Microbiological Quality of Fresh Chicken Breast Meat after Rosemary Essential Oil Treatment and Vacuum Packaging

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Abstract
The objective of the present research was to develop vacuum packaging incorporated with *Rosmarinus officinalis* L. essential oil treatment of fresh chicken breast meat. For this purpose, fresh chicken's meat breast samples were divided into three groups. First group was kept as a control group with air packaging, others one was with vacuum packaging of samples and last one group was treated with rosemary essential oil. All fresh chickens' breast meat samples were stored at 4°C and microbiological evaluation was conducted at intervals of 0, 4, 8, 12 and 16 days post-storage for lactic acid bacteria and *Pseudomonas aeroginosa* counts. *Rosmarinus officinalis* L. essential oil 2%, significantly reduced lactic acid bacteria and *Pseudomonas aeroginosa* counts in the fresh chickens breast meat samples. The results indicated that 2% essential oil improved the microbiological quality and prolonged the shelf-life of the fresh chicken's breast meat to sixteen days of retail displayed at 4°C. The results obtained in this study point to the necessity of continuing investigations to determine the dose of rosemary preparations that would inhibit the growth of microflora being the most frequent cause of raw materials and products spoilage.

Keywords: fresh chicken's breast meat, microbiological quality, rosemary essential oil

1. Introduction

Meat is recognized as one of the most perishable foods. This is due to its chemical composition that favours microbial growth to unacceptable levels contributing significantly to meat deterioration or spoilage. When large numbers of microorganisms are present in raw meat, there will be changes such that it becomes unappealing and unsuitable for human consumption [1, 2]. The initial microbial load of meat depends on the physiological status of the animal at slaughter, the spread of contamination into slaughterhouses and during processing, while temperature and other conditions of storage during distribution can also influence the rate of spoilage [3].

The assurance of inventory and the shelf life of meat represent an important challenge for the meat industries. The spoilage of refrigerated meat is caused in part by *Pseudomonas* species bacteria which are responsible for the offodours, off-flavours, discoloration, gas production and slime production. Antimicrobial agents, including food preservatives have been used to inhibit foodborne bacteria and extend the shelf life of processed food. Many naturally occuring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a

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source for antimicrobial agents against food spoilage and pathogens [4, 5].
More particularly, essential oils and their components are known to be active against a wide variety of microorganisms, including Gram-negative bacteria [6, 7].
Essential oils are the odorous, volatile products of an aromatic plant's secondary metabolism, normally formed in special cells or groups of cells, found in many leaves and stems. They are commonly concentrated in one particular region such as leaves, bark or fruit, and when they occur in various organs in the same plant, they frequently have different composition profiles. Essential oils have long served as flavoring agents in food and beverages, and due to their versatile content of antimicrobial compounds, they possess potential as natural agents for food preservation [8].
Several references on the antimicrobial activity of essential oils are available in the literature [9-12]. The antimicrobial activity of essential oils is assigned to a number of small terpenoid and phenolic compounds, which also in pure form have been shown to exhibit antibacterial or antifungal activity [8, 13, 14]. The antibacterial properties of these compounds are in part associated with their lipophilic character, leading to accumulation in membranes and to subsequent membrane-associated events such as energy depletion [8].
Considering the above, the aim of the present study was to investigate the combined effect of rosemary essential oil and vacuum packaging VP on the shelf-life extension of fresh breast chicken meat, stored at 4 °C.

2. Materials and methods

The experiment was implemented in the local poultry station (Hydinaren a.s., Zamostie). The tested chickens were Cobb. At the end of the fattening period (day 42) were chickens slaughter for analysis. To evaluate the microbiological properties was taken breast muscle (*musculus pectoralis major*) without skin of each experimental group.
The treatments of chicken fillets examined in the present study were the following: Air-packaged (C, control samples on air), vacuum-packaged (VP) and VP with EDTA–with rosemary (R) essential oil 2% v/w.
Rosemary essential oil (Calendula, Nova Lubovna, Slovakia) was added to the coated chicken surface (two sides) of each sample using a micropipette so as to achieve a 2% v/w final concentration of EO.
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, 4, 8, 12 and 16 days.
Microbiological analyses were conducted using standard microbiological methods. For *Pseudomonas aeroginosa* 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. Pseudomonads were determined on Pseudomonas agar (Oxoid, UK) after incubation at 48 h at 35°C. Lactic Acid Bacteria (LAB) enumerations, a 1.0 ml sample were inoculated into Rogosa agar (Oxoid, UK) after incubation 48 h at 37°C. All plates were examined for typical colony types and morphology characteristics associated with each growth medium.
Data from each replication were averaged and log transformed (microbiological analysis). Results of microbiological analyses are reported as mean values standard deviation (S.D). Differences in mean log CFU/g among treatments or storage times were determined by the Students t-test (significance was defined at $P<0.05$).

3. Results and discussion

Pseudomonads (Fig. 1) are Gram negative bacteria sensitive to CO_2, comprising the main spoilage microorganisms of meat [15]. Thus, VP, inhibited the growth of Pseudomonads, as compared to air packaging. It is believed that VP extends the lag phase of aerobic microbial growth and decreases growth rate during the logarithmic phase [16].
For example, VP reduced the Pseudomonads by 1.3 log $cfu.g^{-1}$ on day 16 of storage and kept their populations under 2 log $cfu.g^{-1}$ until day 16 of storage. On the other hand, rosemary essential oil had a small but not statistically significant controlling effect on the Pseudomonads. It has been reported that due to their cell wall composition, Gram negative bacteria are less

sensitive to EOs than Gram positive bacteria even though various studies claim the opposite [17]. Likewise, the combination of VP and REO showed the same pattern of Pseudomonads' inhibition as VP alone. With regard to VP, results of the present study are in agreement with those of Santos et al. [18] who reported MAP those Gram negative bacteria such as the Pseudomonads and *Enterobacteriaceae* are more sensitive to VP than Gram positive bacteria such as LAB. With regard to EOs, Deans and Ritchie [19] showed that thyme oil was very effective against *Pseudomonas aeruginosa* while Ouattara et al. [20] found no substantial effect of thyme oil on growth of meat spoilage microorganisms including *Pseudomonas fluorescens*, *Brochothrix thermosphacta* and *Lactobacillus sakei*. Finally Skandamis et al. [21, 22] reported that Pseudomonads were the most resistant bacterial group to oregano oil.

LAB behave as facultative anaerobes and are able to grow under high concentrations of CO_2. They thus constitute a substantial part of the natural microflora of VP meats. The initial LAB counts (Fig. 2) were 2.1 log cfu.g^{-1} (day 0) increasing progressively with time. On day 16 of storage the use of C, VP and the combination of VP plus R (2%) resulted in a reduction in LAB counts by 3.36; 3.26 and 2.57 log cfu.g^{-1} respectively. With regard to the use of REO, present results are in agreement to those of Zaika et al. [23] who reported a reduction of 4 log cfu.g^{-1} in LAB populations in pure culture after the addition of 4 g/l (0.4%) of oregano oil, considering the differences in EO concentration used (0.4 vs. 0.1%) and the fact that foodstuff components always act protectively on microorganisms as compared to pure cultures [17]. With regard to the combined use of EOs and VP, present results are in general agreement with those of Chouliara et al. [24] and Chouliara and Kontominas [25] for chicken breast meat.

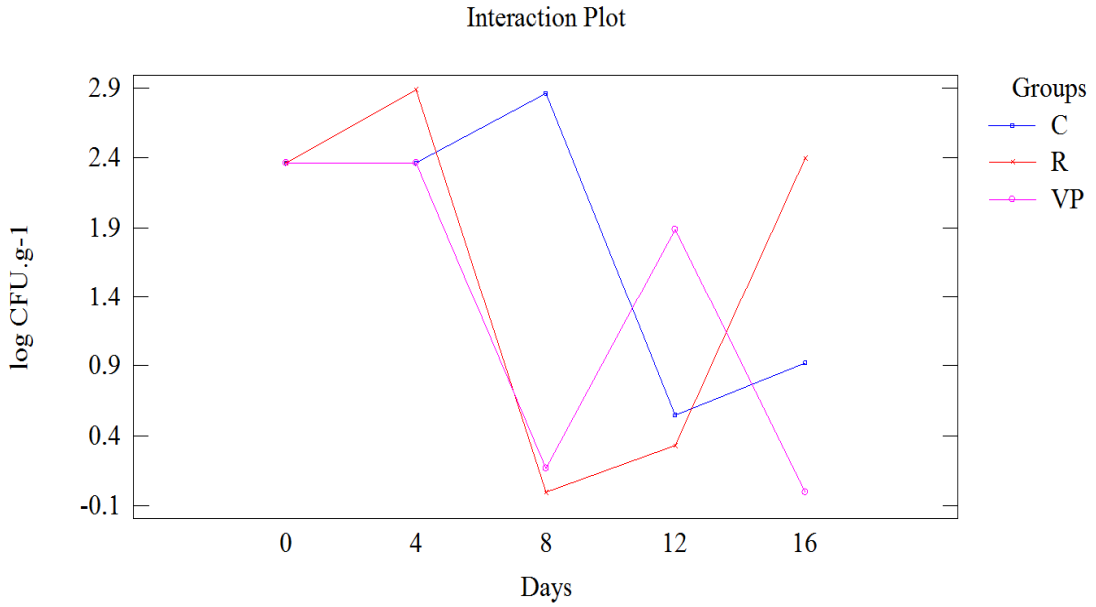

Figure 1. Changes (log CFU.g^{-1}) in population of *Pseudomonas aeroginosa* in chicken breast meat samples stored in air (C); stored under vacuum (VP); with EDTA and rosemary oil stored under vacuum (R).

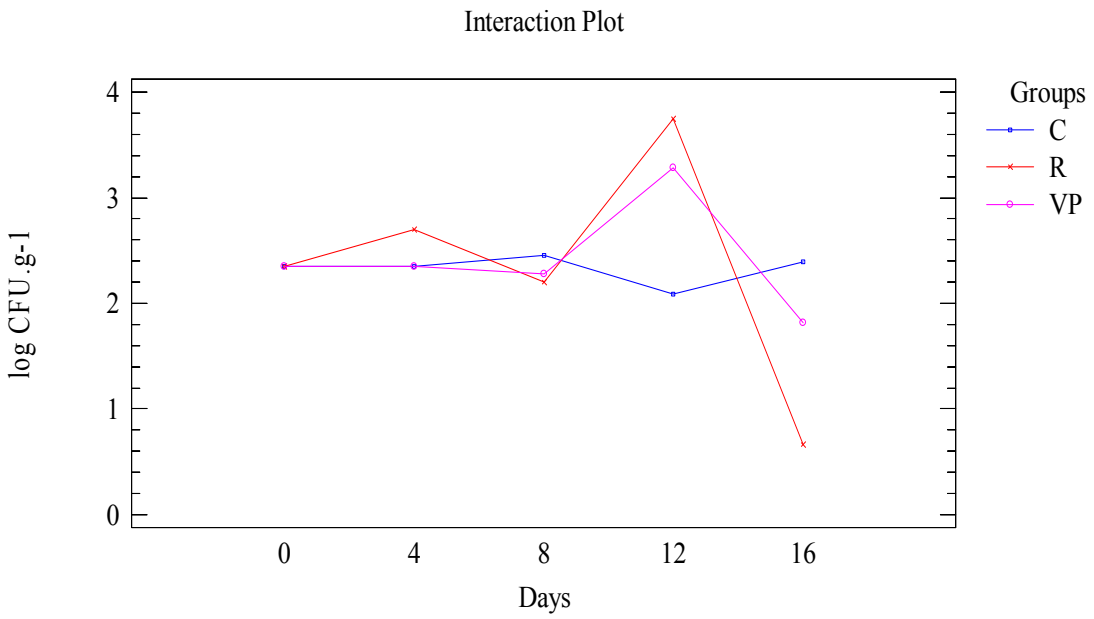

Figure 2. Changes (log CFU.g^{-1}) in population of Lactic acid bacteria in chicken breast meat samples stored in air (C); stored under vacuum (VP); with EDTA and rosemary oil stored under vacuum (R).

4. Conclusions

Of the antimicrobial combination treatment examined in the present study, the use of treatment, EDTA–rosemary oil (VP+R) were the most effective against the growth of Pseudomonads and Lactic acid bacteria. Based on microbiological (Pseudomonads data) analyses, treatment VP+R produced a shelf-life extension of 12–16 days, as compared to the control samples.

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