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Control of *Listeria monocytogenes* in Ground Chicken Breast Meat under Aerobic, Vacuum and Modified Atmosphere Packaging Conditions with or without the Presence of Bay Essential Oil at 4°C

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In this study the combined effect of bay (*Laurus nobilis* L.) essential oil (0.5% v/w) with modified atmosphere (MAP) (20%CO_2/80%N_2) and vacuum packaging in ground chicken breast meat stored at 4°C was investigated. Microbial populations of inoculated *Listeria monocytogenes* AUFE 39237 in chicken meat and *Escherichia coli* and total viable counts (TVC) were also monitored during 0,1,3,5,7 th days of storage. MAP packaging with/without bay oil gave significantly ($p<0.01$) higher reductions of microbial populations. Vacuum packaging and bay oil combination was also found significantly effective ($p<0.01$) against *E. coli* in ground chicken meats. The preservative effect of MAP and vacuum packaging against some food pathogens can be increased with bay essential oil in chicken meats.

Keywords: *Listeria monocytogenes*, chicken meat, bay (*Laurus nobilis* L.) essential oil, antimicrobial

Introduction

Poultry meat is very popular around the world because of the low cost of production and the high nutritional value. Refrigeration is the most common preservation method of raw meat and meat products but nowadays application of hurdle technology (packaging systems with natural additives, changing pH or temperature) to foods maintain minimal processing and also to preserve foods from spoilage and pathogen microorganisms (Chouliara *et al.*, 2007; Goulas and Kontominas, 2007).

The listeriae are gram-positive, non-spore forming rods and widely distributed in nature. *L. monocytogenes* is most often found and received much attention because of early outbreaks. In general the organism is found in raw milk, soft cheeses, fresh and frozen meat, poultry, sea food and in fruits and vegetable products (Sandasi *et al.*, 2008; Singh *et al.*, 2003). This psychrotrophic bacterium can cause meningitis, abortion and perinatal septicemia in humans. It can grow under refrigeration, in anaerobic conditions and in low O_2 atmosphere and it tolerates freezing (Solomakos *et al.*, 2008; Bonilauri *et al.*, 2004). The ability of this pathogen to grow on vacuum-packaged raw and industrially processed poultry and meat has been well described by many authors. Best growth occurred in chicken and turkey products, in part due to the higher initial pH of these products (Jay *et al.*, 2005).

The use of natural preservatives to inhibit the growth of serious pathogens such as *Listeria monocytogenes* is of great interest to the meat industry (Zhang *et al.*, 2009). Antimicrobial activities of essential oils have been reported by numerous workers (Irkin and Korukluoglu, 2009a; Irkin and Korukluoglu, 2009b). It has been suggested that *Laurus nobilis* oil contains 1,8 –cineole, sabinene, α and β –pinenes, linalool compounds primarily and these compounds could be very useful in preserving foods from contaminations (Simic *et al.*, 2004; Bouzouita *et al.*, 2003).

Essential oils (EO) can extend the shelf-life of food when used alone or in combination with other preservation techniques. EO can be added to foods by simple tumbling or spraying. As compared to several other mild preservation procedures such as smoking, low dose irradiation, addition of protective cultures, or high pressure treatment, EO is inexpensive and uncomplicated method of inhibiting pathogens and also extending shelf-life of MAP or vacuum packaged

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products (Mejholm and Dalgaard, 2002). However, some food components and techniques decrease the antimicrobial effect of EO and some of the essential oils have strong flavor (Raybaudi- Massillia *et al.*, 2009; Gutierrez *et al.*, 2008). For these reasons using lower concentrations of essential oils in combination with other preservation technologies give more proper results. There are limited numbers of studies on the use of EO with combination of packaging technique in a food model (Chouliara *et al.*, 2007; Nedorostova *et al.*, 2009).

MAP technology has been an effective method of food preservation in the last years. It is known as a method for extending the shelf-life of foods including fresh meats and poultry and optimization of gas composition is still important to ensure the quality and safety of foods. Application of carbondioxide (to inhibit bacteria growth) and nitrogen (to avoid oxidation of fats) atmospheres in poultry meat packages can generate optimum preservation effect (Goulas, 2008; Narasimha and Sachindra, 2002).

In this study it was aimed to determine the inhibition activity of bay oil with packaging vacuum and MAP techniques in ground chicken breast meat against inoculated *L. monocytogenes*, to present microflora of *E. coli* and total viable counts and to compare their counts with air packaged groups.

Materials and Methods

Bacteria *Listeria monocytogenes* AUFE 39237 culture was obtained from Ankara University Food Engineering Department. Stock culture was maintained on Nutrient Agar (NA) slants at 4°C. *L. monocytogenes* AUFE 39237 was incubated at 35°C for 24 h by inoculation into Nutrient Broth. After incubation 1 ml of bacteria culture (app. 10^6 cfu/ml) was inoculated in to ground chicken breast meat and mixed with a stomacher (Tekmar, STO-400, US) 30 s at low speed.

Chicken breast ground meat Chicken meat was purchased from a local market in Balikesir, Turkey. The skin and external fat tissue was removed from the muscles and the lean muscle was diced into approximately 1-cm cubes, and ground through a 3-mm diameter orifice using a mincer. Chicken ground meat samples were weighted ca. 250 g each and the ratio between volume of gas and weight of food product (G/P ratio) was 3:1 (v/w). Modified atmosphere packaging was performed by using Tiromat Compact M380 (Greece) packaging machine. Ground chicken breast samples were packed in Poly Ethylene terephytalate (PET)/ Ethylene Vinily Alcohol (EVOH)/ Low Density Polyethylene (LDPE) trays of 750 µm thickness. A film of oriented poly propylene (OPP)/ Low density polyethylene (LDPE)/ Ethylene vinyl alcohol (EVOH)/ Low density poly ethylene (LDPE) was used having a 72 µm in thickness and an oxygen permeability of 4.5 cm^3/m^2-day-1atm at 75% relative humidity (RH), 23°C and a water vapor permeability 3.5 g m^2 /day at 90% RH, 38°C. Vacuum packaging was performed with PA/PE film having 80 µm in thickness, and an oxygen permeability of 90 cm^3/m^2-day-1 atm at 75% relative humidity (RH), 23°C and a water vapor permeability of 5 g/ m^2-day at 90% RH, 38°C by using VC 999/K12NA (Switzerland) packaging machine.

All chicken ground meat was inoculated with *L. monocytogenes* (app.10^6 cfu/ml) and mixed with a stomacher (Tekmar, STO-400, US) 30 s at low speed immediately and then lots of samples were prepared as follows: The first lots of samples were denoted as control group with aerobic packaging. Essential oil of bay was purchased from Yakatarla Food Botanic Company (Istanbul, Turkey). Its quality parameters (appearance, color, purity, odor, density -20°C, refraction index -20°C) were described in an accompanying technical report. Bay oil was pipetted to the second, fourth and sixth lots so as to obtain 0.5% (v/w) concentrations in samples. Second lot was packaged under aerobic conditions with essential oil. Third and fourth lots were sealed under MAP using a mixture of 20%/80% (CO_2/N_2). Fifth and sixth lots were vacuum-packed.

Carbondioxide and nitrogen concentrations in the MAP packages headspace were monitored periodically by using PBI Dansensor Check Pointer O_2/CO_2 (Ringsted, Denmark) analyzer. All samples were kept at 4°C and sampling was carried out on 0,1,3,5,7 th days of storage.

pH Determination The pH value was recorded employing Hanna Instruments model HI221 Microprocessor (US), pH meter. Chicken samples were thoroughly homogenized with 10 ml of distilled water and the homogenate was used for pH determination. Fat and moisture contents of minced meats were determined according to AOAC (1980) and ISO (1973), respectively.

Microbial counts Chicken minced samples (25 g) were transferred aseptically into a medium containing 225 ml of sterile Buffered Peptone Water (BPW) solution (0.1% w/v) and homogenized in a Lab Blender Braun MP80 (Germany). For each sample, appropriate serial decimal dilutions were prepared in BPW solution (0.1%). Total viable counts (TVC) were determined using Plate Count Agar (PCA), after incubation for 48 h at 35°C (Gonzales- Montalvo *et al.*, 2007). *L. monocytogenes* was determined by Brain Heart Infusion (BHI) agar (Oxoid) at 35°C for 24 h (Menon and Garg, 2001). *E. coli* was counted on Eosin Methylene Blue Agar (EMBA) at 37°C for 24 h (The Oxoid Manual, 1998). All count data were written as logarithms before their statistical treatment.

Statistical analysis Experiments were replicated twice on different occasions with different ground chicken meat samples. Analysis were run in triplicate for each replicate

(*n*=3×2). The data were statistically subjected to univariate analysis of variance (General Linear Model) using SPSS 10.0 and Tukey HSD test was used at a significance level of 0.05 (Ozdamar, 2004).

Results and Discussion

Proximate analysis Proximate analysis of chicken ground meats generated average moisture 7.3±0.15% and fat 1.56±0.12 % (%, weight basis). Average pH values of chicken ground meats are presented in Fig.1. Slight changes in gas concentrations in the MAP packages were determined during the storage period.

Microbiological analysis Microorganism values of samples are presented in Tables 1, 2 and 3. TVC count was 5.1 log cfu/g on 0th day. At the end of the 7-day storage higher TVC ($p<0.01$) values were detected for air packaged samples in comparison with all other lots of chicken meat samples. Some significant differences were observed ($p<0.05$) between MAP, VP, VP and oil, AP, AP and oil after 7 days of

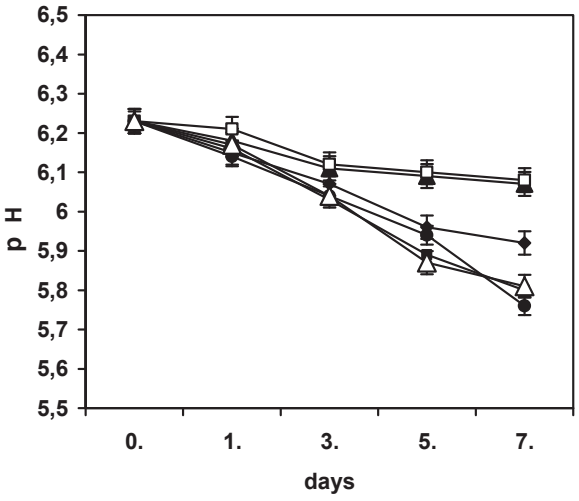

Fig. 1. Effect of packaging conditions and bay essential oil on pH in ground chicken meat during storage at 4°C. (●): Air packaged (control), (◆) B: (Air packaged+Essential oil), (▲): (MAP, $20CO_2+80N_2$%), (□) D: (MAP + Essential oil), (■): (Vacuum packaged), (△): (Vacuum Packaged + Essential oil). Data represent mean values of (*n*=2×3) measurements and error bars are indicated.

Table 1. The total viable counts in ground chicken meats in different packaging systems with/without presence of 0.5 % (v/w) bay oil stored in refrigeration condition.

Packages	$0^{ß}$	1	3	5	7
AP	5.1* ± 0.3**	$6.96 \pm 0.3_{B}{}^{b}$	$6.5 \pm 1.2_{C}{}^{a}$	$7.08 \pm 0.6_{C}{}^{b}$	$7.77 \pm 0.8_{B}{}^{c}$
AP+O	5.1 ± 0.3	$6.28 \pm 0.6_{A}{}^{a}$	$6.34 \pm 1.2_{A}{}^{a}$	$6.98 \pm 1.5_{AB}{}^{b}$	$7.63 \pm 1.1_{A}{}^{c}$
MAP	5.1 ± 0.3	$6.55 \pm 1.2_{A}{}^{b}$	$6.0 \pm 0.3_{A}{}^{a}$	$6.5 \pm 1.1_{A}{}^{b}$	$7.34 \pm 1.2_{A}{}^{b}$
MAP+O	5.1 ± 0.3	$6.54 \pm 1.1_{B}{}^{b}$	$5.97 \pm 1.4_{A}{}^{a}$	$5.86 \pm 0.7_{A}{}^{a}$	$6.87 \pm 0.5_{A}{}^{b}$
VP	5.1 ± 0.3	$6.47 \pm 0.5_{A}{}^{a}$	$6.55 \pm 0.5_{A}{}^{a}$	$6.59 \pm 0.5_{A}{}^{a}$	$7.62 \pm 0.7_{A}{}^{b}$
VP+O	5.1 ± 0.3	$6.43 \pm 0.2_{A}{}^{a}$	$6.57 \pm 0.4_{A}{}^{a}$	$6.55 \pm 0.6_{A}{}^{a}$	$7.68 \pm 1.4_{A}{}^{b}$

AP: Air packaging (Control); AP+O: Air packaging and essential oil; MAP: $20\%CO_2/80\%N_2$; MAP+O: $20\%CO_2/80\%N_2$ and essential oil; VP: Vacuum packaging; VP+O: Vacuum packaging and essential oil.
A, means with different capital letters indicate differences ($p<0.01$) among the packaging treatments.
a, means with different lower-case letters show significant differences ($p<0.05$) of the storage times.
* log cfu/g.
** S.D. (*n*=6.)
ß storage time (d)

Table 2. The counts of *L. monocytogenes* in ground chicken meats in different packaging systems with/without presence of 0.5 % (v/w) bay oil stored in refrigeration condition.

Packages	$0^{ß}$	1	3	5	7
AP	6.21* ± 0.4**	$7.07 \pm 1.2_{A}{}^{a}$	$7.23 \pm 0.2_{B}{}^{a}$	$6.98 \pm 1.2_{A}{}^{a}$	$7.06 \pm 1.5_{A}{}^{a}$
AP+O	6.21 ± 0.4	$6.85 \pm 1.0_{B}{}^{b}$	$6.18 \pm 1.6_{A}{}^{a}$	$6.7 \pm 1.0_{B}{}^{b}$	$6.82 \pm 0.2_{B}{}^{b}$
MAP	6.21 ± 0.4	$6.89 \pm 0.2_{B}{}^{b}$	$6.07 \pm 0.8_{A}{}^{a}$	$6.62 \pm 0.7_{B}{}^{b}$	$5.74 \pm 0.3_{A}{}^{a}$
MAP+O	6.21 ± 0.4	$6.19 \pm 0.1_{A}{}^{a}$	$5.96 \pm 0.3_{A}{}^{a}$	$6.46 \pm 0.5_{B}{}^{b}$	$5.38 \pm 0.8_{A}{}^{a}$
VP	6.21 ± 0.4	$6.5 \pm 0.7_{A}{}^{a}$	$6.44 \pm 0.9_{A}{}^{a}$	$6.98 \pm 0.8_{B}{}^{b}$	$6.85 \pm 0.5_{B}{}^{b}$
VP+O	6.21 ± 0.4	$6.37 \pm 0.8_{A}{}^{a}$	$6.28 \pm 0.2_{A}{}^{a}$	$6.93 \pm 1.1_{B}{}^{b}$	$6.78 \pm 0.4_{B}{}^{b}$

AP: Air packaging (Control); AP+O: Air packaging and essential oil; MAP: $20\%CO_2/80\%N_2$; MAP+O: $20\%CO_2/80\%N_2$ and essential oil; VP: Vacuum packaging; VP+O: Vacuum packaging and essential oil.
A, means with different capital letters indicate differences ($p<0.05$) among the packaging treatments.
a, means with different lower-case letters show significant differences ($p<0.05$) the storage times.
* log cfu/g.
** S.D. (*n*=6.)
ß storage time (d)

Table 3. The counts of *E.coli* in ground chicken meats in different packaging systems with/without presence of 0.5 % (v/w) bay oil stored in refrigeration condition.

Packages	$0^{ß}$	1	3	5	7
AP	1.78* ± 0.3**	$1.95 \pm 0.3_{A}{}^{a}$	$2.33 \pm 1.2_{A}{}^{a}$	$3.59 \pm 0.6_{B}{}^{a}$	$4.82 \pm 0.8_{B}{}^{b}$
AP+O	1.78 ± 0.3	$1.83 \pm 0.6_{A}{}^{a}$	$2.25 \pm 1.2_{A}{}^{a}$	$3.17 \pm 1.5_{B}{}^{b}$	$3.6 \pm 1.1_{B}{}^{b}$
MAP	1.78 ± 0.3	$1.65 \pm 1.2_{A}{}^{a}$	$1.74 \pm 0.3_{A}{}^{a}$	$2.89 \pm 1.1_{A}{}^{b}$	$3.44 \pm 1.2_{B}{}^{c}$
MAP+O	1.78 ± 0.3	$1.54 \pm 1.1_{A}{}^{a}$	$1.79 \pm 1.4_{A}{}^{a}$	$2.67 \pm 0.7_{A}{}^{b}$	$3.23 \pm 0.5_{B}{}^{c}$
VP	1.78 ± 0.3	$1.76 \pm 0.5_{A}{}^{a}$	$2.2 \pm 0.5_{A}{}^{a}$	$3.23 \pm 0.5_{B}{}^{b}$	$3.64 \pm 0.7_{B}{}^{c}$
VP+O	1.78 ± 0.3	$1.7_{A} \pm 0.2_{A}{}^{a}$	$2.1 \pm 0.4_{A}{}^{a}$	$3.19 \pm 0.6_{B}{}^{b}$	$3.52 \pm 1.4_{B}{}^{c}$

AP: Air packaging (Control); AP+O: Air packaging and essential oil; MAP: 20%CO_2/80%N_2; MAP+O: 20%CO_2/80%N_2 and essential oil; VP: Vacuum packaging; VP+O: Vacuum packaging and essential oil.
A, means with different capital letters indicate differences ($p<0.01$) among the packaging treatments.
a, means with different lower-case letters show significant differences ($p<0.05$) the storage times.
* log cfu/g.
** S.D. (n=6.)
ß storage time (d)

storage. TVC value of MAP with bay oil sample was 6.87 log cfu/g and it is very close to maximum permissible limit of 7 log cfu/g TVC for good quality fresh poultry meat but other samples exceeded this limit on the 7th day. It can be concluded that 20% carbondioxide and low oxygen concentrations in MAP packages with bay oil can decrease the microbial counts. In parallel with our findings, Chouliara *et al.* (2007) found that MAP (70 CO_2+30 N_2) and oregano oil of 1% had inhibitory effects on TVC in fresh chicken meats. Scandamis and Nychas (2001) reported a decrease in TVC in minced beef meat with oregano oil at 1% addition.

The effects of modified atmosphere packaging with/without bay oil in terms of the survival rate of *L. monocytogenes* in samples are presented in Table 2. Significant differences between treatments of MAP and MAP with bay oil were found to be significant ($p<0.05$). During the first 3 days of storage at 4°C, the behavior of *L. monocytogenes* was different for every package. Populations on day 7 significantly increased in all samples except MAP and MAP with bay oil samples, which displayed a logarithmic reduction of 0.47 and 0.83, respectively. But *Listeria* count increased about 0.57 log cfu/ g in vacuum packaged with bay oil samples. The effect of bay oil in air packaged samples was not enough to inactivate *L. monocytogenes* and counts was 6.82 log cfu/g in air packaged with bay oil samples at the end of the 7 days. Since *L. monocytogenes* is a psychrotroph and a facultative anaerobe microorganism, it can be concluded that the atmospheric composition had some inhibitory effect on it and the lower counts can be obtained with high carbondioxide concentrations.

The observations above are in agreement with the results reported by other authors. Rutherford *et al.* (2007) reported that CO_2 packaging was the most effective one at controlling growth of *L. monocytogenes* while vacuum packaging was the second most effective and air packaging was the least effective. Moreover, they found that under these conditions *L. monocytogenes* could not be controlled in RTE shrimps stored at the temperatures higher than 3.3°C and additional hurdles would be needed. Sheridan *et al.* (1995) reported that *Listeria* spp. did not grow in vacuum packs of lamb meat at 5°C. Duffy *et al.* (2000) reported that the packaging atmosphere had a significant effect on growth rate of *Listeria* and it couldn't grow in vacuum packs, even at the higher temperature (10°C). Nevertheless, Gonzales-Fandos *et al.* (2001) reported that the growth and survival of *L. monocytogenes* was not affected with modified atmosphere packaging and additional hurdle systems would be required for this purpose. Mejholm *et al.* (2008) didn't found any evident effect on *L. monocytogenes* in MAP packages (40%CO_2+60%N_2) of brined shrimps.

Bay oil was found to be an effective inhibitory oil in this study and similarly, Smith-Palmer *et al.* (1998) determined the bacteriocidal effects of MIC (Minimum Inhibitory Concentration) values for *L. monocytogenes* and *E. coli* with bay oil of 0.04 and 0.1% (v/v) respectively and they found the bay oil is the most effective oil among the oils studied by them against the tested bacteria.

With respect to *E. coli* -considered as a hygiene indicator (Edwards and Fung, 2006), the initial counts were 1.78 log cfu/g and this count indicate good quality of chicken meat. *E. coli* grew at a slower rate in MAP, MAP with bay oil and VP with bay oil samples than aerobic and vacuum packaged samples, significantly ($p<0.01$). This result is in aggrement with the results of Holley and Patel (2005) who reported that essential oils had a strong reduction effects on *E. coli* counts. On the 7th day, *E. coli* counts of MAP with bay oil samples are less than the one of aerobic packages by 1.59 log cfu/ g. Hammer *et al.* (1999) reported that bay oil inhibited all the

tested microorganisms and they found MIC values for *E. coli* is 0.12% (v/v). Maccioni *et al.* (2002) determined that *Laurus nobilis* essential oil have a good inhibition activity to *E. coli*.

Reductions in the pH values of the chicken meat samples were recorded during the 7 days of storage, which could be attributed to the production of lactic acid through the lactic acid bacteria growth. The addition of bay oil resulted in a slight increase in pH values according to inhibition effect of oil on the growth of lactic acid bacteria groups (Gonzales-Montalvo *et al.*, 2007). Correlation of the pH values to the development of lactic acid bacteria was also reported by Whitley *et al.* (2000). The pH levels (5.76−6.08) of samples were within the growth range of *L. monocytogenes* and did not affect its growth during storage period.

Bay essential oil in combination with MAP was the most effective treatment for the inhibition of *L. monocytogenes* and *E. coli* in ground chicken meat was followed by MAP. Also long shelf-life can be obtained with the decrease of TVC in chicken ground meats.

Bay leaves are used very often in chicken meals to give tasty aroma in Turkey. The sensory analysis couldn't be designed because of the inoculated pathogen microorganism in this research.

It should be noted that the inoculation level used in these study was too much higher (6.21 log cfu/g) than that expected level to contaminate ground chicken meat by natural ways. It can be concluded that the use of "bay" essential oil with MAP is an innovative and useful tool as controlling *L. monocytogenes* and *E. coli* and also extending shelf-life of naturally contaminated ground chicken meat. Active packaging is an emerging and exciting concept in food technology conferring many benefits which fulfills consumer demand for safe products as a means of preservation.

A limitation of the current study is the application of one strain of *L. monocytogenes* so that further testing against additional strains must be performed in future studies. Although microbial safety of chicken ground meat was proved in this study, some sensory analysis will be required about the use of the bay oil with the MAP technique in the chicken ground meats by evaluation of organoleptic properties in the future.

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