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THE MICROBIOLOGICAL QUALITY OF MINCED PORK TREATED WITH GARLIC IN COMBINATION WITH VACUUM PACKAGING

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ABSTRACT
The present study aimed to evaluate the microbial quality of minced pork treated with fresh garlic, dried garlic, and garlic oil in combination with vacuum packing. The growth of Total Viable Counts (TVC), Coliform Bacteria (CB), and *Pseudomonas* spp. were evaluated. The microbiological analyses were performed by the plate dilution method. The average value of TVC was 8.45 log CFU.g^{-1} in aerobically packed samples, 5.59 log CFU.g^{-1} in samples treated with garlic oil, 5.36 log CFU.g^{-1} in vacuum packed samples, and samples treated with dried garlic, and 4.98 log CFU.g^{-1} in samples treated with fresh garlic on 8th day of storage. The number of TVC was significantly lower in samples treated with fresh garlic compared to samples treated with dried garlic and garlic oil on the 8th day of storage (p <0.05). The average value of CB was 4.13 log CFU.g^{-1} in aerobically packed samples, 1.82 log CFU.g^{-1} in samples treated with garlic oil, the value of CB in vacuum packed samples, in samples treated with fresh garlic and also with dried garlic was lower than 1.00 log CFU.g^{-1} on 8th day of storage. The number of CB was significantly higher in aerobically packed samples and samples treated with garlic oil compared to vacuum-packed samples, samples treated with fresh garlic and dried garlic on the 8th day of storage (p <0.05). The average number of *Pseudomonas* spp. was 2.45 in aerobically packed samples, count of *Pseudomonas* spp. was lower than 1.00 log CFU.g^{-1} in vacuum packed samples and in the sample with garlic on the 8th day of storage. The number of *Pseudomonas* spp. was significantly higher in aerobically packed samples in comparison with vacuum-packed samples, samples with fresh garlic, dried garlic, and garlic oil (p <0.05).

Keywords: packaging; total viable counts; coliform bacteria; *Pseudomonas* spp.; minced pork

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 **(Sebranek et al., 2005)**. Meat and meat products are highly subject to microbial deterioration, which ultimately leads to safety and quality issues if the meat is not opportunely handled and preserved **(Najjaa et al., 2020)**. This is especially the case for ground meat since ground meat is more sensitive to oxidation because it has more susceptibility to microbial spoilage due to the grounding process **(Esmer et al., 2011)**.

The microbial population that colonizes and spoils minced pork meat is highly variable and depending on which groups of microbial taxa the product has been exposed to **(Zhao et al., 2015)**. The presence of microorganisms on the surface of meat and meat products determines meat spoilage upon their interaction and growth under optimal conditions **(Doulgeraki et al., 2012)**. The most prevailing spoilage organisms in meat are bacteria, yeast, and molds. Due to the ubiquity of microorganisms, they are mostly incorporated in meat from the environment. These organisms can cause spoilage by producing off-odors in meat products. Bacterial spoilage of meat is more prominent as compared to others like yeast and molds **(Sohaib et al., 2016)**. The most common bacteria causing spoilage of refrigerated beef and pork are *Brochothrix thermosphacta*, *Carnobacterium* spp., Clostridium spp., *Enterobacteriaceae*, *Lactobacillus* spp., *Leuconostoc* spp. and *Pseudomonas* spp. Their metabolic activity can cause the defects such as sour flavors, discoloration, gas or slime production, and decreases in pH **(Casaburi et al., 2015)**.

Food packing aims to optimize food quality, to ensure food hygiene and safety, to increase its storage stability, to reduce storage costs, and directly lead to lower use of preservatives during storage **(Łopusiewicz, Jedra and Mizieińska, 2018)**. Vacuum packaging is used in the conditioning of whole pieces or small parts and it protects the meat product from contact with oxygen from the air. Vacuum packaging and storage under refrigerated conditions are often used to prolong the shelf life of fresh meat or cooked meat products **(Pennacchia et al., 2011)**.

The emerging problematics regarding the negative impact exerted by some synthetic preservatives on the health of consumers versus the benefits imparted by natural antimicrobials and their specificity to foodborne pathogens, all these considerations are leading to the requirement for more systemic research to evaluate the toxicity and mechanism of action of natural preservatives **(Lee and Paik, 2016)**.

Garlic (*Alium sativum* L.) holds an important value due to its prophylactic and therapeutic actions. Sulfur and polyphenols present in garlic respond to the antibacterial, antifungal, and antioxidant activity **(Queiroz et al., 2009)**. Allium vegetables, particularly garlic exhibit broad antibiotic activity against Gram-positive and Gram-negative bacteria **(Whitemore and Naidu, 2000)**. Garlic has a wide spectrum of actions, not only antibacterial, antifungal, and antioxidant, but also it has beneficial effects on the cardiovascular and immune systems **(Harris et al., 2001)**. During the last decade, the antimicrobial activity of garlic, garlic-derived organosulfur compounds, and essential oil was widely investigated against both food spoilage bacteria and foodborne pathogens **(Benkeblia, 2004)**. However, the organosulfur compound volatility and low physic-chemical stability limit the possibility of its application as a food-functional ingredient. Dried garlic powder contains an important amount of alliin (sulfoxide S-allyl cysteine) (approximately 1%). The highest rate of alliin to allicin conversion has been observed in the temperature range of 35–36 °C **(Lanzotti, 2006)**. The antimicrobial activity of freeze-dried garlic and essential oils against various pathogens such as *Bacillus subtilis*, *Pseudomonas aeruginosa, S. aureus*, *E. coli,* and *Proteus* spp. was investigated **(Viswanathan et al., 2014)**.

Scientific hypothesis

Garlic is known to have numerous beneficial effects on human health. Garlic can be used as a natural preservative to prevent meat spoilage due to bacterial growth during storage. Minced meat treated with garlic, dried garlic, and garlic oil has a longer shelf life compared to untreated meat. Fresh galic, dried garlic, and garlic oil in combination with vacuum packing are effective against many microbial species.

MATERIAL AND METHODOLOGY

Samples

The microbiological quality of minced pork meat treated with crushed fresh garlic, dried garlic, and garlic oil was evaluated. Samples were stored for 8 days at a temperature of 4 °C. Analyzes were performed on the 0th, 2nd, 4th, 6th the 8th day of storage.

Growth medium

Plate Count Agar (PCA)

Plate count agar (PCA) is a bacteriological substrate used for the determination of the total number of live, aerobic bacteria in a sample. The amount of bacteria is expressed as colony-forming units per gram ($CFU.g^{-1}$), in solid samples and per mL ($CFU.mL^{-1}$) in liquid samples. The recommended technique is the pour plate technique. The samples are diluted and appropriate dilutions are added in Petri plates. Sterile molten agar is added to these plates and plates are rotated gently to ensure uniform mixing of the sample with agar. The plates are incubated at 20 or 30 °C in three days. After incubation, the number of colonies is counted on the plate with 25 – 250 colonies, which is considered to give the most accurate result. When calculating the actual number of bacteria in the sample, the dilution factor should be taken into consideration **(Atlas and Snyder, 2014)**.

Violet Red Bile Lactose Agar (VRBL)

VRBL, containing Bile and Violet Red dye, is based on MacConkey Agar for the detection and enumeration of lactose-fermenting bacteria and the differentiation of coliforms from non-lactose fermenting organisms in dairy products, water, and foods. Peptone provides nitrogen, vitamins, minerals, and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group.

Pseudomonas Agar (PA)

Pseudomonas Agar Base is designed so that by the addition of the appropriate supplement the medium becomes selective for *Pseudomonas aeruginosa* or *Pseudomonas* spp. generally. Pseudomonas CFC Supplement (SR0103) is recommended for the selective isolation of Pseudomonas spp. generally. **Mead and Adams (1977)** showed that reducing the cetrimide content to 10 $mg.mL^{-1}$ allowed the growth of all pigmented and non-pigmented psychrophilic pseudomonads. To improve its selective action they added cephaloridine 50 $\mu g.mL^{-1}$) and fucidin (10 $\mu g.mL^{-1}$). This combination of agents gave a new and more specific medium for isolating pseudomonads from chilled foods and processing plants.

Laboratory methods

Microbiological analysis

The following groups of microorganisms were determined in samples of minced pork meat:

- Total Viable Count (TVC).
- Coliform Bacteria (CB).
- *Pseudomonas* spp.

The plate dilution method was used for the determination of the number of colonies forming units (CFU) of each group of microorganisms. An amount of 5 g of the minced pork meat was transferred into a sterile stomacher bag containing 45 mL of 0.1% physiological solution (pH 7.0) and homogenized for 30 minutes. Microbiological analyses were conducted under standard microbiological methods.

Determination of microorganisms

Plate Count Agar (PCA, Oxoid, UK) was used to isolation of Total Viable Counts (TVC). PCA agar was after inoculation incubated at 30 °C for 48 hours **(STN EN ISO 7218, 2000; STN ISO 4833, 2014)**. Dilutions 10^{-4} and 10^{-5} were used to determination of TVC.

Violet Red Bile Lactose Agar (VRBL, Oxoid, UK) was used to isolation of Coliform Bacteria (CB). Inoculated agar was incubated at 37 °C for 24 hours **(STN EN ISO 9308 – 1, 2015)**.

Pseudomonas Agar (PA, Oxoid, UK) was used for isolation of *Pseudomonas* spp. Inoculated agar was incubated at 35 °C ±1 °C for 48 hours **(STN 560100, 1968)**. Dilutions 10^{-1} and 10^{-2} were used to determination of CB and Pseudomonas.

Samples preparation

Preparation of garlic oil

- 300 mL of sunflower oil,
- 300 mL of extra virgin olive oil,
- 10 cloves (50 g) of garlic.

Sunflower oil and olive oil were poured into a glass cup. The garlic cloves were cleaned and crushed in a mortar. Crushed garlic was added to the oils. Garlic oil was stored in a dark place at room temperature for 7 days. After storage, the oil was filtered and applied to meat samples.

Dried garlic was purchased in the supermarket.

Fresh garlic was extruded using a garlic press.

Samples of meat were prepared in amount 5 g and fresh garlic, dried garlic, and garlic oil were added to the amount 1 g.1 mL^{-1}. A total of 150 samples were analyzed (6 samples in each group).

Individual samples were prepared as follows:

- Aerobically packed samples – control group.
- Vacuum-packed samples – control group.
- Vacuum-packed samples with fresh garlic.
- Vacuum-packed samples with dried garlic.
- Vacuum-packed samples with garlic oil.

Vacuum-packed samples were packaged by vacuum packing machine (Concept VA 0010, Czech Republic).

Enumeration of microorganisms count

Individual colonies of microorganisms were calculated after cultivation on Petri dishes. The number of microorganisms was calculated according to the following formula:

$$N = \frac{\Sigma c}{V.(N1 + 0{,}1N2).d}$$

Where:

N – CFU in 1 g of sample; Σc – the sum of all colonies on selected Petri dishes; V – the volume of inoculum in ml inoculated on each petri dish; N_1 - the number of dishes used for the calculation from the first dilution; N_2 – the number of dishes used for the calculation from the second dilution; d – dilution factor corresponding to the first dilution.

Statistical Analysis

The experiments were performed in triplicate and standard deviations for replication were calculated (MS Excel). Analysis of variance (ANOVA) was used to evaluate the results. Comparison of the treatment means was based on Tukey's Honest Significant Difference (HSD) test.

RESULTS AND DISCUSSION

Minced pork is the basis for different meat preparations (e.g. sausages, meatballs, burgers), but also consumed raw. Contamination of ground meat with microorganisms can occur during the whole processing, though in particular in meat cutting plants or at retail when the meat is cut or minced with more surfaces exposed **(Ejeta et al., 2004)**. Biopreservation is focused on the utilization of natural preservatives from sources like bacteria, fungi, plants, animals, having the ability to ensure food safety due to their antimicrobial activity exerted against a wide spectrum of foodborne pathogens **(Gyawali and Ibrahim, 2014)**.

Plant-based derivatives containing different bioactive compounds can be divided into two major categories: (1) vegetable or essential oils (obtained from the olive, canola, soy, sunflower, linseed, avocado, grape seed, oregano, rosemary, coriander seed), and (2) extracts (obtained from grape seed, green tea, olive leaf, cranberry, pomegranate, broccoli, cocoa leaf, lemon balm) **(Hygreeva et al., 2014)**. Some plants can contain both major derivatives, and it has been reported that garlic can be used like fresh extract, freeze-dried powder, or oil extract **(Sallam et al., 2004)**. **Kačániová et al. (2016)** suggest the possibility of applying the *Pimpinella anisum*, *Mentha spicata* var. *crispa*, *Thymus vulgaris* L., *Origanum vulgare* L. essential oil as natural food preservatives and potential sources of antimicrobial ingredients for the food industry.

The average value of Total Viable Counts (TVC) ranged from 4.59 log CFU.g^{-1} on 0. day to 8.45 log CFU.g^{-1} on 8. day in aerobically packed samples, from 4.59 log CFU.g^{-1} to 5.36 log CFU.g^{-1} in vacuum-packed samples. The average value of TVC ranged from 4.59 log CFU.g^{-1} to 4.98 log CFU.g^{-1} on the 8th day of storage in samples treated with fresh garlic. The highest value of TVC was 4.65 log CFU.g^{-1} on 2. day in samples treated with dried garlic and 5.64 log CFU.g^{-1} on the 4th day in samples treated with garlic oil (Table 1).

Freeze-dried fresh garlic and the spray-dried microencapsulated essential oil can be effectively used in meat and meat products as natural alternatives to synthetic food additives, particularly as effective antimicrobial agents **(Najjaa et al., 2020)**.

Park and Chin (2014) compared the antimicrobial activity of 1.4% and 2.8% fresh garlic extract against TVC in minced pork stored at 4 °C for 28 days. They found out, that values of TVC were lower in the samples containing 1.4 and 2.8% of fresh garlic than in the control.

Gheisari and Ranjbar (2012) investigated the antimicrobial effects of equivalent concentrations of garlic derivatives in ground camel meat during storage at 4 °C. The antioxidant activities of the various ingredients added followed the order of fresh garlic (FG), garlic powder (GP), and garlic oil (GO). After 14 days of storage, the value of TVC of both FG and GP formulated meat was significantly lower than that of either the control samples. However, the addition of GO resulted in no significant difference in TVC when compared with the control.

Sallam et al. (2004) reported that the antimicrobial effects of the fresh garlic were the best out of three preparations, followed by garlic powder and garlic oil. They reported that the low activity of garlic oil and garlic powder compared to fresh garlic can be caused by the losses of organosulfur compounds during sample preparations.

However, **Benkeblia (2004)** reported, that the essential oil extracts of garlic had a marked antibacterial activity against certain pathogens, including *Staphylococcus aureus, Salmonella Enteritidis, Aspergillus niger, Penicillium cyclopium*, and *Fusarium oxysporum*.

Cao et al. (2013) examined the effects of 1% or 0.5% chitosan, 10% or 5% aqueous extract of ginger, onion, and garlic and their composite solutions on the quality and shelf life of stewed pork. Microbiological (total viable counts) characteristics were analyzed at 4 °C for 12 days. They found out that chitosan and extract of ginger, onion, and garlic treatments retarded the increases in Total Viable Counts. Chitosan showed better antibacterial activity, but weaker antioxidation compared to ginger, onion, and garlic extract.

The number of TVC was significantly higher in aerobically packed control samples in comparison with samples treated with fresh garlic, dried garlic, and garlic oil during 8 days of storage. There were significant differences in TVC values between vacuum-packed samples and samples treated with garlic oil on the 4^{th} day of storage. The number of TVC was significantly lower in samples treated with fresh garlic compared to samples treated with dried garlic and garlic oil on the 4^{th}, $6^{th,}$ and 8^{th} day of storage (p <0.05) (Table 1).

Huang et al. (2017) investigated the effect of 2.5% and 5% rosemary powder against TVC in minced pork stored for 7 days at 4 °C. They have shown that a 5% concentration of rosemary powder has a stronger antimicrobial effect than a 2.5% concentration of rosemary powder, and in combination with a supercritical CO_2 level may be involved in prolonging shelf life.

Park et al. (2008) investigated the addition of onion and garlic powder to pork meat. The value of Total viable counts was lower in samples containing garlic and onion powder compared to the control samples (p <0.05). The garlic powder showed a stronger antimicrobial effect against *Enterobacteriaceae* in comparison with onion powder (p <0.05).

Krisch et al. (2010) evaluated the effect of 1% dried garlic and 1% onion powder on TVC in minced pork, which was stored at 5 °C for 72 hours. They reported that the dried garlic would eliminate the contamination of minced pork after 24 hours by 2.00 log $CFU.g^{-1}$. Dried garlic and onion powder also show excellent antimicrobial effects against *Candida* sp. and *E. coli.*

Cold-tolerant species of the family *Enterobacteriaceae,* such as *Serratia liquefaciens, Serratia proteamaculans, Hafnia alvei, Enterobacter agglomerans, Proteus* spp., *Klebsiella* spp., and *Pantoea* spp., contribute to spoilage of chilled meat stored under inadequate conditions **(Odeyemi et al., 2020)**.

The average number of CB ranged from 1.28 log $CFU.g^{-1}$ on the 0^{th} day to 4.13 log $CFU.g^{-1}$ on the 8^{th} day in aerobically packed samples. The highest number of CB was 1.82 log $CFU.g^{-1}$ on the 8^{th} day in samples treated with garlic oil. The number of CB was lower than 1.00 log $CFU.g^{-1}$ in vacuum-packed samples, samples treated with fresh garlic, and in samples treated with dried garlic (Table 2).

Kaczmarek et al. (2017) monitored the effect of plant extracts added to fresh pork against different groups of microorganisms. They found out, that the highest inhibitory effect against *Enterobacteriaceae* and *Pseudomonas* bacteria was noted in the meat samples treated with fresh onion.

Michalczyk et al. (2015) analyzed the effect of oregano essential oil, freeze-dried garlic, tomato concentrate, and their combination on the shelf life of minced pork meat during 16 days of storage. The effect of oregano essential oil against Total Viable Counts and *Enterobacteriaceae* was was very weak. Adding tomato concentrate, combined with other additives, slowed the rate of microbiological changes. The addition of freeze-dried garlic did not cause a substantial reduction of detectable bacteria to count. The addition of a combination of all three additives resulted in a product with a distinctly longer shelf life.

Leong et al. (2014) reported that organosulfur compounds derived from allicin are very unstable, reactive, and decompose rapidly. Therefore, garlic oil prepared by combining fresh garlic and vegetable oil contains a minimal concentration of allicin. On the other hand, essential garlic oil obtained by steam distillation contains compounds soluble in oil, especially alylmetyltrisulphid, which affects antimicrobial activity.

The number of CB was significantly higher in aerobically packed samples compared to vacuum-packed samples, samples treated with fresh garlic, dried garlic, and also garlic oil. The count of CB was significantly higher in samples treated with garlic oil in comparison with vacuum-packed samples, samples treated with fresh garlic, and samples treated with dried garlic on the 4^{th}, $6^{th,}$ and 8^{th} day of storage (p <0.05) (Table 2).

Stojanović-Radić et al. (2018) investigated the effect of the basil and rosemary essential oils as well as their combination on the growth of *Salmonella enterica* subspecies enterica serovar Enteritidis (*Salmonella Enteritidis*) in chicken meat, together with their spoilage protective potential at 4 °C and 18 °C. Food model experiments included investigation of their effect on accompanying microbial flora on fresh meat, while anti-salmonella activity was evaluated on artificially inoculated raw and thermally processed meat. Changes in microflora pointed to a significant effect of both oils against microbial meat spoilage, where various groups were affected by different treatments.

Park and Chin (2014) evaluated the effects of fresh garlic on microbial growth in pork patties. The microbial counts for *Enterobacteriaceae* in pork patties without garlic showed growth higher than 7 log $CFU.g^{-1}$ at 28 days of storage, whereas, samples treated with fresh garlic demonstrated growth lower than 6 log $CFU.g^{-1}$ at 28 days of storage.

Kim et al. (2011) investigated the antimicrobial effect of dried garlic, at a concentration of 0.5% in pork sausages. found the inhibitory effect of garlic powder against *B. cereus, S. aureus,* and *L. monocytogenes*.

Fujisawa et al. (2008) analyzed the instability of garlic (allicin) in aqueous and ethanolic solutions as well as in vegetable oil. Allicin was more stable in 20% alcohol than in water, but unstable in vegetable oil, with an activity half-life of 0.8 h, as estimated from its antibacterial activity toward *Escherichia coli*.

Stellato et al. (2017) analyzed the main microflora responsible for the deterioration of raw meat. They reported, that *Pseudomonas* spp. belongs to the dominant aerobic contaminating microflora, especially in pork. *P. fragi* and *P. fluorescens* are responsible for meat degradation by the production of extracellular proteases and lipases at low temperatures.

The average number of *Pseudomonas* sp. ranged from 1.21 log $CFU.g^{-1}$ on the 4^{th} day to 2.45 log $CFU.g^{-1}$ on the 8^{th}. day in aerobically packed samples. The number of *Pseudomonas* sp. was lower than 1.00 log $CFU.g^{-1}$ in vacuum packed samples, samples treated with fresh garlic, with dried garlic, and also with garlic oil (Table 3).

Pseudomonas spp. are recognized as the main agents of meat spoilage and are very often isolated from fresh and spoiled meat products **(Rajmohan et al., 2010)**.

The value of *Pseudomonas* sp. was significantly higher on the 4^{th}, $6^{th,}$ and 8^{th} day in aerobically packed samples compared to vacuum-packed samples, samples treated with fresh garlic, dried garlic, and also samples treated with garlic oil (p <0.05) (Table 3).

Table 1 Average values of Total Viable Microorganisms in minced meat samples and significant differences.

Total Viable Counts					
	0. day	**2. day**	**4. day**	**6. day**	**8. day**
CA	4.59 ±0.34	5.86 ±0.20	6.81 ±0.13	7.54 ±0.23	8.45 ±0.20
CV	4.59 ±0.34	4.60 ±0.24	5.24 ±0.22	5.34 ±0.19	5.36 ±0.17
FG	4.59 ±0.34	4.51 ±0.40	5.04 ±0.13	5.00 ±0.11	4.98 ±0.15
DG	4.59 ±0.34	4.65 ±0.29	5.43 ±0.12	5.44 ±0.13	5.36 ±0.20
GO	4.59 ±0.34	4.64 ±0.10	5.64 ±0.19	5.62 ±0.20	5.59 ±0.15
Tukey test	-	CA:CV+, CA:FG+, CA:DG+, CA:GO+	CA:CV+, CA:FG+, CA:DG+, CA:GO+, CV:GO+, FG:DG+, FG:GO+	CA:CV+, CA:FG+, CA:DG+, CA:GO+, FG:DG+, FG:GO+	CA:CV+, CA:FG+, CA:DG+, CA:GO+, FG:DG+, FG:GO+

Note: CA – control aerobically packed samples, CV – control vacuum packed samples, FG – samples treated with fresh garlic, DG – samples treated with dried garlic, GO – samples treated with garlic oil, + significant differences (p <0.05).

Table 2 Average values of Coliform Bacteria in minced meat samples and significant differences.

Coliform Bacteria					
	0. day	**2. day**	**4. day**	**6. day**	**8. day**
CA	1.28 ±0.20	2.03 ±0.28	2.59 ±0.37	3.29 ±0.21	4.13 ±0.01
CV	1.28 ±0.20	<1.00	<1.00	<1.00	<1.00
FG	1.28 ±0.20	<1.00	<1.00	<1.00	<1.00
DG	1.28 ±0.20	<1.00	<1.00	<1.00	<1.00
GO	1.28 ±0.20	<1.00	1.53 ±0.35	1.78 ±0.14	1.82 ±0.15
Tukey test		CA:CV+, CA:FG+, CA:DG+, CA:GO+	CA:CV+, CA:FG+, CA:DG+, CA:GO+, CV:GO+, FG:GO+, DG:GO+	CA:CV+, CA:FG+, CA:DG+, CA:GO+, CV:GO+, FG:GO+, DG:GO+	CA:CV+, CA:FG+, CA:DG+, CA:GO+, CV:GO+, FG:GO+, DG:GO+

Note: CA – control aerobically packed samples, CV – control vacuum packed samples, FG – samples treated with fresh garlic, DG – samples treated with dried garlic, GO – samples treated with garlic oil, + significant differences (p <0.05)

Table 3 Average values of *Pseudomonas* sp. in minced meat samples and significant differences.

Pseudomonas sp.					
	0. day	**2. day**	**4. day**	**6. day**	**8. day**
CA	<1.00	<1.00	1.21 ±0.07	1.72 ±0.77	2.45 ±0.47
CV	<1.00	<1.00	<1.00	<1.00	<1.00
FG	<1.00	<1.00	<1.00	<1.00	<1.00
DG	<1.00	<1.00	<1.00	<1.00	<1.00
GO	<1.00	<1.00	<1.00	<1.00	<1.00
Tukey test			CA:CV+, CA:FG+, CA:DG+, CA:GO+	CA:CV+, CA:FG+, CA:DG+, CA:GO+	CA:CV+, CA:FG+, CA:DG+, CA:GO+

Note: CA – control aerobically packed samples, CV – control vacuum packed samples, FG – samples treated with fresh garlic, DG – samples treated with dried garlic, GO – samples treated with garlic oil, + significant differences (p <0.05).

Zhang et al. (2009) investigated the antimicrobial effect of clove extract, rosemary extract, licorice extract, and combined rosemary and licorice extract against *Pseudomonas* spp. in raw pork during 28 days. Combined rosemary and licorice extract (RL) showed the strongest inhibitory effect against *Pseudomonas* spp.

Kunová et al. (2017) evaluated the effect of essential oils against *Pseudomonas* spp., *Enterobacteriaceae,* and anaerobic plate counts during storage of chicken meat. They found out, that the caraway and thyme essential oils can be used as natural food preservatives and they are also a good source of antimicrobial ingredients for meat.

CONCLUSION

Within the food industry, meat is one of the main raw materials, which is a valuable source of nutrients for humans. For this reason, it is essential to ensure the availability of high-quality and safe meat for consumers.

Minced meat is an ideal growth medium for spoiling and pathogenic microorganisms. Due to the health problems associated with the use of synthetic preservatives, consumers appeal to the use of natural antimicrobial preservatives. Our results show that fresh garlic in combination with vacuum packaging showed the highest efficacy against all groups of microorganisms, garlic oil was not effective against coliform bacteria. Fresh garlic and dried garlic in combination with vacuum packaging are a suitable method of eliminating microorganisms in minced pork.

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