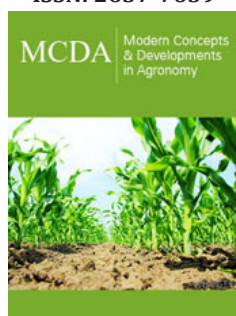


Microbiological Quality of Chicken's Thigh After Vacuum Packaging and Fir Essential Oil Treatment

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Abstract

The aim of the present study was to monitor the fresh chicken thighs microbiological quality after treatment by sunflower oil and *Abies alba* essential oil in two concentrations, stored under vacuum packing (VP), at 4±0.5 °C for a period of 16 days. There were used the following treatments of chicken thighs: Air-packaged (AC, control samples), vacuum-packaged (VPC, control samples), vacuum-packed with sunflower oil (SOC, control samples), VP with *Abies alba* essential oil at concentrations 0.1%v/w (VP+A0.1) and concentration 0.2%v/w (VP+A0.2). The quality assessment of VP of the product in accordance with the terms above and sunflower oil treatment, *Abies alba* oil was established by microbiological analyze. The microbiological properties as the total viable counts and coliform bacteria, lactobacilli and *Pseudomonas* spp. were monitored. The using of *Abies alba* oil and sunflower oil with combination of vacuum packing has significant effect to reduction of microorganisms compared with control group without vacuum packing and untreated control group.

Keywords: *Abies alba*; Essential oil; Sunflower oil; Antimicrobial; Chicken thigh

Introduction

Essential oils (EOs) obtained from various herbs are widely used in cosmetics and food manufacturing and can be used for prolonging the shelf-life of food for their antibacterial, antifungal and antioxidant activities. EOs has inhibitory affect against microorganisms, are aromatic oily liquids obtained from the different plants material, which can be preparing by expression, fermentation, extraction etc. [1] and can contain aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones [2]. Beside essential oil from oregano, thyme, sage, rosemary, which are the most active in this respect against a number of food spoilage and pathogen microorganisms [1,3,4] is possible use EOs from different resources as trees Pinaceae, which includes many of the well-known conifers of commercial importance. Pinaceae obtained polyphenolic compounds [5]. The chemical substances are terpenoids [6,7]. The composition of *Abies* sp. essential oil was observed many authors [8-12]. The main components of essential oils of *Abies* species are: α -pinene, β -pinene, camphene, limonene and δ -3-carene. These substances have significant antibacterial activities [13,14]. It is possible that these essential oils identified from the coniferous trees can be used antibacterial or antifungal agents in foods or in other products.

Guidelines specifically for marketing active and intelligent materials and articles intended for contact with food were published on May 29, 2009 (Regulation No. 450/2009) in the Official Journal of the EU and came into effect on June 18, 2009. These provisions were based on the general requirements established in 2004 in Framework Regulation (Regulation (EC)

No 1935/2004) for the safe use of active and intelligent packaging materials. According to this regulation, 'active materials and articles' are defined as "materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food" [15]. The topic of this study was to examine the conjunct effect of sunflower oil and *Abies alba* essential oil at two different concentrations, on the microbiological quality of fresh chicken thighs stored under vacuum packaging (VP), at 4 ± 0.5 °C for a period of 16 days.

Material and Methods

Preparation of samples

The experiment was realized in the Slovak poultry farm (Hydinaren a.s., Zamostie). The used animals were Cobb chickens. Chickens were fattening for period of 42 days and transported to the laboratory of Slovak University of Agricultural in Nitra. Chicken meat of each experimental group was taken to evaluate their properties. The chicken fresh meat samples with essential oils were prepared:

- Air-packaged (AC, control samples): Chicken thigh fresh meat was packed to polyethylene backs and stored under aerobic conditions in refrigerator.
- Vacuum-packaged (VPC, control samples): Chicken thigh fresh meat was packed to polyethylene backs and stored under aerobic conditions in vacuum and in refrigerator.
- Vacuum-packed with sunflower oil (SOC, control samples): Chicken thigh fresh meat was treated with sunflower oil for 1min and packed to polyethylene backs and stored under aerobic conditions in vacuum and in refrigerator.
- Vacuum-packed with *Abies alba* oil 0.10% v/w (VP+A0.1): Chicken thigh fresh meat was treated with *Abies alba* oil for 1 min and packed to polyethylene backs and stored under aerobic conditions in vacuum and in refrigerator.
- Vacuum-packed with *Abies alba* oil 0.20 % v/w, (VP+A0.2): Chicken thigh fresh meat was treated with *Abies alba* oil for 1min and packed to polyethylene backs and stored under aerobic conditions in vacuum and in refrigerator.

Each sample was packaged using a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic) immediately after dipping. Sunflower oil was purchased in a shop. *Abies alba* essential oil (Calendula, Nova Lubovna, Slovakia) was added to the coated chicken thigh surface (both sides) of each sample using a micropipette so as to achieve a 0.1% and 0.2%v/w final concentration of EO in sunflower oil.

Essential oil sample preparing and chemical composition

The medicinal plant for essential oil isolation was donated by successful and established growers. Essential oil was distilled in the large-scale distillation apparatus specifically designed for aromatic and medicinal plants. There are known two types: Type HV-3000

(height: 5.250mm, width: 2.180mm, with container for 200 or 250kg of dried matter of 400 or 500kg of fresh matter of plant material) and Type HV-300 (height: 3.400mm, width: 1.300mm, with container for 40 or 50kg of dry matter and 100 or 120kg of fresh matter of plant material). This large-scale technology of essential oil distillation in this Slovak company consists from the main distillatory apparatus, a steam condenser, and additional apparatuses (steam boiler and apparatus for improving of used water). Analysis of the essential oils was carried out using a Hewlett-Packard 5890/5970 GC-MSD system. Chemical composition of *Abies alba* oil was as follow: bornyl acetate (30%), camphene (18%), α -pinene (3%), borneol (1.5%), α -terpinene (1.2%).

Microbiological analysis

Approximately 10g (10cm²) of the chicken meat was sampled in sterile condition, add to a sterile stomacher bags, with 90mL of 0.1% peptone water (pH 7.0), and homogenized for 60s in a Stomacher at room temperature. Time intervals for measuring samples were 0, 4, 8, 12 and 16 day. Meat from chickens were stored under vacuum packing, at 4 ± 0.5 °C. For microbiological analyses were using standard microbiological methods. Total viable counts (TVC) were evaluated on Plate Count Agar (PCA, Oxoid, UK), with incubation for 2 days at 37 °C. For *Pseudomonas* spp. enumerations, 0.1mL from 1:10 prepared serial dilutions (0.1% physiological solution) of chicken homogenates was spread onto the surface of solid media. *Pseudomonas* were evaluated on Pseudomonas Isolation agar (PIA, Oxoid, UK) after incubation at 48h at 25 °C with formation of blue or blue-green pyocyanin pigment for *Pseudomonas aeruginosa* identification. *Lactobacillus* spp. were enumerated in 1.0mL of samples were on Rogosa and Sharpe agar (MRS, Oxoid, UK) after incubation 48-78h at 37 °C in an aerobic atmosphere supplemented with carbon dioxide (5%CO₂). For members of the family Enterobacteriaceae, a 1.0mL sample was inoculated into 10mL of molten (45 °C) violet red bile glucose agar (VRBL, Oxoid, UK). After setting, a 10mL overlay of molten medium was added and samples incubated at 37 °C for 24h. The large colonies with purple haloes were counted. All plates were examined for typical colony types and morphology characteristics associated with each growth medium.

Statistical analysis

Statistical analysis of each replication were enumerated to log. The data obtained from each evaluation was implemented by means with Statgraphics Plus version 5.1 (AV Trading, Umex, Dresden, Germany). A statistical analysis was performed with Student's t-test. Differences were expressed as $P<0.05$; $P<0.01$; $P<0.001$.

Results and Discussion

Many centuries we are know about the antimicrobial effect of essential oil. The number of total anaerobe bacteria count (TAB) values for the tested groups of chicken meat are in Figure 1. The initial TAB value of chicken meat was 2.90 ± 0.23 log cfug⁻¹ (day 0), what can show good quality under the limit for poultry products of 10^7 cfug⁻¹ [16]. Ismail et al. [17] found in TVC number from 3.32 to 5.77 log cfug⁻¹ for chicken products. A high fat content appears to

markedly reduce the action of EOs in meat products. The highest TVC in control samples and vacuum packaging control samples ranged from $3.71 \pm 0.11 \log \text{cfug}^{-1}$ to $3.75 \pm 0.17 \log \text{cfug}^{-1}$ (day 16). In the study of Economou et al. [18] was evaluated, that total viable counts (TVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, lactic acid bacteria (LAB) and Enterobacteriaceae counts for all EDTA-treated chicken samples were similar to the control samples with no statistically significant differences among combination nisin-EDTA treatments. EDTA treatments did not affect TVC growth in agreement [19-21] Dawson et al. [22] reported a reduction in growth of aerobic bacteria by 1-1.5 $\log \text{cfug}^{-1}$ in ground chicken meat after 14 days of storage under modified atmosphere packaging. Hyeusoo et al. [23] reported that the essential oils from the three *Pinus* species (*P. densiflora*, *P. thunbergii* and *P. rigida*) showed higher antimicrobial activity against Gram-negative bacteria than Gram-positive bacteria.

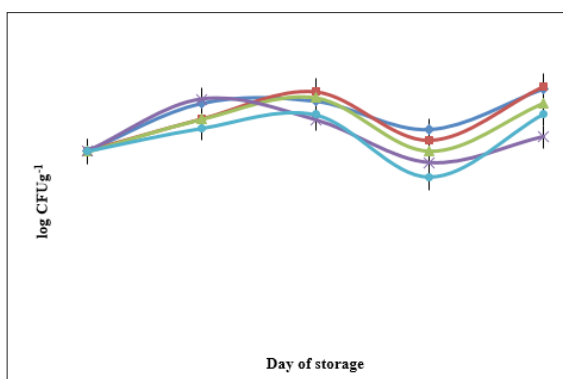


Figure 1: Changes ($\log \text{cfug}^{-1}$) in population of Total Viable Count in chicken thigh stored in air (AC, \blacklozenge); stored under vacuum (VP, \blacksquare); stored under vacuum packaging with sunflower oil (SOC, \blacktriangle); stored under vacuum packaging with *Abies alba* 0.1% essential oil (VP+A0.1, \times); stored under vacuum packaging with *Abies alba* 0.2% essential oil (VP+A0.2, \bullet). Each point is the mean of three samples taken from two replicate experiments ($n=3 \times 2=6$). Error bars show SD.

Bağcı and Diğrak [24] found that the antimicrobial activity of essential oils of *Abies* species varied at different concentrations against bacteria and yeasts. The antimicrobial activities of essential oils used were found to be more active against yeasts than bacteria. Statistically significant difference ($P < 0.05$) was found between AC and VP+A0.1 and AC; AC and VP+A0.2. Other authors found bacteria from the Enterobacteriaceae family on raw beef, lamb, pork, and poultry products, as well as on offal meats [25]. The essential oil of *Pinus densiflora* did not inhibit the activity of *E. coli*, while the essential oils from *P. thunbergii* and *P. rigida* displayed antimicrobial activity against *E. coli*. Previous work showed that the essential oils from *P. densiflora* and *P. thunbergii* had moderate inhibitory activity, and were observed that the essential oil from *P. roxburghaii* did not affect the growth of *E. coli* [26,27].

Bağcı and Diğrak [24] tested antimicrobial activity of essential oils of nine *Abies* species (include *A. alba*) against nine bacteria (include *E. coli* and *P. aeruginosa*) and two yeasts. The essential oil of *Abies alba* classed into group which has higher effective on

microorganisms. Microorganisms *E. coli* and *S. aureus* were the most resistant bacteria except for against *A. koreana*, *A. cilicica* subsp. *isaurica* and little *A. alba* and *A. firma* essential oils. Little antibacterial activity, α of silver essential oil-pinene was found against the growth of microorganism [28] and borneol and p-cymene show antibacterial effect too [29,30]. Di Pasqua et al. [31] reported that antimicrobial compounds including limonene has antibacterial activity against *E. coli*, *S. aureus*, *S. enterica*, *P. fluorescens*, and *B. thermosphacta* cells. Antimicrobial activity against *S. aureus* was found with silver fir oil. Bacterial species from Enterobacteriaceae family as a hygiene indicator were evaluated [32,33]. In our study (day 0) the number of counts were $0.33 \pm 0.81 \log \text{cfu/g}$ what is indicator of well chicken's meat. On day 16 of storage Enterobacteriaceae genera reached $4.75 \pm 0.19 \log \text{cfug}^{-1}$ in control samples. In the case VP, the count of Enterobacteriaceae ranged from $0.33 \pm 0.81 \log \text{cfug}^{-1}$ (day 0) to $3.90 \pm 0.57 \log \text{cfug}^{-1}$ (day 16). The number of Enterobacteriaceae $0 \log \text{cfug}^{-1}$ was found all the time of testing in the VP group with 0.1 and 0.2% *Abies alba* essential oil was (Figure 2).

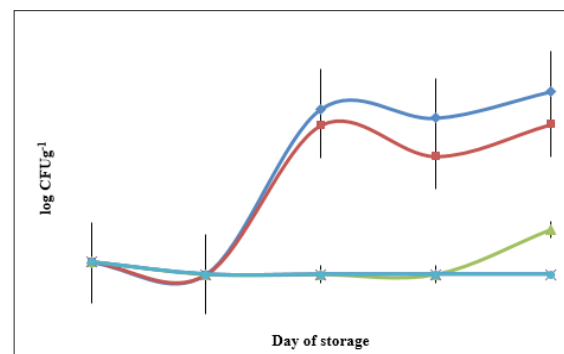


Figure 2: Changes ($\log \text{cfug}^{-1}$) in population of Enterobacteriaceae in chicken thigh stored in air (AC, \blacklozenge); stored under vacuum (VP, \blacksquare); stored under vacuum packaging with sunflower oil (SOC, \blacktriangle); stored under vacuum packaging with *Abies alba* 0.1% essential oil (VP+A0.1, \times); stored under vacuum packaging with *Abies alba* 0.2% essential oil (VP+A0.2, \bullet). Each point is the mean of three samples taken from two replicate experiments ($n=3 \times 2=6$). Error bars show SD.

The number of Enterobacteriaceae $1.16 \pm 1.80 \log \text{cfug}^{-1}$ only in 16 day of evaluation was indicated in the case of the storage under the package with sunflower oil groups. Statistically significant difference ($P < 0.05$) was found between all tested group without VP+A0.1 and VP+A0.2; VP+A0.1 and SOC and VP+A0.2 and SOC. Lactic acid bacteria are facultative anaerobes and grow under concentrations of CO_2 . These bacteria are part of the microflora of meats used anaerobic condition. LABs are the important strains of the spoiled microbial species with anaerobic conditions [33-35]. Particularly, *Lactobacillus* spp., *Carnobacterium* spp. and *Leuconostoc* spp. are microorganisms with spoiled character [36,37]. Lot of species of lactobacilli can be found during the storage under the vacuum condition at 4°C including *Lb. algidus* beyond *Lb. sakei*. Ntzimani et al. [37] obtained that LAB was an important microbiota of the chicken meat in anaerobic condition. The rapid growth of LABs between days 0 and 2 of storage under MAP at 5°C

were evaluated [38]. The total counts of *Lactobacillus* sp. (Figure 3) was 1.88 ± 0.49 log cfug⁻¹ (day 0). The number of *Lactobacillus* sp. in control group was ranged from 1.88 ± 0.49 log cfug⁻¹ (day 0) to 4.16 ± 0.07 log cfug⁻¹ (day 8).

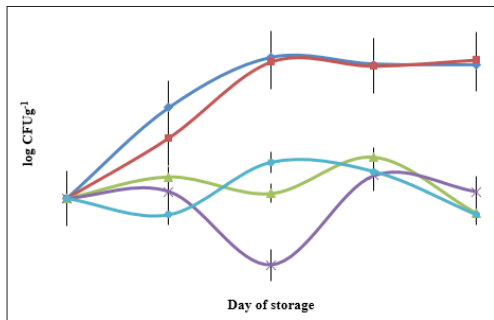


Figure 3: Changes (log cfug-1) in population of *Lactobacillus* sp. in chicken thigh stored in air (AC, ◆); stored under vacuum (VP, ■); stored under vacuum packaging with sunflower oil (SOC, ▲); stored under vacuum packaging with *Abies alba* 0.1% essential oil (VP+A0.1, ×); stored under vacuum packaging with *Abies alba* 0.2% essential oil (VP+A0.2, ●). Each point is the mean of three samples taken from two replicate experiments (n=3×2=6). Error bars show SD.

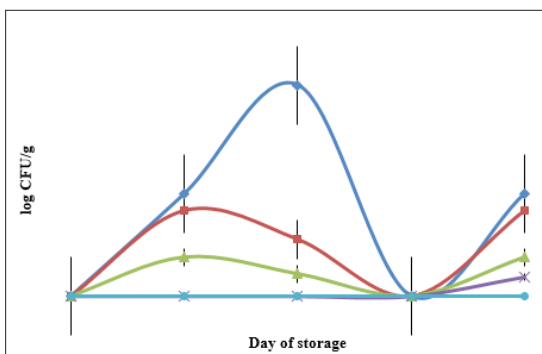


Figure 4: Changes (log cfug-1) in population of *Pseudomonas* spp. in chicken thigh stored in air (AC, ◆); stored under vacuum (VP, ■); stored under vacuum packaging with sunflower oil (SOC, ▲); stored under vacuum packaging with *Abies alba* 0.1% essential oil (VP+A0.1, ×); stored under vacuum packaging with *Abies alba* 0.2% essential oil (VP+A0.2, ●). Each point is the mean of three samples taken from two replicate experiments (n=3×2=6). Error bars show SD.

In the case VP group, the highest count of *Lactobacillus* sp. 4.12 ± 0.04 log cfug⁻¹ was marked on day 16 of storage; in SOC group 2.55 ± 0.30 log cfug⁻¹ (day 12); in VP+A0.1 group 2.25 ± 0.38 log cfug⁻¹ (day 12) and VP+A0.2 2.46 ± 0.60 log cfug⁻¹ (day 8) [39]. Statistically significant difference (P<0.05) was found between AC and SOC; SOC and VP; VP+A0.1 and AC; VP+A0.1 and VP; VP+A0.2 and AC; VP+A0.2 and VP. Several gram-negative bacteria, including *Pseudomonads*, and *P. aeruginosa*, are not very resistant against of EOs [40]. Bioactive components of plant-origin antimicrobials are

relatively weak against *Pseudomonas* spp. Various studies show the opposite effect of essential oils against gram-positive bacteria [1]. The essential oils from leaves of the three *Pinus* species inhibited the bacterial growth. The essential oils from *P. thunbergii* and *P. rigida* showed bactericidal activity at 4h. Previous work showed that the essential oils from *P. densiflora* and *P. thunbergii* had slight inhibitory activity and the essential oil from *P. caribaea* exhibited antimicrobial activity [40]. In our study the initial count of *Pseudomonas* spp. was 0 ± 0.00 log cfug⁻¹ (day 0) and the highest numerous amount was 3.65 ± 0.12 log cfug⁻¹ (day 8) in the control group; 1.47 ± 1.15 log cfug⁻¹ (day 4) in VP group; 0.67 ± 1.03 log cfug⁻¹ (day 4) in SOC group; 0.33 ± 0.82 log cfug⁻¹ (day 4) in VP+A0.1 group and in VP+A0.2 group was no presented *P. aeruginosa* during all testing period. It is very interesting, that all tested groups the numerous of *P. aeruginosa* was 0 log cfu/g on 12th day of the study. The statistically significant differences (P<0.05) were found between AC and SOC; AC and VP; VP+A0.1 and AC; AP+A0.1 and VP; VP+A0.2 and AC; AP+A0.2 and VP.

Conclusion

Our results show the potential antimicrobial effect of the *Abies alba* essential oil be natural food preservatives and antimicrobial sources for chicken meat. The use of our treatment with sunflower oil and *Abies alba* essential oil have good antimicrobial activity against the growth of *Pseudomonas* spp., *Lactobacillus* spp. and Enterobacteriaceae genera and decrease total viable count. Microbiological analyses of *Abies alba* essential oil treatments show better microbiological quality of chicken's meat compare with control samples. Combined effect of essential oil and vacuum packaging should be investigated on the safety and hygienic quality of chicken's meat.

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