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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