Journal of Applied Science and Agriculture, 9(2) February 2014, Pages: 471-473

AENSI Journals

Journal of Applied Science and Agriculture

ISSN 1816-9112

Journal home page: www.aensiweb.com/jasa/index.html

# Microbiological Quality of Fresh Beef Meat after *Salvia officinalis L.* Essential Oil Treatment and Vacuum Packaging

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ARTICLE INFO

*Article history:*
Received 18 November 2013
Received in revised form 20 February 2014
Accepted 22 February 2014
Available online 20 March 2014

*Keywords:*
fresh beef meat microbiological quality Salvia essential oil

ABSTRACT

**Objective:** The objective of the present research was to develop vacuum packaging incorporated with *Salvia officinalis L.* essential oil treatment of fresh beef meat. **Method:** For this purpose, fresh beef meat 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 Salvia essential oil. All fresh beef meat samples were stored at 4°C and microbiological evaluation was conducted at intervals of 0, 4, 8, 12 and 16 days poststorage for lactic acid bacteria and *Pseudomonas aeroginosa* counts. *Salvia officinalis* L. essential oil 2%, significantly reduced lactic acid bacteria and *Pseudomonas aeroginosa* counts in the fresh beef meat samples. **Results:** The results indicated that 2% essential oil improved the microbiological quality and prolonged the shelf-life of the fresh beef meat to sixteen days of retail displayed at 4°C.



**To Cite This Article:** Hossein Safarpour., Microbiological Quality of Fresh Beef Meat after *Salvia officinalis L.* Essential Oil Treatment and Vacuum Packaging. ***J. Appl. Sci. & Agric.,*** *9(2): 471-473, 2014*

## INTRODUCTION

Meat is one of the most perishable foods. This is due to its chemical composition that favors 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 (Fung, D.Y., 2010; Gram, L., *et al*., 2002). 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 (Nychas, G.J.E., 2008). 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, offflavours, 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 occurring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Bagamboula, C.F., *et al*., 2004; Dorman, H.J.D., S.G. Deans, 2000). More particularly, essential oils and their components are known to be active against a wide variety of microorganisms, including Gramnegative bacteria (Helander, I.M., *et al*., 1998; Sivropoulou, A., *et al*., 1996). 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 (Conner, D.E., 1993). Several references on the antimicrobial activity of essential oils are available in the literature (Araújo, C., *et al*., 2003; Burt, S.A., R.D. Reinders, 2003; Cox, S.D., *et al*., 2000; Delaquis, P.J., *et al*., 2002). 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 (Conner, D.E., 1993). 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 (Conner, D.E., 1993). Considering the above, the aim of the present study was to investigate the combined effect of Salvia essential oil and vacuum packaging VP on the shelf-life extension of fresh breast chicken meat, stored at 4 °C.

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*Methodology:*

The treatments of beef meat examined in the present study were the following: Air-packaged (C, control samples on air), vacuum-packaged (VP) and VP with EDTA–with Salvia (S) essential oil 2% v/w. Salvia essential oil (Calendula, Nova Lubovna, Slovakia) was added to the coated beef 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 cm2) of the beef meat(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 beef meat 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 $P<0.05$).

## RESULTS AND DISCUSSION

*Pseudomonads* (Fig. 1) are Gram negative bacteria sensitive to CO2, comprising the main spoilage microorganisms of meat. 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. 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, Salvia 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. 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*. 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 showed that thyme oil was very effective against *Pseudomonas aeruginosa* while Ouattara *et al*. 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*. reported that *Pseudomonads* were the most resistant bacteria group to oregano oil. LAB behaves as facultative anaerobes and is able to grow under high concentrations of CO2. 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*. 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*. and Chouliara and Kontominas for beef meat.

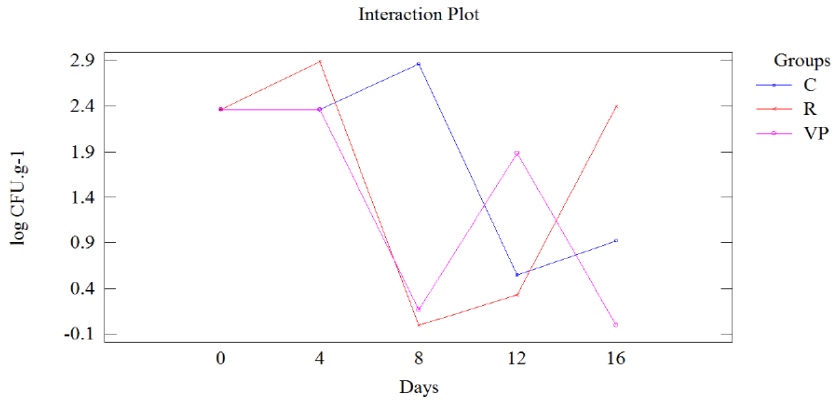


**Fig. 1:** Changes (log CFU.g-1) in population of *Pseudomonas aeroginosa* in beef meat samples stored in air (C); stored under vacuum (VP); with EDTA and Salvia oil stored under vacuum (R).

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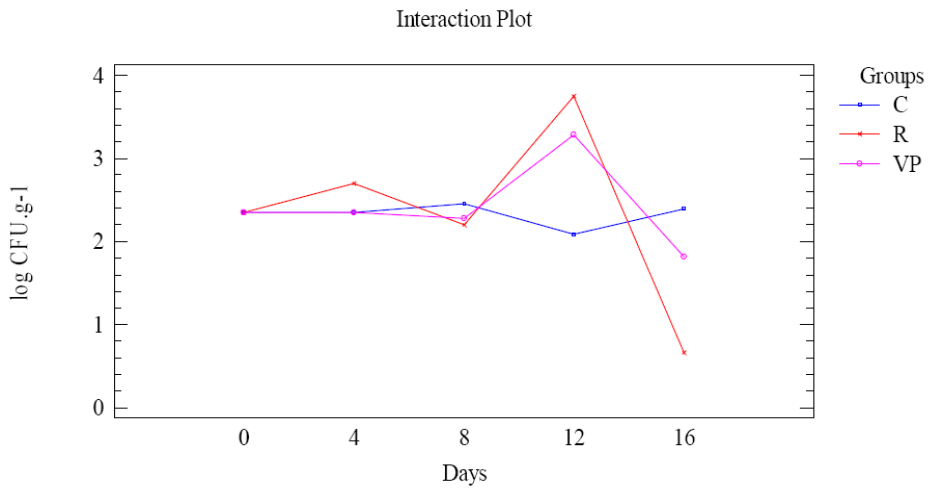


**Fig. 2:** Changes (log CFU.g-1) in population of Lactic acid bacteria in beef meat samples stored in air (C); stored under vacuum (VP); with EDTA and Salvia oil stored under vacuum (R).

*Conclusion:*

Of the antimicrobial combination treatment examined in the present study, the use of treatment, EDTA–Salvia 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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