage after Application of Garlic

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Abstract

The aim of the present study was to analyze the antimicrobial effect of garlic to the various groups of microorganisms during storage of ground pork. In the experiment, samples of ground pork were used. The total viable counts (TVC), *Pseudomonas* spp. and coliform bacteria (CB) after application of fresh garlic, garlic oil and dried garlic and vacuum packaging were evaluated. The samples were analyzed at 0., 2nd., 4th., 6th and 8th day of storage of meat at temperature 4 ° C. There were analyzed 120 samples of ground meat (60 samples aerobically packed, 60 samples vacuum packed). Dried and fresh garlic were added to the ground pork in the amount 1 g per 5 g of sample and 1 mL of garlic oil per 5 g of sample. Plate dilution method was used to microbiological analysis of ground pork. The average values of total viable counts (TVC) after 8th day of storage were 4.98 log CFU.g^{-1} in the samples treated with fresh garlic in the samples with vacuum packaging, 5.36 log CFU.g^{-1} in control group with vacuum packaging and in samples treated with dried garlic in vacuum packaging, 5.59 log CFU.g^{-1} in the samples treated with garlic oil with vacuum packaging, 6.16 log CFU.g^{-1} in the samples treated with fresh garlic in air condition, 6.31 log CFU.g^{-1} in samples treated with dried garlic in air condition, 6.50 log CFU.g^{-1} in samples treated with garlic oil in air condition, 8.45 log CFU.g^{-1} in control group without treatment in air condition. The average values of coliform bacteria (CB) were after 8th day of storage 4.13 log CFU.g^{-1} in the control group without treatment in air condition, 2.82 log CFU.g^{-1} in the samples treated with garlic oil, 2.22 log CFU.g^{-1} in the samples treated with dried garlic in air condition, 1.82 log CFU.g^{-1} in samples treated with garlic oil in vacuum condition, 1.78 log CFU.g^{-1} in the samples treated with fresh garlic in air condition. The presence of coliform bacteria was not detected in control groups without treatment in vacuum packaging, in the samples treated with fresh garlic in vacuum packaging and in the samples treated with dried garlic in vacuum packaging. The average values of *Pseudomonas* spp. after 8 days were 1.78 log KTJ.g^{-1} in control group without treatment in air condition and in the samples treated with dried garlic in air condition. *Pseudomonas* spp. was not detected in another samples.

Keywords: ground pork, microbiological quality, fresh garlic, dried garlic, garlic oil.

1. Introduction

Raw meat is a rich nutrient matrix and is highly perishable. Spoilage of raw meat may occur in two ways during storage: microbial growth and oxidative rancidity [1]. This is especially the case for ground meat, since ground meat is more sensitive to oxidation because of its porous structure and it has more susceptibility to microbial spoilage due to the grounding process [2].

Pork and its products are rich in protein, lipids and have suitable moisture content, which make them 'natural media' of microorganisms. Pork also contains high level of unsaturated fatty acids which are prone to oxidation. Microbial growth and lipid oxidation are the two leading factors for quality deterioration of meat [3].

Garlic is traditional edible and medicinal plants. It is regularly used as seasonings to enhance the sensory quality of foods. Besides its health benefits have been studied widely, the extracts

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garlic have also been individually studied on its antioxidant properties in food preservation [4]. In vitro and in vivo studies have shown a great effectiveness of garlic and onion against a broad spectrum of fungi and yeasts, including certain *Candida, Torulopsis, Cryptococcus* and *Aspergillus* species. Garlic has been proven to inhibit the growth of gram positive and gram-negative bacteria including strains of, e.g. *Pseudomonas, Proteus, Escherichia,, Staphylococcus, Salmonella* [5]. Garlic (*Allium sativum*) contains a number of sulfur and phenolic compounds, which have excellent antioxidant and antimicrobial activity. Sulfur containing compounds are the most characteristic constituents of garlic, especially alliin and allicin. The amino acid, alliin, is the most representative sulfur compound in fresh garlic, and is converted to allicin by alliinase when garlic is crushed. Furthermore, the allicin produced is instantly decomposed to other sulfur compounds, such as, dimethyl sulfide, dimethyl disulfide, diallyl disulfide, dithiines and ajoene. However, the proportion of these constituents are significantly affected by the methods of preparation during manufacturing, resulting in different pharmacologic activities. Grinding garlic bulbs activates alliinase, which converts alliin to allicin, hence fresh garlic includes allicin and its derivaties including diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) [6].

2. Material and methods

The aim of the present study was to monitor the effect of fresh garlic, dried garlic and garlic oil to microbiological quality of ground pork during storage. Samples of breast muscles were used to experiment. The samples of meat were storage 8 days at temperature 4 °C. The microbiological analysis were performed on 0., 2., 4., 6. and 8. days of storage, there were analyzed 120 samples. The ground pork samples were prepared as follow:
- control group (CG) – ground pork samples were packaged to polyethylene bags and stored aerobically at 4 ±0.5°C;
- control group vacuum-packaged - ground pork samples were packaged to polyethylene bags and stored anaerobically at 4 ±0.5°C;
- vacuum-packaged samples with *fresh garlic (1 g of fresh garlic per 5 g of meat)* - ground pork samples were treated with *fresh garlic* and samples were packaged to polyethylene bags and stored anaerobically at 4 ±0.5°C;
- vacuum-packaged samples with *dried garlic (1 g of dried garlic per 5 g of meat)* - ground pork samples were treated with dried garlic and samples were packaged to polyethylene bags and stored anaerobically at 4 ±0.5°C;
- vacuum-packaged samples with *garlic oil (1 mL of fresh garlic per 5 g of meat)* - ground pork samples were treated with garlic oil and samples were packaged to polyethylene bags and stored anaerobically at 4 ±0.5°C.

Preparation of garlic oil

Garlic oil was prepared by mixing 600 mL of sunflower oil and 10 cloves of crushed garlic. Garlic oil was stored in a glass bottle at temperature 20 °C.

Microbiological analysis

The following groups of microorganisms were determined in samples of chicken breast,
- Total viable counts (TVC),
- Coliform bacteria (CB),
- *Pseudomonas* spp.

Plate dilution method was used for the quantitative determination of the number of colony forming units (CFU) of each group of microorganisms. Microbiological analyses were conducted with accordance to standard microbiological methods. Basic dilutions (10^{-1}) was obtained by mixing 5 g of the sample (ground pork) and 45 mL of physiological solution (0.85% NaCl). Plate Count Agar (PCA, Oxoid, UK) was used for determine of TVC in samples. Dilutions of 10^{-3} and 10^{-4} were used to determine of TVC. Petri dishes were cultivated upside-down in a thermostat at 30 °C for 48-72 hours. Violet red bile agar (VRBA, Oxoid, UK) was used for determine of CB in samples. Dilutions of 10^{-1} and 10^{-2} were used to determine of CB. Petri dishes were cultivated upside-down in a thermostat at 37 °C for 24 -48 hours. Pseudomonas Isolation Agar (PIA, Oxoid, UK) was used for isolation of Pseudomonas spp. Inoculated agar was incubated at 35 °C ±1 °C during 48 hours.

Calculation of microorganisms

The number of microorganisms in 1 g samples (N) were calculated using the following formula:

$$N = \Sigma C / [(n_1 + 0{,}1n_2) .d]$$

ΣC – sum of characteristic colonies on selected plates,
$\mathbf{n_1}$ – number of dishes from 1. dilutions used to calculate,
$\mathbf{n_2}$– number of dishes from 2. dilutions used to calculate,
d – dilution factor identical with 1. used dilution.

Statistics

Arithmetic mean, standard deviation, coefficient of variation (%) were performed using Excel.

3. Results and discussion

The average value of TVC in samples stored in air condition ranged from od 4.59 log CFU.g^{-1} on 0th day to 8.45 log CFU.g^{-1} on 8th day of storage. The average value of TVC were in range from 4.59 log CFU.g^{-1} on 0th day to 5.36 log CFU.g^{-1} on 8th day in the samples in vacuum packaging. The average value of TVC ranged from 4.59 log CFU.g^{-1} on 0th day to 6.16 log CFU.g^{-1} on 8th day in the samples treated with fresh garlic in air condition. The average value of TVC ranged from 4.59 log CFU.g^{-1} on 0th day to 4,98 log CFU.g^{-1} on 8th day of storage in the samples treated with fresh garlic in vacuum packaking (Figure 1).

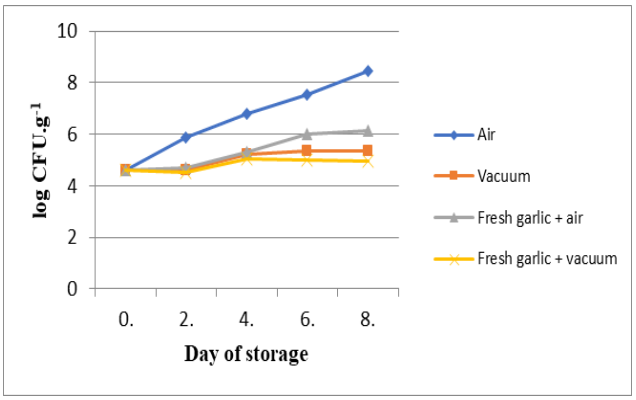

Figure 1. Average values of TVC in control samples and samples treated with fresh garlic

Yin and Cheng [7] investigated the antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds (diallyl sulfide, diallyl disulfide, S-ethyl cysteine, and n-acetyl cysteine) in ground beef, and noted that four garlicderived compounds reduced lipid oxidation, and diallyl sulfide and diallyl disulfide having hydrophobic properties inhibited the growth of pathogenic bacteria such *as Salmonella Typhimurium, E. coli* O157:H7, *L. monocytogenes*, *Staphylococcus aureus* and *Campylobacter jejuni.*

The average value of TVC in samples treated with dried garlic in air condition was 6.31 log CFU.g^{-1} on 8th day of storage, the average value of TVC treated with dried garlic in vacuum packaking was 5.36 log CFU.g^{-1} on 8th day of storage (Figure 2).

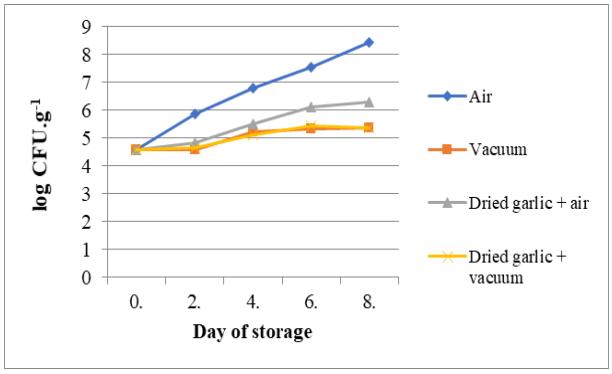

Figure 2. Average values of TVC in control samples and samples treated with dried garlic

Effect of fresh and dried garlic, onion, thyme and oregano on the bacterial germ count of minced pork stored at 5°C was evaluated. All spices were added in 1% concentration. Furthermore, MIC (Minimal Inhibitory Concentration) values of marjoram and thyme essential oils on *Escherichia coli* were determined in vitro, and their antibacterial effect was tested in minced pork inoculated with *E. coli*. In general, fresh spices showed weak or no inhibition on the total cell count of minced pork, or even caused increased microbe count. On the contrary, dried garlic and thyme decreased total cell count with 1 or two orders of magnitude. MIC values for marjoram and thyme essential oils were 0.5 µl/mL and 2 µl/mL, respectively. The essential oils decreased *E. coli* cell number in minced pork with 1 log CFU after 24 h storage at 5 °C [8].

The average value of TVC in samples treated with garlic oil in air condition ranged from 4.59 log CFU.g^{-1} on 0 day to 6.50 log CFU.g^{-1} on 8th day of storage. The average value of TVC treated with garlic oil in vacuum packaging ranged from 4.59

log CFU.g^{-1} on 0 day to 5,59 log CFU.g^{-1} on 8th day of storage (Figure 3).

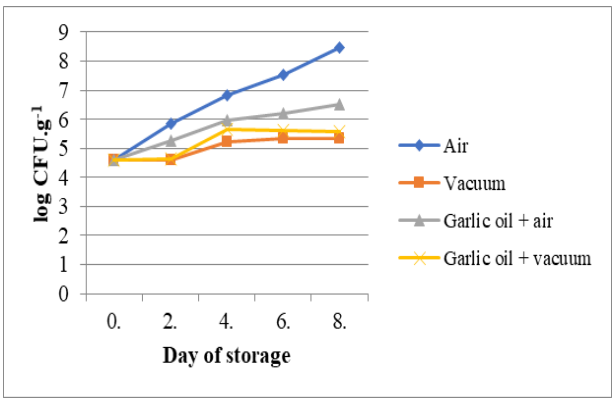

Figure 3. Average values of TVC in control samples and samples treated with garlic oil

Benkbelia [9] investigated antimicrobial activity of different concentrations (50, 100, 200, 300 and 500 mL/L) of essential oil extracts of three type of onions (green, yellow and red) and garlic against two bacteria, *Staphylococcus aureus*, *Salmomella Enteritidis*, and three fungi, *Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum*. In the dose response study, the inhibition zone increased with increasing concentration of extracts. Low concentrations (50 and 100 mL/L) inhibited weakly the development of bacteria; however was more sensitive than *S. aureus*. At high concentrations (200, 300 and 500 mL/l), EO extracts exhibited marked inhibition activity against bacteria, and inhibition of EO extract of garlic was strongest than those of onions EO extracts. Comparatively, *S. aureus* was less sensitive to the inhibitory activity of the onions and garlic extracts than *S.* Enteritidis which was more inhibited at same concentrations of EO extracts.

The average value of coliform bacteria (CB) in samples stored in air conditions ranged from 1.28 log CFU.g^{-1} on 0th day to 4.13 log CFU.g^{-1} on 8th day of storage. The average value of CB in vacuum packaging was detected only on 0th day of storage and it was 1.28 log CFU.g^{-1}. The average value of CB in samples in air condition treated with fresh garlic ranged from 1.28 log CFU.g^{-1} on 0th day to 1.78 log CFU.g^{-1} on 8th day of storage. CB were not detected in samples in vacuum packaging treated with fresh garlic (Figure 4).

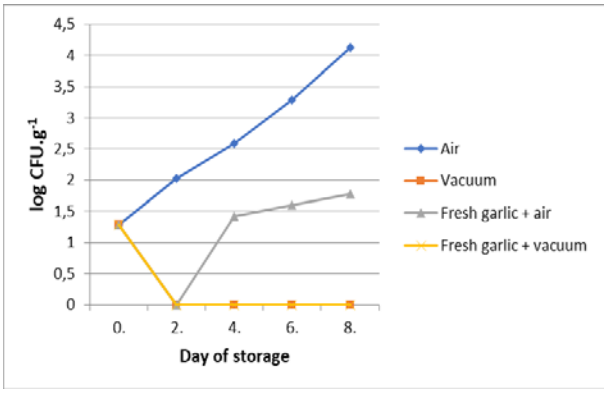

Figure 4. Average values of CB in control samples and samples treated with fresh garlic

Park and Chin [6] evaluated the effects of two levels (1.4 vs. 2.8 %) of fresh garlic on lipid oxidation and microbial growth in pork patties. Total bacterial counts were lower in the patties containing 1.4 and 2.8 % of fresh garlic than the control. In addition, fresh garlic also showed potent antimicrobial effects against *Enterobacteriaceae*. The microbial counts for *Enterobacteriaceae* in pork patties without fresh garlic showed the growth of > 7 log CFU.g^{-1} during 28 days of storage, whereas, pork patties with fresh garlic demonstrated growth < 6 log CFU.g^{-1} at 28 day of storage.

The average value of CB in samples in vacuum packaging was detected only on 0th day of storage and it was 1.28 log CFU.g^{-1}. The average value of CB in samples in air condition treated with dried garlic ranged from 1.28 log CFU.g^{-1} on 0th day to 2.22 log CFU.g^{-1} on 8th day of storage. CB were not detected in samples in vacuum packaging treated with dried garlic (Figure 5).

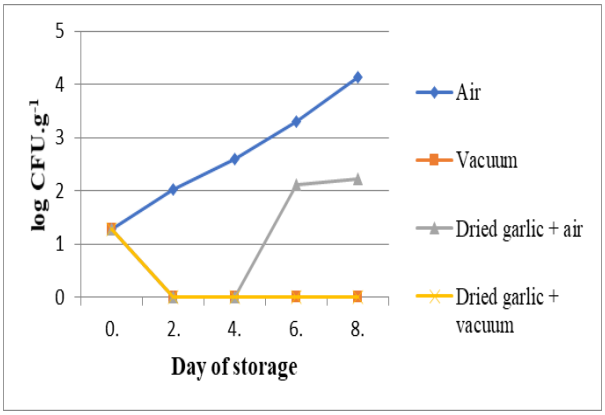

Figure 5. Average values of CB in control samples and samples treated with dried garlic

Sallam et al. [10] cited by Park and Chin [6] reported that antimicrobial effects of the fresh garlic were the best out of three preparations tested, followed by garlic powder and garlic oil, and suggested that the low activity of garlic oil and garlic powder in comparison with fresh garlic might be due to the losses of organosulfur compounds during sample preparations.

The average value of CB in samples in air condition treated with garlic oil ranged from 1.28 log CFU.g^{-1} on 0th day to 2.82 log CFU.g^{-1} on 8th day of storage. The average value of CB ranged from 1.28 log CFU.g^{-1} on 0th day to 1.82 log CFU.g^{-1} on 8th day of storage in samples in vacuum packaging treated with garlic oil (Figure 6).

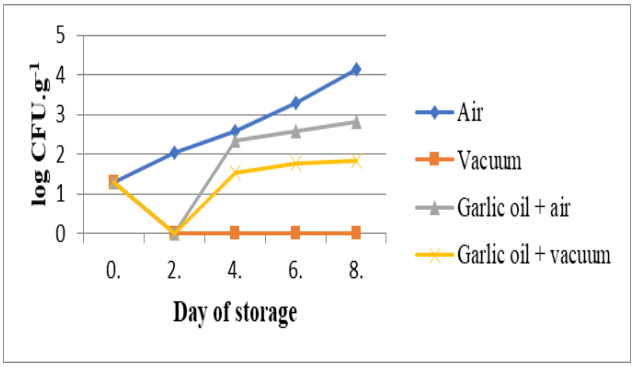

Figure 6 Average values of CB in control samples and samples treated with garlic oil

According to the results of Benkeblia [9]**,** the essential oil extracts of garlic had a marked antibacterial activity against certain pathogens, including *Staphylococcus aureus, Salmomella Enteritidis, Aspergillus niger, Penicillium cyclopium*, and *Fusarium oxysporum*.

The growth of E. coli and S. aureus are dose-dependently inhibited by the addition of allicin [11].

The antimicrobial activities of the pork meat containing fresh garlic might be partially due to allicin, which has a potent antimicrobial activity. Allicin, one of the active compounds of freshly crushed garlic homogenates is know to have antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria. Antimicrobial activities of allicin are mainly due to its chemical reaction with thiol-containing enzymes such as thioredoxin reductase, RNA polymerase, and alcohol dehydrogenase [12].

The average value of *Pseudomonas* was 2.45 log CFU.g^{-1} on 8th day of storage in samples in air condition, genus Pseudomonas was not detected in samples in vacuum packaging, in samples treated with fresh garlic (Figure 7).

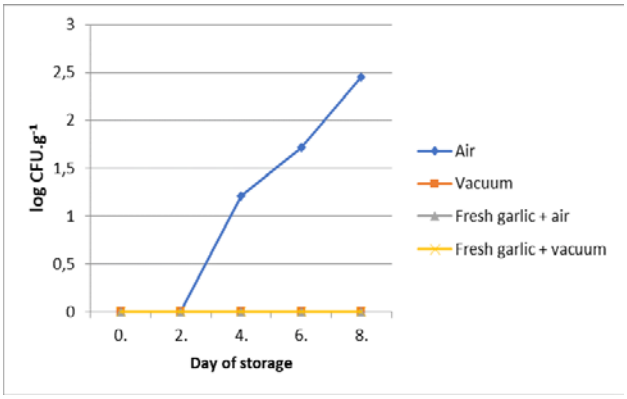

Figure 7 Average values of *Pseudomonas* in control samples and samples treated with fresh garlic

Garlic juice or aqueous extract from fresh garlic, both of which include a large amount of allicin are known to have high antibacterial effects against many pathogenic bacteria and bacteria causing food poisoning including the following; *S. aureus, S. saprophyticus, Streptococcus pneumonia, Enterococcus faecalis, E. coli, Enterobacter cloacae, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter haemolyticus, Bacillus cereus, Clostridium perfringens, Salmonella, Shigella* and *L. monocytogenes* [6, 13].

The average value of Pseudomonas was 2.45 log CFU.g^{-1} on 8th day of storage in samples in air condition, the average value of *Pseudomonas* was 1.33 log CFU.g^{-1} on 8th day of storage in samples in air condition treated with dried garlic (Figure 8).

The occurrence of bacteria in the food processing environments plays a key role in food contamination and development of spoilage. Species of the genus Pseudomonas are recognized as major food spoilers and the capability to actually determine spoilage can be species- as well as strain-dependent. Stellato et al. [14] showed that the most frequently found oligotypes belonged to *Pseudomonas fragi* and *P. fluorescens*, that the most abundant oligotypes co-

occurred, and were shared between the meat and dairy datasets.

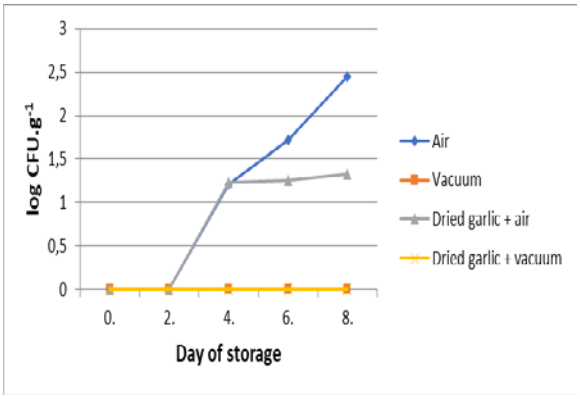

Figure 8. Average values of *Pseudomonas* in control samples and samples treated with dried garlic

The average value of *Pseudomonas* was 2.45 log CFU.g^{-1} on 8th day of storage in samples in air condition, *Pseudomonas* was not detected in vacuum packed samples, in samples treated with garlic oil (figure 9).

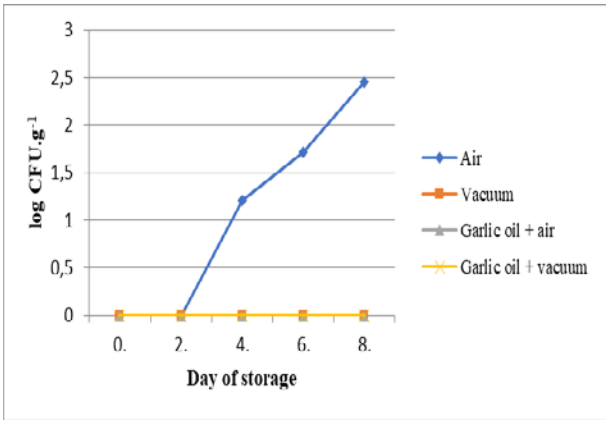

Figure 9. Average values of *Pseudomonas* in control samples and samples treated with garlic oil

Kong et al. [15] observed the inhibitory effects of extracts from honeysuckle, *Scutellaria*, *Forsythia* suspensa (Thunb), cinnamon, and rosemary with 75% ethanol and from clove oil dissolved in 75% ethanol on the growth of *Escherichia coli*, *Pseudomonas fluorescens*, and *Lactobacillus plantarum*. All the extracts suppressed the growth of these bacteria; *Scutellaria* exhibited the strongest effect against *E. coli*. An orthogonal test revealed that the most effective antimicrobial composite extracts were equal-volume mixtures of 0.125 g/mL Scutellaria + 0.5 g/mL honeysuckle + 0.125 g/mL Forsythia + 0.25 g/mL cinnamon and 0.25 g/mL cinnamon + 0.125 g/mL rosemary + 0.25% clove oil. These mixed extracts also produced strong antimicrobial effects in vacuum-packaged fresh pork, with 1.81- to 2.32-log reductions in microbial counts compared with the control when stored for up to 28 days.

In the work of Concagul and Ayaz [16], a range of plant pathogenic bacteria and fungi were inhibited by garlic bulb extracts that were obtained under various conditions. The conditions included different solvents (distilled water, methanol and ethanol), and water at different pH values (pH 3.0, 5.0, 7.0, 9.0 and 10.7). Water extraction produced the best antimicrobial activity, compared to methanol and ethanol, and the greatest activity was obtained by extraction under strongly acidic condition (pH 3.0). Subsequent analysis using HPLC and GC–MS revealed that the major active ingredients were 3-vinyl-1,2-dithiacyclohex-5-ene and 3-vinyl-1,2-dithiacyclohex-4-ene. In addition, changes observed in membrane permeability, protein leakage and by scanning electron microscopy suggested that the antimicrobial activity of garlic extracts may be due to destruction of the structural integrity of cell membranes, leading to cell death.

4. Conclusion

The values of microorganisms in meat samples during storage were significantly lower in samples treated with garlic in combination with vacuum in comparison with samples stored in air condition.

The combination of fresh garlic with vacuum packaging and dried garlic with vacuum packaging has shown the highest efficacy against microbial contamination during storage. It is therefore essential to continue to use natural antimicrobials in the food industry to reduce health problems accompanying the use of synthetic preservatives.

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