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The effect of vacuum packaging, EDTA, oregano and thyme oils on the microbiological quality of chicken's breast

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ABSTRACT

The effect of ethylenediaminetetraacetate (EDTA), oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) oils, on the chicken breast fillets was examined in this study. The chicken breast fillets were stored under vacuum packaging (VP), at 4 ± 0.5 °C for a period of 18 days. There were used the following treatments of chicken breast fillets: Air-packaged (AC, control samples), vacuum-packaged (VPC, control samples), VP with EDTA solution 1.50% w/w (VPEC, control samples), VP with oregano oil 0.20% v/w (VP + O) and VP with thyme oil 0.20% v/w, (VP + T). The quality assessment for vacuum packaging of the product in accordance with the terms above and EDTA treatment, oregano and thyme oil was established by microbiological analyzes. The microbiological properties as the total viable counts on Plate Count Agar, after incubation for 2 days at 37 °C and coliform bacteria on Violet Red Bile Glucose agar incubated at 37 °C for 24 h, lactobacilli on Rogosa and Sharpe agar after incubation 48–78 h at 37 °C in an aerobic atmosphere supplemented with carbon dioxide (5% CO_2) and *Pseudomonas aeruginosa* on Pseudomonas Isolation agar (PIA, Oxoid, UK) after incubation at 48 h at 35 °C were monitored. The using of oregano, thyme oil and EDTA with combination of vacuum packaging has significant effects to reduction of all followed groups of microorganisms compared with control group without vacuum packaging and untreated control group. The natural preservatives can be used as alternatives to chemical additives which could extend the meat and meat products shelf life. The knowledge about them can have an important economic feedback by reducing losses attributed to spoilage and by allowing the products to reach distant and new markets. This study shows how using of natural antimicrobials can extend the shelf-life of the meat product.

1. Introduction

Essential oils (EOs) well known as inhibitors of microorganisms, are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots), which can be obtained by expression, fermentation, enfleurage, extraction and method of steam distillation [1]. EOs and their components commonly used as flavoring in the food industry also present some antibacterial, antifungal, and antioxidant properties. The primary constituents of EOs are terpenoids and terpenes. EOs can also contain aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones [2]. One of the most commonly used spices in the food industry is oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*), well known for its antioxidative and antimicrobial properties [1,3].

The most representative compounds in oregano and thyme essential oil were carvacrol and thymol [4]. Carvacrol and thymol, the major components of oregano essential oil, are mainly responsible for its antimicrobial activity [4–6]. The mode of action of carvacrol and thymol, appears to have received the most attention from researchers. Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different location on the phenolic ring. Due to their hydrophobic nature, carvacrol and thymol interact with the lipid bilayer of cytoplasmic membranes

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causing loss of integrity and leakage of cellular material such as ions, ATP and nucleic acid.

Poultry meat is a highly perishable food commodity providing an almost perfect medium for microbial growth including both spoilage and pathogenic microorganisms [7,8].

In the meat industry, vacuum packaging and storage at strictly controlled temperatures of −1.5 °C are widely used to store and export raw meat [9]. Spoilage of fresh poultry products is an economic burden to the producer and in some cases may also present a health hazard, since poultry meat may harbor pathogenic microorganisms [10]. Consequently, developing methods to increase shelf-life and overall safety/quality represents a major task of the poultry processing industry. Several preservation approaches have been investigated including modified atmosphere packaging (MAP), vacuum packaging (VP) alone or in combination with other procedures including treatment with acids [11], EDTA–nisin treatment [12], addition of phosphates [13], essential oils [14] and irradiation [15].

The aim of the present study was to investigate the combined effect of ethylenediaminetetraacetate (EDTA), oregano (*O. vulgare*) and thyme (*T. vulgaris*) essential oil, on the shelf-life extension of fresh chicken breast fillets stored under vacuum packaging, at 4 ± 0.5 °C for a period of 18 days.

2. Material and methods

2.1. Preparation of meat samples

The experiment was implemented into the local poultry station (Hydinaren a.s., Zamostie). The tested chickens were Cobb. The chickens were slaughtered for analysis at the end of the fattening period (day 42). The breast muscle (*musculus pectoralis major*) without skin was taken to evaluate the microbiological properties from each experimental group. The chicken breast fresh samples with weight 25 g were prepared:

Air-packaged (AC, control samples): 25 g of chicken breast fresh meat were packaging to polyethylene backs and stored aerobically in refrigerator;

Vacuum-packaged (VPC, control samples): 25 g of chicken breast fresh meat were packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;

VP with EDTA solution 1.50% w/w (VPEC, control samples): 25 g of chicken breast fresh meat were treated with EDTA for 1 min and packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;

VP with oregano oil 0.20% v/w (VP + O): 25 g of chicken breast fresh meat were treated with oregano oil for 1 min and packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;

VP with thyme oil 0.20% v/w, (VP + T): 25 g of chicken breast fresh meat were treated with thyme oil for 1 min and packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;

Immediately after dipping, each sample was packaged using a vacuum packaging machine type VB-6 (RM Gastro, Czech republic).

EDTA was ($C_{10}H_{14}N_2O_8.Na_2.2H_2O$), 99.5% purity, analytical grade, (Invitrogen, USA). A stock solution of 500 mM concentration was prepared by diluting 186.15 g in 1 L distilled water. A final concentration of 50 mM EDTA solution was prepared from the stock solution. The pH of the solution was adjusted to 8.0 with the addition of the appropriate quantity of NaOH solution. The amount of EDTA added to the semi cooked coated chicken fillets was 0.28 g/kg.

Oregano (*O. vulgare*) and thyme (*T. vulgaris*) essential oils (Calendula, Nova Lubovna, Slovakia) were added to the coated chicken surface (both sides) of each sample using a micropipette so as to achieve a 0.2% v/w final concentration of EO. Our results with chemical composition of essential oils with GC/GC-MS analysis of oregano and thyme essential oils showed that the predominant antibacterial compounds were thymol (5.82%, respectively 16.79%) and carvacrol (35.21%, respectively 15.62%).

2.2. Microbiological analysis

Approximately 10 g (10 cm^2) of the chicken fillet (of uniform area) 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 at predetermined time intervals namely: 0, 3, 6, 9, 12, 15 and 18 days. Chicken breast fillets were stored under vacuum packaging, at 4 ± 0.5 °C.

Microbiological analyses were conducted by using standard microbiological methods. Total viable counts (TVC) were determined using Plate Count Agar (PCA, Oxoid, UK), after incubation for 2 days at 37 °C. For *Pseudomonas aeruginosa* enumerations, 0.1 ml from 1:10 prepared serial dilutions (0.1% physiological solution) of chicken homogenates was spread onto the surface of solid media. *Pseudomonas* were determined on Pseudomonas Isolation agar (PIA, Oxoid, UK) after incubation at 48 h at 35 °C. This medium is selective and formulated to enhanced formation of blue or blue-green pyocyanin pigment by *P. aeruginosa*. The pigment diffuses into the medium surrounding growth. *Lactobacillus* sp. enumerations, a 1.0 ml sample were inoculated into Rogosa and Sharpe agar (MRS, Oxoid, UK) after incubation 48–78 h at 37 °C in an aerobic atmosphere supplemented with carbon dioxide (5% CO_2). For members of the family *Enterobacteriaceae*, a 1.0 ml sample was inoculated into 10 ml of molten (45 °C) violet red bile glucose agar (VRBL, Oxoid, UK). After setting, a 10 ml overlay of molten medium was added and samples incubated at 37 °C for 24 h. The large colonies with purple haloes were counted. All plates were examined for typical colony types and morphology characteristics associated with each growth medium.

2.3. Statistically analysis

Data from each replication were averaged and log transformed. The statistical processing of the data obtained from number of microorganisms was implemented by mean with STATGRAPHICS 5 software. The experimental results of microorganisms' number were expressed as mean, standard deviation (SD) and coefficient of variability (CV). A statistical analysis was performed with Student's *t*-test. Confidence limits were added at $P < 0.05$; $P < 0.01$; $P < 0.001$.

3. Results and discussion

Generally, EOs exhibit the strongest antibacterial properties against food borne pathogens and spoilage organisms as a result of high percentage of phenolic compounds such as carvacrol, thymol, p-cymene, γ-terpinene. Other researchers reported a shelf-life extension of 4 days after the application of oregano oil on minced beef stored aerobically under refrigeration [16].

Total viable count (TVC) values for the tested groups of chicken breast meat are shown in Fig. 1. The initial TVC value of control chicken breast was 4.72 log cfu/g. Dawson et al. [17] indicated the acceptable poultry meat quality at the initial TVC 4.14 log cfu/g in their study. Our results regarding to the use of oregano essential oil, are in general agreement with those of Chouliara et al. [14]. These authors found out an extension of 1 day in microbiological shelf life of raw chicken meat by the addition of 0.1 ml/100 g of oregano essential oil. These results are also in agreement with those of Zhang et al. [18], who reported a reduction in TVC of pork chops by

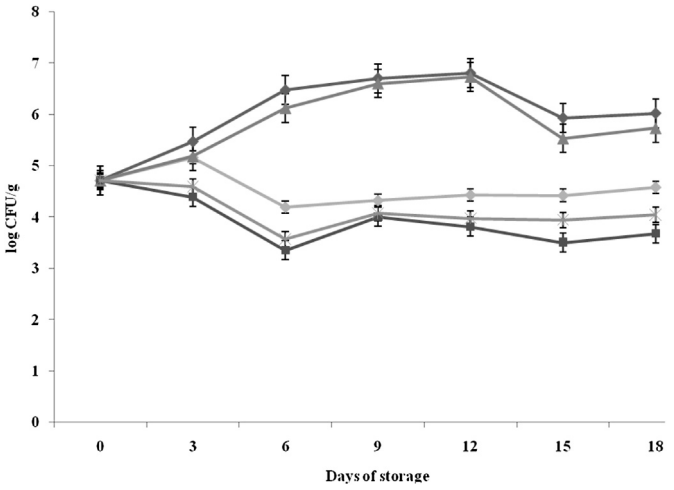

Fig. 1. Changes (log CFU/g) in population of Total Viable Count in chicken breast meat stored in air (AC, ♦); stored under vacuum (VPC, ▲); stored under vacuum packaging with EDTA (VPEC, ●); stored under vacuum packaging with oregano essential oil (VP + O, ■); stored under vacuum packaging with thyme essential oil (VP + T). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

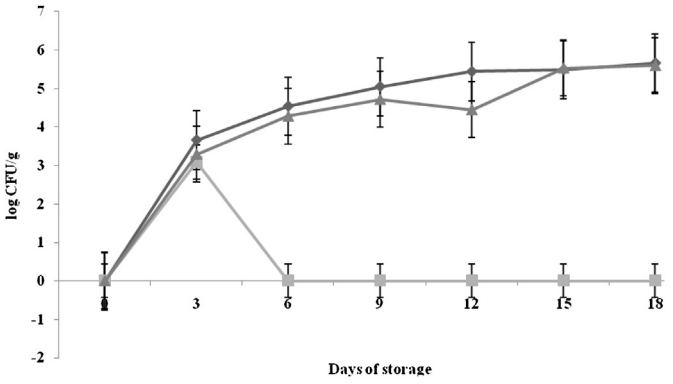

Fig. 2. Changes (log CFU/g) in population of *Enterobacteriacae* genera in chicken breast meat stored in air (AC, ♦); stored under vacuum (VPC, ▲); stored under vacuum packaging with oregano essential oil (VP + O, ■). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

1.2–2.6 log cfu/g with the addition of a mixture of spice extracts including oregano essential oil after 7 days of storage. With the respect to the use of oregano oil, present results are in agreement with those of Tsigarida et al. [19], who reported a reduction in initial microflora of beef meat fillets by 2–3 log cfu/g with the addition of 0.8% oregano essential oil. They are also in agreement with those of Scandamis and Nychas [16] who reported an immediate suppression of TVC in minced beef meat by 1 log cfu/g when oregano oil was added at concentration of 1%. Differences in TVC reduction values between the literature and the present work may be related to different concentrations of applied essential oil, different food substrates in relation to fat content as well as different composition of individual essential oils used. We observed the lowest TVC in the storage under the vacuum packed with oregano essential oil group 3.68 log cfu/g and in the storage under the vacuum packed with thyme essential oil group 4.05 log cfu/g after 18 day storage at 4 ± 0.5 °C. Dawson et al. [17] reported a reduction in growth of aerobic bacteria by 1–1.5 log cfu/g in ground chicken meat after 14 days of storage under MAP. Statistically significant differences were between all tested groups ($P < 0.05$). Khanjari et al. [20] reported that oregano essential oil had a significant controlling effect on TVC and a microbiological shelf life were extension of 4 days using oregano essential oil.

The structures of thymol and carvacrol were similar; they had the hydroxyl group at a different location on the phenolic ring. The relative position of the hydroxyl group on the phenolic ring did not appear to strongly influence the degree of antibacterial activity; the action of thymol against *Bacillus cereus, Staphylococcus aureus* and *P. aeruginosa* appeared to be comparable to that of carvacrol [21–23]. In study of the Ultee et al. [22], the antibacterial activity of thymol against *S.* Typhimurium was also similar to that of carvacrol. Thymol and carvacrol were able to disintegrate the outer membrane of gram-negative bacteria, release lipopolysaccharides (LPS), increase the permeability of the cytoplasmic membrane to adenosine triphosphate (ATP) and allow ions to leave the cytoplasm [21,23]. By the antibacterial mechanisms of these three components, we proposed two hypotheses: First, thymol or carvacrol could increase the permeability of the cytoplasmic membrane, and probably enable cinnamaldehyde to be more easily transported into the cell. Second, thymol or carvacrol could increase the number, size or duration of existence of the pores created by cinnamaldehyde binding to proteins in the cell membrane, so that a synergistic effect is achieved when the two are used together.

Several authors have detected many members of the *Enterobacteriaceae* on raw beef, lamb, pork, and poultry products, as well as on offal meats [24]. However, the genera *Serratia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Proteus* and *Hafnia*, often contribute to meat spoilage [25,26]. With regard to their meat spoilage potential, the most important *Enterobacteriaceae* are the species *Serratia liquefaciens*, *Hafnia alvei* and *Enterobacter* (*Pantoea*) *agglomerans* [27]. Among the *Enterobacteriaceae*, *Serratia* spp. is the most commonly found genus in meat. *Serratia grimesii* and *Serratia proteamaculans* occur in meat stored in air, MAP and VP storage; although *S. grimesii* is often found at late stages of storage [28–30]. *S. proteamaculans* was recovered from fresh meat, while *Citrobacter freundii* and *Proteus vulgaris* were recovered from minced beef stored aerobically and under MAP, respectively [31]. *S. liquefaciens* has been found to be the most common member of the *Enterobacteriaceae* in meat stored in different atmospheres [31,32], while *Hafnia alvei* is very frequently encountered in minced beef stored under MAP or VP [23,31,32], *Rahnella* spp. has been shown to potentially play an important role in the spoilage of meat and was found as the dominant enterobacterium in the late phases of refrigerated storage of beef in MAP and VP [28,29].

In our study the number of *Enterobacteriaceae* ranged from 0 log cfu/g in first day to 5.66; 5.60 log cfu/g in last day of testing, respectively in the control group and the group stored under the vacuum. The number of *Enterobacteriaceae* 3.09 log cfu/g only in 3 day of evaluation was indicated in the case of the storage under the package with EDTA groups. Number of *Enterobacteriaceae* 0 log cfu/g was found all the time of testing in the group with oregano and thymol essential oils (Fig. 2). Statistically significant difference was found between all testing groups ($P < 0.05$) without VP + O and VP + T.

LAB as facultative anaerobic bacteria can grow under the high concentrations of CO_2 and thus constitute a substantial part of the natural microflora of MAP meats. LAB (lactic acid bacteria) are recognized as the important competitors of the other spoilage related microbial groups under VP/MAP conditions [19,32–35]. Particularly, *Lactobacillus* spp., *Carnobacterium* spp. and *Leuconostoc* spp. are associated to the spoilage of refrigerated raw meat [36], while they can also become dominant throughout storage in reduced O_2 availability [37]. Similarly, *Lactobacillus* was the major component of the microbiota in chilled pork for VP [38–40]. More species of lactobacilli can be found during the storage under the vacuum at 4 °C including *Lactobacillus algidus* beyond *Lactobacillus*

sakei. In addition, at 1 °C *Lactobacillus* spp., *Weissella* spp. and *Leuconostoc mesenteroides* can occur indicating an influence of the temperature in the development of different species under the same packaging conditions. Holzapfel [41] reported that more rarely *Lactobacillus plantarum* and *Lactobacillus casei* are associated with meat systems and in lower frequency and numbers than *Lactobacillus curvatus* and *L. sakei*. Additionally, leuconostocs have been identified as predominant organisms in beef stored aerobically [33] and under VP/MAP [32,42] while their presence in the initial mesophilic bacterial microbiota is very frequent [25,31]. Zaika et al. [43], reported a reduction of 4 log cfu/g in LAB populations in pure culture, after adding oregano oil at a concentration of 4 g/l. Chouliara et al. [14] found the initial LAB counts were ca. 3.7 log cfu/g (on day 0) and reached 7 log cfu/g on day 9 of storage for the air packaged samples. On the same day of storage, the use of oregano oil (0.1%) resulted in a reduction in LAB counts by almost 1 log cfu/g ($P < 0.05$) while the concentration of 1% of oregano oil completely inhibited the growth of LAB until day 12 of storage. MAP 1 (CO_2 30%, N_2 70%) resulted in a reduction of LAB counts by ca. 0.5 log cfu/g (day 9 of storage) ($P < 0.05$) and MAP 2 (CO_2 70%, N_2 30%) in a reduction of 1.8 log cfu/g ($P < 0.05$). The combination of oregano oil at concentrations of 0.1 and 1% and MAP 1 resulted in a reduction of LAB by 1.7 log cfu/g ($P < 0.05$) and more than 6 log cfu/g, respectively, while the combination of MAP 2 and oregano oil at concentrations of 0.1% and 1% resulted in a reduction of LAB by 1.9 log cfu/g ($P < 0.05$) and more than 6 log cfu/g on the same day. Oregano oil at the concentration of 1% was more effective than the concentration of 0.1% in reducing the populations of LAB while the combination of MAP and oregano oil at a concentration 1% had the greatest effect.

In our study, the number of *Lactobacillus* sp. (Fig. 3) in control group ranged from 2.62 log cfu/g (day 15) to 4.31 log cfu/g (day 3). The number of *Lactobacillus* sp. in the vacuum packing group was 2.83 log cfu/g (day 15)—4.31 log cfu/g (day 3). The lowest number of *Lactobacillus* sp. of vacuum packaging with EDTA group was 1.76 log cfu/g (day 6) and the highest 4.27 log cfu/g (day 0). In VP + O group value of *Lactobacillus* sp. ranged from 1.34 (day 15) to 4.32 log cfu/g (day 3). The highest number 4.29 log cfu/g (day 3) and the lowest 1.43 log cfu/g (day 6) of *Lactobacillus* sp. was detected in VP + T group. Statistically significant difference ($P < 0.05$) was found between AC and VPEC, VP + O, VP + T; between VPEC and VPC, VP + O; between VPC and VP + O, VP + T and between VP + O and VP + T.

Pseudomonads are Gram-negative bacteria sensitive to CO_2, comprising the main spoilage microorganisms in meat [44]. Thus CO_2-enriched atmospheres such as MAP inhibit the growth of pseudomonads, as compared to air packaging. Chouliara et al. [14] reported that on the 9. day of storage, the combined effect of MAP 1 and oregano oil 0.1% was more pronounced resulting in a reduction of 1.4 log cfu/g ($P < 0.05$), while MAP 1 and oregano 1% resulted in a reduction of 4.4 log cfu/g. Respectively, the combination of MAP 2 and oregano oil 0.1% resulted in a reduction of 2.4 log cfu/g ($P < 0.05$) and the combination of MAP 2 and oregano oil 1% in a reduction of more than 5 log cfu/g. oregano oil was more effective than MAP in reducing pseudomonads counts. Deans and Richie [45] showed that thyme oil, an essential oil containing similar components as oregano oil was very effective against *P. aeruginosa*, in the study where the inhibitory properties of ten plant essential oils were tested using an agar diffusion technique, while Scandamis et al. [46] reported that pseudomonads were the most resistant bacterial group to oregano oil. VP resulted in a 2.2 log cfu/g reduction of pseudomonads, while oregano oil only in 0.5 log cfu/g reduction. This is in agreement with Elgayyar et al. [47] who reported that oregano essential oil was less effective in inhibiting *P. aeruginosa* compared to other microorganisms. Lastly, Ouattara et al. [48], reported low inhibition effects of both oregano and thyme oils on a number of meat spoilage bacteria such as *Pseudomonas fluorescens*, *Brochothrix thermosphacta* and *L. sakei*. Oussalah et al. [49] tested the inhibitory effect of 60 different essential oils at concentrations from 0.003 to 0.8% (wt/v) on *Pseudomonas putida* strain of meat origin, associated with meat spoilage. They reported that *Origanum compactum*, *Origanum heracleoticum*, *T. vulgaris carvacroliferum*, *T. vulgaris thymoliferum* have shown a strong antimicrobial activity against *P. putida* and *Origanum majorana*, *Thymus satureoides*, *Thymus serpyllum* showed a high antimicrobial activity. Conner [50] showed that cinnamon, clove, pimento, thyme, oregano and rosemary had strong and consistent inhibitory effects against various pathogens and spoilage microorganisms. Hammer et al. [51] showed that oregano, lemongrass and pimento inhibited the growth of *P. aeruginosa* at a concentration 1.0% (vol/vol).

Our results also indicate that oregano and thyme essential oils had significant effect on *P. aeruginosa* at 0.20% v/w (Fig. 4). The number of *P. aeruginosa* were found only in the air packaging control group AC (6, 9, 12 day) and in the vacuum packaging control group VPC (9, 12 day). In the groups with EDTA, essential oils oregano and thymus were not found the pseudomonads. The statistically significant differences were found between all tested

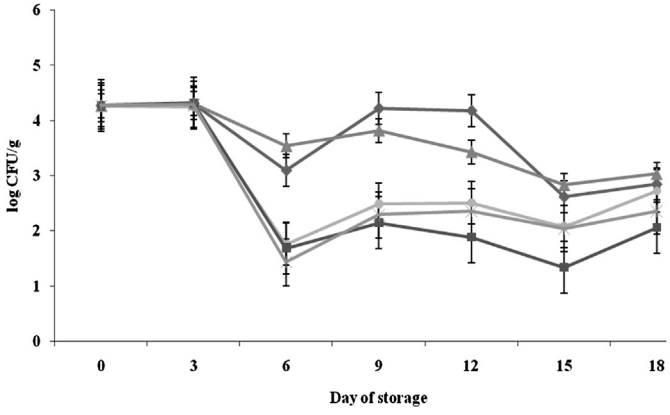

Fig. 3. Changes (log CFU/g) in population of *Lactobacillus* sp. in chicken breast meat stored in air (AC, ♦); stored under vacuum (VPC, ▲); stored under vacuum packaging with EDTA (VPEC, ●); stored under vacuum packaging with oregano essential oil (VP + O, ■); stored under vacuum packaging with thyme essential oil (VP + T). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

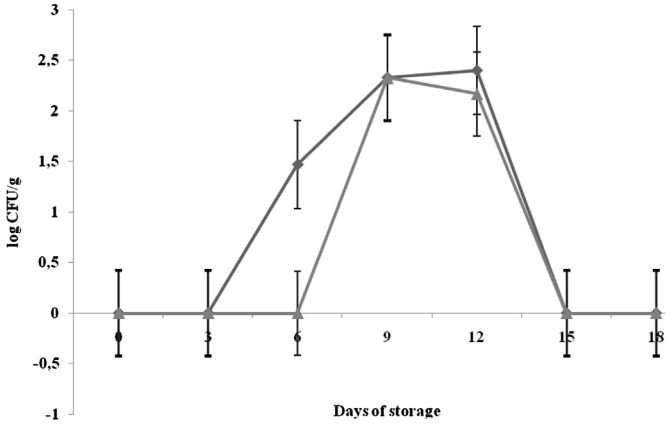

Fig. 4. Changes (log CFU/g) in population of *Pseudomonas aeruginosa* in chicken breast meat stored in air (AC, ♦); stored under vacuum (VPC, ▲). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

groups except VPEC and VP + O, respectively VP + T and between VP + O and VP + T ($P < 0.05$).

4. Conclusion

Of the antimicrobial combination treatments examined in the present study, the use of treatments, EDTA, oregano oil ad thymus oil were the most effective against the growth of Gram-negative bacteria and to a lesser extent on total viable count and lactobacilli. Based on microbiological analyses, treatments oregano oil and thymus oil produced a shelf-life extension of 8–9 days, as compared to the control samples. The ability of vacuum packaging to inhibit spoilage organisms is well documented, but many pathogenic organisms are less affected. Therefore, the combined effect of essential oils as oregano and thymus and vacuum packaging on the safety of the meat could be investigated.

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