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THE EXTENSION OF SHELF-LIFE OF CHICKEN MEAT AFTER APPLICATION OF CARAWAY AND ANISE ESSENTIAL OILS AND VACUUM PACKAGING

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ABSTRACT

The effect of caraway (CEO) and anise (AEO) essential oils as well as vacuum packaging (VP) in extending of the shelf life of fresh chicken breast meat stored at 4 °C was investigated. CEO and AEO were used at concentrations 0.2% v/w with and without VP. Microbiological properties of chicken breast meat were monitored over a 16 day period. The microbiological parameters as the anaerobic plate count (AC), *Enterobacteraceae*, lactic acid bacteria and *Pseudomonas* spp. counts were detected. The anaerobic plate counts ranged from 2.77 log CFU.g^{-1} in all tested group on 0 day to 5.45 log CFU.g^{-1} on 16 day in control group stored in air condition. The number of lactic acid bacteria ranged from 3.20 log CFU.g^{-1} in all tested group on 0 day to 4.75 log CFU.g^{-1} on 16 day in control group stored in air condition. *Enterobacteriaceae* counts ranged from 0.00 to 4.25 log CFU.g^{-1} on 16 day in control group stored in air condition. The number of *Pseudomonas* spp. ranged from 0.00 log CFU.g^{-1} in all tested group on 0 day to 2.65 log CFU.g^{-1} on 16 day in control group stored in air condition. Statistically significant differences ($p \leq 0.001$) were found among tested group in all tested microorganisms. Among the antimicrobial combination treatments were examined in the study, the as application of vacuum packaging, EDTA, and essential oils were the most effective against the growth of lactic acid bacteria and *Enterobactericeae* and to a less extent on anaerobic plate count. The results of this present study suggest the possibility of using the essential oil of caraway and anise as natural food preservatives and potential source of antimicrobial ingredients for chicken breast meat.

Keywords: bacteria; caraway and anise essential oils; vacuum; EDTA; chicken breast

INTRODUCTION

Special attention in poultry meat production is paid to the fact that live animals are hosts of a large number of different microorganisms residing on their skin, feathers or in the alimentary tract. Majority of these microorganisms are eliminated during the slaughter, but subsequent contamination is possible at any stage of the production process. Contamination may occur from feather plucking and evisceration equipment, washing prior to storage as cooling, or during the freezing. Microorganisms from the environment, equipment and operators' hands can contaminate meat. During the slaughter, the changes in composition of microflora occur from, in general, Gram-positive rods and micrococci to, most frequently, Gram-negative bacteria, including *Enterobacteriaceae*, *Pseudomonas* spp. Industrial poultry slaughterhouses have a particular technological process, the individual stages of which are not in conformity with modern principles of hygienic meat production and processing so there are various possibilities for contamination of chicken meat **(Kozačinski et al., 2006)**. Poultry meat is a highly perishable food commodity providing an almost perfect medium for microbial growth including both spoilage and pathogenic microorganisms **(Jay et al., 2005**) therefore the microbial contamination during the poultry meat processing is very crucial.

Meat production is one of the major activities in Europe. The main type of meat produced is pork (48.7%) followed by poultry (23.6%) and bovine (23.3%). Meat and meat products present an ideal substrate supporting the growth of several spoilage and pathogenic bacteria. Moreover, meat and poultry products have frequently been found to be contaminated with pathogens **(Mor-Mur and Yuste, 2010)**. The pathogens ability to grow at refrigerator temperatures helps the organism to evolve from a low initial to an infective dose level during the storage of refrigerated foods, including those originally harbouring the pathogen and those, post-heat treatment, contaminated **(Ray, 2001)**.

It is well known that packaging makes food more convenient and gives the food greater safety assurance from microorganisms, biological and chemical changes so that the packaged foods may have a longer shelf life. As a result, packaging has become an indispensable element in the food manufacturing process. In order to meet the huge demand of the food industry, there has been a remarkable growth in the development of food packaging in the past decades **(Tsigarida and Nychas, 2001)**.

Aromatic plants and herbal products have been used worldwide as natural additives for medicinal purposes because they have been accepted by consumers. Various biological activitive compounds sharing antioxidative, anticoccidial, immunostimulating or antimicrobial properties have been identified in these plants **(Ivanišová et al., 2013; Ivanišová et al., 2015 a,b)**.

Carum carvi, which is also known as caraway, is one of the oldest spices cultivated in Europe. Nowadays, it is cultivated from northern temperate to tropical climates, including countries such as Jamaica, India, Canada, the United States of America and Australia. In India, this spice is known as *Kashmiri jeera*. The dried ripe fruits (schizocarp) of *C. carvi* L. family *Apiaceae* (*Umbelliferae*) are extensively being used in folk medicine as a carminative, found to be effective against spasmodic gastrointestinal complaints, irritable stomach, indigestion, lack of appetite and dyspepsia in adults, and in relieving flatulent colic of infants. The volatile oils from *C. carvi* have also been used as an anti ulcerogenic, antitumor, antiproliferative and antihyperglycemic agent. The seeds of *C. carvi* have been used in alternative medicine as a laxative, in colic treatment, and as a mouth freshener **(Thippeswamy et al., 2013)**.

Anise (*Pimpinella anisum* L.) a member of the *Apiaceae* family, is an annual aromatic plant, native to Iran, India, Turkey and many other warm region in the world. Anise seed possesses eugenol trans-anethole, methylchavicol, anisaldehyde, estragole, coumarins, scopoletin, umbelliferone, estrols, terpene hydrocarbons, polyenes, and polyacetylenes. Most of the plant parts such as fruits, seeds, and essential oil contain compounds with proven antiparasitic and digestion stimulating, antifungal and antipyretic, antioxidant, antimicrobial, anthelmintic and hypocholesterolemic properties **(Yazdi et al., 2014)**.

The present study was undertaken to determine the effect of vacuum packaging combined with caraway or anise essential oil tratment on microbiological properties of chicken breast meat stored at 4 °C.

MATERIAL AND METHODOLOGY

Preparation of samples

Chicken breast samples (totally 30) for microbiological analysis were used in this study.

To evaluate the antimicrobial activity of essential oils the chicken breast with skin of each experimental group was taken. The chicken breast fresh samples were prepared as follow: for air-packaging (AC, control samples) chicken breast fresh meat was packaged to polyethylene bags and stored aerobically at 4 °C; for vacuum-packaged (VC, control samples) chicken breast fresh meat was packaged to polyethylene bags and stored anaerobically in vacuum at 4 °C; for vacuum-packed samples with EDTA solution 1.5% w/w (VPEC, control samples) chicken breast fresh meat was treated with EDTA for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum at 4 °C; for vacuum-packed samples with *Carvum carvi* 0.20% v/w (VP+CEO) chicken breast fresh meat was treated with caraway oil for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum at 4 °C; for vacuum-packed samples with *Pimpinella anisum* L. 0.20% v/w, (VP+AEO) chicken breast fresh meat was treated with anise oil for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum at 4 °C. For sample packaging a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic) was used and each sample were packed immediately after treatment.
EDTA solution (pH 8.0, 99.5% purity, analytical grade, Invitrogen, USA) was prepared at final concentration of 50 mM Caraway and anise essential oils (Calendula, Nová Ľubovňa, Slovakia) was added to coat chicken breast surface (both sides) of each sample using a micropipette.

Microbiological analysis

An amount of 10 g (10 cm^2) of the chicken breast was sampled using sterile scalpels and forceps, immediately transferred into a sterile stomacher bag, containing 90 mL of 0.1% peptone water (pH 7.0), and homogenized for 60 s in a stomacher at room temperature. Sampling was carried out on 0, 4, 8, 12 and 16 days of experiment. Microbiological analyses were conducted by using standard microbiological methods. Anaerobic plate count (AC) was determined using Plate Count Agar (PCA, Oxoid, UK) after incubation for 48 h at 35 °C under anaerobically condition. For *Pseudomonas* spp., 0.1 mL from serial dilutions of chicken homogenates was spread onto the surface of Pseudomonas Isolation agar (PIA, Oxoid, UK). *Pseudomonas* spp. enumerated after incubation for 48 h at 25 °C. For lactic acid bacteria, Rogosa and Sharpe agar (MRS, Oxoid, UK) was inoculated with a 1.0 mL of sample suspension. Inoculated plates were incubated for 48-78 h at 37 °C in an aerobic atmosphere supplemented with carbon dioxide (5% CO_2). For *Enterobacteriaceae*, a 1.0 mL of sample was transferred into 10 mL of molten (45 °C) Violet Red Bile Glucose agar (VRBL, Oxoid, UK). Inoculated plates were incubated at 37 °C for 24 h. All plates were examined for typical colony types and morphology characteristics associated with each medium applied for incubation. Enumeration of all tested groups of bacteria was performed in triplicate.

Figures were created Microsoft® EXCEL 2013. Data for the mean from each replication was calculated and all data were log transformed. Statistical analysis were done with STATGRAPHICS 5 software (UMEX GmbH Dresden, Germany). Confectionary Student's Tukey HSD test was calculated for differences in numbers of bacteria and samples were accepted as significantly different at $p \leq 0.001$.

RESULTS AND DISCUSSION

Food contamination by microorganisms and their development and, hence, the food decontamination possibilities represent a serious problem. Chemical agents to prevent microbial growth and various additives that are used in food industries are considered to be potentially harmful to human health. In seeking of possible alternatives, the antimicrobial compounds of natural origin sharing antibacterial activities originated from plants currently are studied intensively worldwide.

Spices are aromatic plants that are widely used in the food industry and culinary food preparation for flavouring. However, their essential oils and extracts can contribute to control of the growth of harmful microorganisms. It is

necessary the spice to be effective enough to ensure that the product is safe and also have acceptable sensory characteristics **(Dimič et al., 2012)**. The primary objective of chilling poultry safety enssurance is to reduce microbial growth to a level that will improve both food safety and shelf life **(Popelka et al., 2014)**. The anaerobic plate count ranged from 2.77 log CFU.g^{-1} in all tested group on 0 day to 5.45 log CFU.g^{-1} on 16 day in control group stored in air condition. In control group stored vacuum packaged AC ranged from 2.77 log CFU.g^{-1} on 0 day to 5.25 log CFU.g^{-1} on 16 day. In control group stored vacuum packaged after EDTA treatment, AC ranged from 2.77 log CFU.g^{-1} on 0 day to 5.21 log CFU.g^{-1} on 16 day. After treatment with caraway essential oil, AC ranged from 2.77 log CFU.g^{-1} on 0 day to 4.20 log CFU.g^{-1} on 16 day and after treatment with anise essential oil ranged from 2.77 log CFU.g^{-1} on 0 day to 4.15 log CFU.g^{-1} on 16 day. Statistically significant differences ($p \leq 0.001$) of anaerobic plate count were found among all tested group at all tested days except AC and VP+CEO, VC and VP+CEO, VC and VP+AEO, VP+CEO and VP+AEO on 4th day; VC and VPEC on 8th and 16th day; VP+CEO and VP+AEO on 16th day. Anaerobic plate count (AC) values for the tested groups of chicken breast are showed in Figure 1.

Many herbs and spices have been recognized for their preservative or medicinal properties for millennia. Essential oils present in plant matter have been attributed as principal sources of compounds exhibiting antimicrobial activity, which has been illustrated against bacteria and fungi. The understanding of antimicrobial mechanisms of action of EO has led to increased interest to the specific compounds responsible for this activity, specifically those phenolic in nature **(Davidson et al., 2013)**.

The initial LAB value of chicken breast was 3.20 log CFU.g^{-1} on 0 day. In AC samples the LAB counts ranged from 3.20 log CFU.g^{-1} to 4.75 log CFU.g^{-1} on 16 day. In control group VC samples LAB count ranged from 3.20 log CFU.g^{-1} on 0 day to 4.52 log CFU.g^{-1} on 16 day. In VPEC samples LAB ranged from 3.20 log CFU.g^{-1} on 0 day to 4.38 log CFU.g^{-1} on 16 day. In VP+CEO samples the counts of LAC ranged from 3.20 log CFU.g^{-1} on 0 day to 3.05 log CFU.g^{-1} on 16 day and in VP+AEO ranged from 3.20 log CFU.g^{-1} on 0 day to 3.00 log CFU.g^{-1} on 16 day. Lactic acid bacteria (LAB) values for the tested groups of chicken breast are showed in Figure 2. Statistically significant differences ($p \leq 0.001$) of lactic acid bacteria numbers were found among all tested group at all tested days except VP+CEO and VP+AEO on 16th day.

Lactic acid bacteria are found to be more resistant to the cytotoxic effects of essential oils. **Rodríguez et al., (2009)** suggest that a fact that LAB are present and grow on phenol containing plants, and therefore have adapted in order to successfully colonize such antagonistic substrates like one of the reason of LAB resistance to phenolics is because. Degradation capabilities of phenolic compounds by LAB have also been described, although the number of studies is still limited.

The use of spices and spice blends in many food products that already contain high levels of similar seasonings has also been examined. Ideally, reengineering of food formulations that contain high levels of spices such as oregano or thyme seasonings could take advantage of the already present sources of essential oils. Unfortunately, some work has shown that spices stimulate the growth and acid production of LAB **(Shelef, 1983)**.

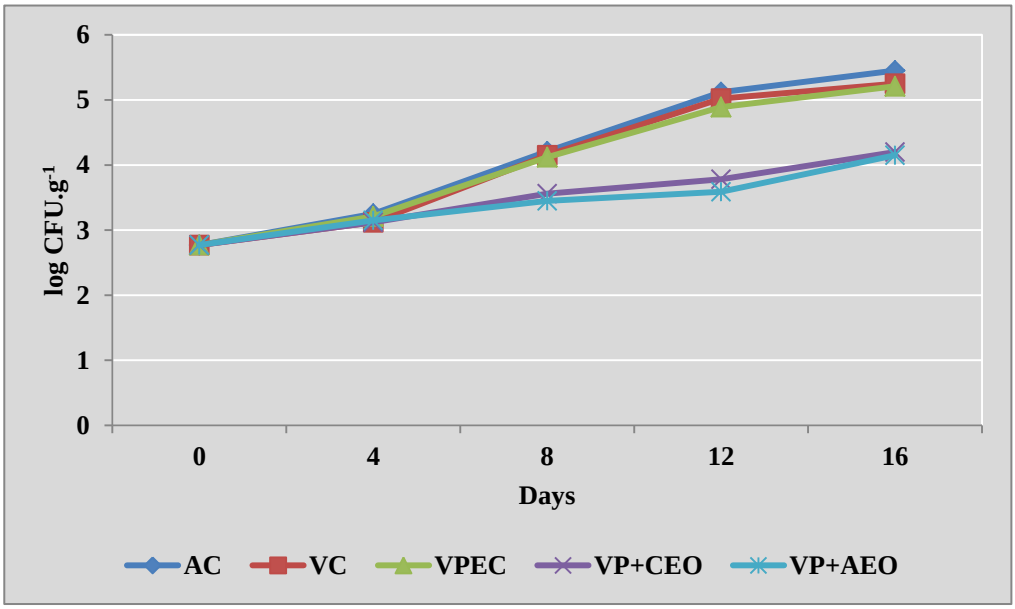

Figure 1 Changes (log CFU.g^{-1}) in population of anaerobic plate count in chicken breast stored in air (AC); stored in vacuum (VC); stored vacuum packaged with EDTA (VPEC); stored under vacuum packaged with *Carvum carvi* 0.20% v/w (VP+CEO); stored vacuum packaged with *Pimpinella anisum* L. 0.20% v/w (VP+AEO).

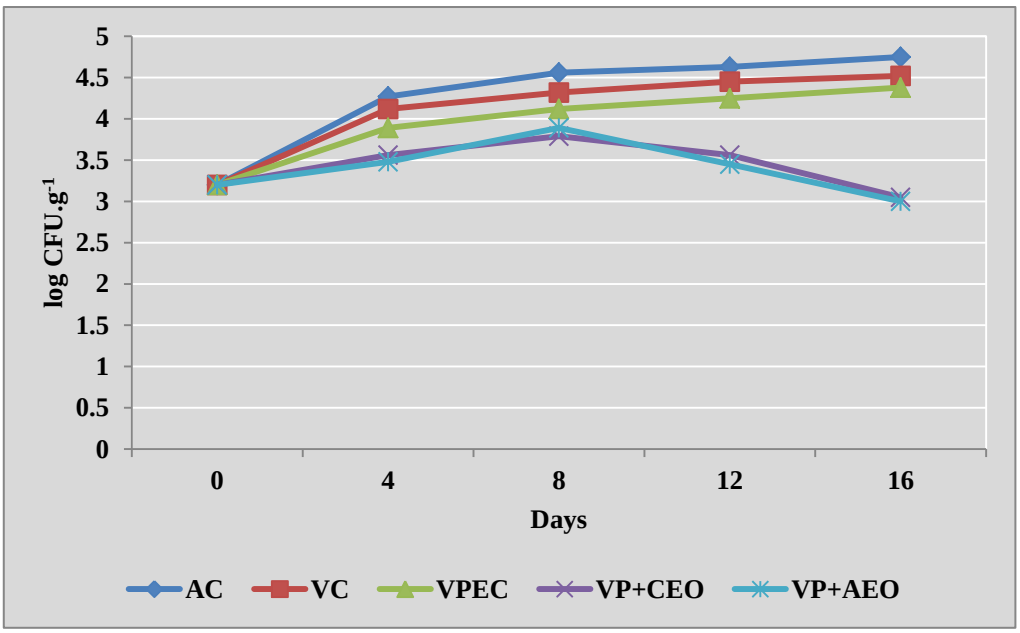

Figure 2 Changes (log CFU.g^{-1}) of lactic acid bacteria in chicken breast stored in air (AC); stored in vacuum (VC); stored vacuum packaged with EDTA (VPEC); stored vacuum packaged with *Carvum carvi* 0.20% v/w (VP+CEO); stored vacuum packaged with *Pimpinella anisum* L. 0.20% v/w (VP+AEO).

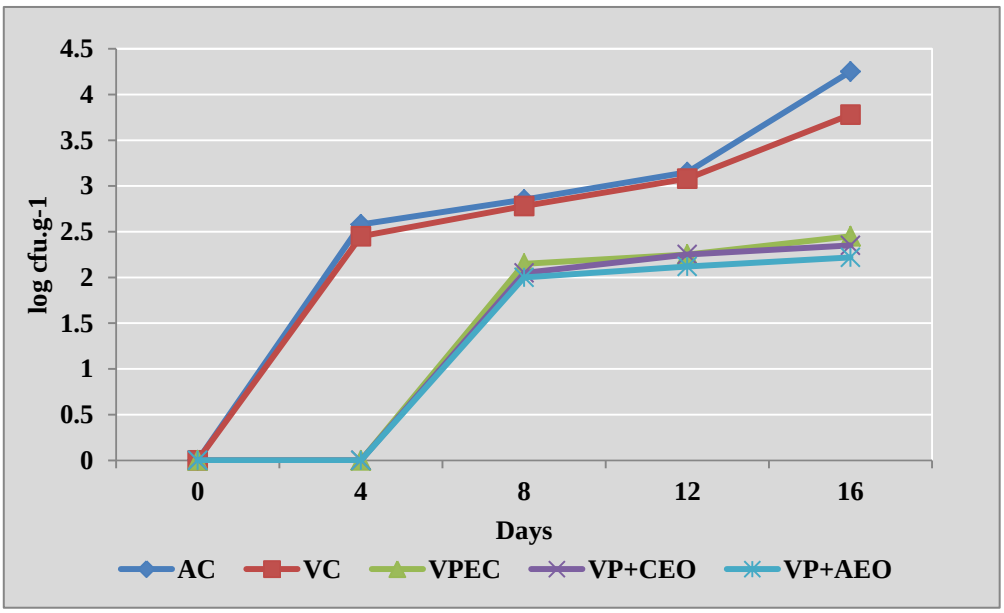

Figure 3 Changes (log CFU.g^{-1}) in *Enterobacteriaceae* counts in chicken breast stored in air (AC); stored in vacuum (VC); stored vacuum packaged with EDTA (VPEC); stored vacuum packaged with *Carvum carvi* 0.20% v/w (VP+CEO); stored vacuum packaged with *Pimpinella anisum* L. 0.20% v/w (VP+AEO).

The chemical composition and physical characteristics of meat makes it a suitable environment for bacterial growth, which includes bacteria such as LAB, *Pseudomonas*, and foodborne pathogens. LAB spoilage in meats is a relevant problem as they are facultative anaerobes that can grow and continue to spoil foods under chilled conditions **(Fratianni et al., 2010; Pyrgotou et al., 2010)**.

Fratianni et al. (2010) treated fresh strips of chicken breast meat with an agar slurry solution containing 0.5% thyme and balm essential oils for 15 min. Samples were stored for 21 days at 4 °C. Thyme was incredibly effective to control the LAB growth for the period of 16 days; 21-day counts were only 0.8×10^3 CFU.mL^{-1}, which was consistent throughout the entire 3 weeks of experiment. The antibacterial effect of balm oil was much less evident until the day 21, with balm oil closely matching the untreated control up until that point. *Salmonella* on the treated chicken was very sensitive to balm oil, while thyme oil very effectively reduced the growth of *E. coli*.

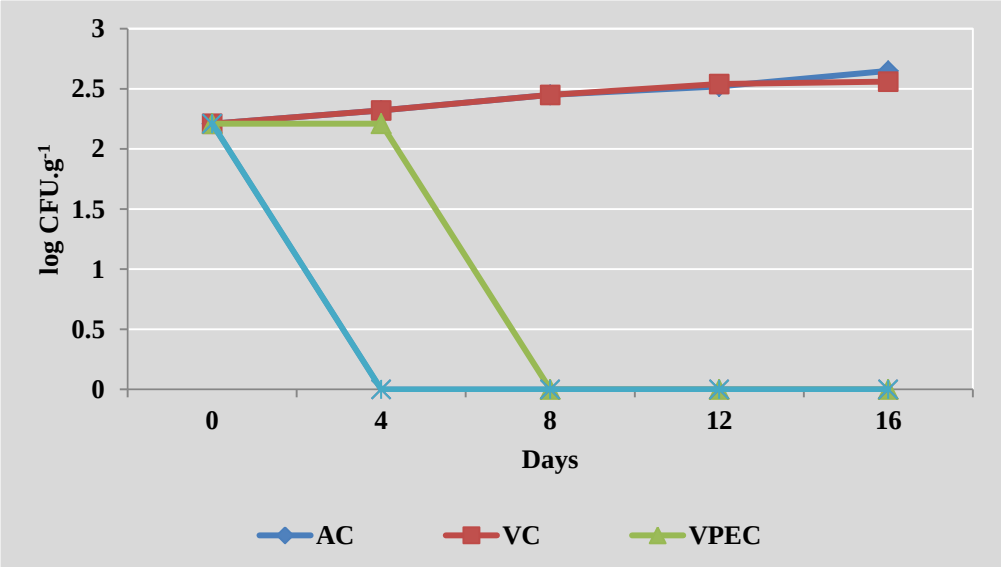

Figure 4 *Pseudomonas* count (log CFU.g^{-1}) in chicken breast stored in air (AC); stored in vacuum (VC); stored in vacuum packaging with EDTA (VPEC); stored vacuum packaged with *Carvum carvi* 0.20% v/w (VP+CEO); stored vacuum packaged with *Pimpinella anisum* L. 0.20% v/w (VP+AEO).

Enterobacteriaceae counts ranged from 0.0 log CFU.g^{-1} in all tested group on 0 day to 4.25 log CFU.g^{-1} on 16 day in AC group. In VP group *Enterobacteriaceae* counts ranged from 0.00 log CFU.g^{-1} on 0 day to 3.78 log CFU.g^{-1} on 16 day. In VPEC group *Enterobacteriaceae* counts ranged from 0.00 log CFU.g^{-1} on 0 day to 2.45 log CFU.g^{-1} on 16 day. In the group with caraway essential oil treatment *Enterobacteriaceae* counts ranged from 0.00 log CFU.g^{-1} on 0 day to 2.35 log CFU.g^{-1} on 16 day and in group treated with anise essential oil from 0.00 log CFU.g^{-1} on 0 day to 2.22 log CFU.g^{-1} on 16 day. Statistically significant differences ($p \leq 0.001$) of *Enterobacteriaceae* genera number were found among all tested group at all tested days except VPEC and VP+CEO, VPEC and VP+CEO, VP+CEO and VP+AEO on 4th day; AC and VC, VPEC and VP+CEO, VPEC and VP+CEO, VP+CEO and VP+AEO on 8th; VPEC and VP+CEO on 12th day. *Enterobacteriaceae* genera values for the tested groups of chicken breast are showed in Figure 3.

Generally, the Gram-positive bacteria were more sensitive to essential oils or antibacterial compounds than Gram-negative bacteria, which is in agreement with previous reports **(Dorman and Deans, 2000; Burt, 2004; Shan et al., 2007)**. This resistance could be ascribed to the structure of the cellular walls of Gram-negative bacteria, mainly with regard to the presence of lipoproteins and lipopolysaccharides that form a barrier to restrict entry of hydrophobic compounds **(Cox and Markham, 2007)**.

Pseudomonas spp. counts ranged from 0.00 log CFU.g^{-1} in all tested group on 0 day to 2.65 log CFU.g^{-1} on 16 day in AC group. In VC group *Pseudomonas* spp. ranged from 0.00 log CFU.g^{-1} on 0 day to 2.56 log CFU.g^{-1} on 16 day. In another tested groups on 16 day *Pseudomonas* spp. were not found. Statistically significant differences ($p \leq 0.001$) of anaerobic plate count were found among all tested group at all tested days except AC and VC on 4th, 8th 12th day; VPEC and VP+CEO, VPEC and VP+CEO on 8th, 12th, 16th day; VP+CEO and VP+AEO on 4th, 8th 12th, 16th day. *Pseudomonas* spp. values for the tested groups of chicken breast are showed in Figure 4.

Numerous studies documented the inhibitory effects of some essential oils and extracts of spices, plants, or their major active constituents on the bacteria - *Escherichia coli*, *Aeromonas* spp., *Enterococcus faecalis*, *Salmonella enterica Typhimurium*, *Staphylococcus aureus*, *Shigella* spp., *Bacillus* spp., *Listeria monocytogenes*, *Micrococcus spp.*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus* spp., *Lactobacillus* spp., *Enterobacter* spp. with Gram-positive bacteria as generally more sensitive than Gram-negative bacteria **(Amensour et al., 2010; Bagamboula et al., 2003; Baidar et al., 2004; Celiktas et al., 2007,Faleiro et al., 2003; Moreira et al., 2005; Skočibušić et al., 2006; Sokmen et al., 2004; Veldhuizen et al., 2007; Viuda-Martos et al., 2008)**.

CONCLUSION

Caraway and anise essential oils exhibited good antimicrobial properties against anaerobic bacteria, lactic acid bacteria and *Enterobacteriacea* at 0.2% concentration. Essential oils and their components may provide a solution for the growing demand of natural preservation methods that require minimal processing of meat. Even more exciting is the fact that these essential oils are already approved for use in foods, meaning that once the issues of application and concentration are resolved and food producers can almost immediately begin using essential oils in their food formulations. Future work must comprise studies that determine which essential oils are most appropriate for preservation, what concentrations and delivery methods are most appropriate and effective, and what foods or packaging methods are most ideal for reformulation or reengineering to take advantage of the antimicrobial activity of essential oils.

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