Combined Effect of Lactic Acid, Bioactive Components and Modified Atmosphere Packaging on the Quality of Minced Meat

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Abstract

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The quality of minced beef was improved. Combinations of modified atmosphere packaging (MAP) and vacuum packaging (VP) with lactic acid (LA), dihydroquercetin (DHQ) and linalool (LIN) mixes were used on fresh minced beef. In the course of the study the total aerobic bacterial, *Escherichia coli,* yeast and mould counts and formation of biogenic amines during 9 days of storage at +4°C temperature were investigated. Mixtures of DHQ+LA+LIN and DHQ+LA solutions statistically significantly reduced the total aerobic bacterial and *E. coli* count and meat pH ($P \leq 0.05$) in comparison with control samples. We have identified a strong positive correlation between pH and total aerobic bacterial ($R = 0.736$, $P < 0.01$) and *E. coli* count ($R = 0.818$, $P < 0.01$). Significant differences in the total biogenic amine content were determined between DHQ+LA+LIN and DHQ+LA treated samples in air packaging (AP) ($P \leq 0.05$). However, there were no significant differences ($P > 0.05$) in the total aerobic bacterial and *E. coli* count between the different packaging techniques, but VP effectively inhibited yeasts and moulds in minced beef ($P \leq 0.05$).

Keywords: beef; dihydroquercetin; linalool; biogenic amines

Many synthetic additives have been used over the years to enhance the quality of minced meat and to extend the period of refrigerated storage. But synthetic additives have been accused of some carcinogenic and toxic properties. This increased the consumer concerns in healthier and higher quality meat products and the demand for natural food additives over the years, which led researchers to examine natural alternatives to synthetic food additives (Mariutti *et al.* 2011; Dinesh 2013).

The combined use of bioflavonoids as natural antioxidants with lactic acid and linalool for treatment of modified atmosphere packaged fresh meat has not been discussed in the available literature. Dihydroquercetin (known also as taxifolin) is a member of the group of flavonons (Vladimirov *et al.* 2009). Satisfactorily pure taxifolin may be extracted from Siberian larch (*Larix sibirica* Ledeb)*.* Taxifolin has a positive

effect on human health, as it prevents accumulation of free radicals (Teselkin *et al.* 2000; Trouillas *et al.* 2004), influences the physical properties of lipids in biological membranes (Theriault *et al.* 2000), ameliorates the cerebral ischemia-reperfusion injury (Wang *et al.* 2006) and activates the formation of collagen fibres (Tarahovsky *et al.* 2007). Application of taxifolin is quite widely distributed in the production of different categories of products. In general, taxifolin can be used as a natural antioxidant and additive with antimicrobial activities in the food industry (Wang *et al.* 2011).

Weak organic acids are among several primary agents used to control microorganisms in both fermented and non-fermented foods (Buchanan *et al.* 2002). For example, lactic acid, citric acid and acetic acid are either naturally produced or added to food or marinades to achieve food safety and meet

quality requirements (Lambert & Stratford 1999; Mani-López *et al.* 2012). Lactic acid has shown antimicrobial activities against many pathogenic organisms because of its abilities to reduce the pH level, exert feedback inhibition and interfere with proton transfer across cell membranes (Davidson *et al.* 2005).

One of the traditional ways of controlling microbial growth in these products, thus improving safety and delaying spoilage, is the application of essential oils (EOs) (Dinesh 2013). Antimicrobial activity of linalool has been reported to possess both fungistatic and antibacterial properties against a wide spectrum of microorganisms such as *Staphylococcus aureus*, *Listeria innocua*, and *Escherichia coli* (Suppakul *et al.* 2003). EOs may be applied as part of a hurdle system to achieve preservative action (Mastromatteo *et al.* 2009). A series of preservative hurdles is established by combined processes (Zhou *et al.* 2010), which in turn improves the microbial stability and the sensory quality of meat and meat products. But the use of EOs as preservatives in food has been limited as they are required in high concentrations in order to achieve the sufficient antimicrobial activity (Celikel & Kavas 2008; Hyldgaard *et al.* 2012). Lower concentrations of EOs can be combined with other antimicrobial compounds and/or other preservative technologies to obtain a synergistic effect without compromising antimicrobial activities (Nguefack *et al.* 2012).

The objective of the study was to enhance the shelf-life of beef using a combination of MAP and vacuum packaging with lactic acid, dihydroquercetin and linalool mixes on microorganisms found in fresh minced beef with regard to acceptability and formation of biogenic amines.

MATERIAL AND METHODS

Meat samples. Meat was purchased from a local establishment in Kedainiai, Lithuania. Samples used for analysis were taken from *pectoralis major* and *minor* muscles of beef carcasses from older than 2-year cattle 48 h after the slaughter and they exceeded the requirements of Commission Regulation (EC) No 1441/2007 for *E. coli* (500 CFU/g) and aerobic colony count (5×10^6 CFU/g). The meat was trimmed of all exterior fat and connective tissue. The samples were transported to a laboratory at 4°C and minced with a sterilized meat mincer in 3 mm size.

Minced meat samples were divided into 9 groups (9 × 0.5 kg) considering different treatments with lactic acid and bioactive substances. The samples were named as follows: (*i*) DHQ+LA+LN, (*ii*) DHQ+LA, (*iii*) untreated control group.

Preparation of bioactive component solutions for analyses of antibacterial properties. Powder concentrate of dihydroquercetin (99.4%), extracted from Siberian larch (*Larix sibirica* Ledeb) and produced by the company Flavit Ltd. (Pushtino, Russia), was used. Dihydroquercetin was diluted into 35°C distilled water to make 10 ml of 0.024% (w/v) dihydroquercetin aqueous solution.

Linalool (97.0% – LN) and lactic acid (50.0% – LA) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and kept at 4°C. All of the solutions (10 ml of each of them) were made on the day of research: 0.003% (w/v) linalool aqueous solution and 0.5% (w/v) lactic acid aqueous solution.

Packaging parameters. The minced meat samples were weighed and packed using three different packaging methods: VP, MAP, and AP. VP and MAP were performed using a Multivac R230 model 542 packaging machine (Multivac, Wolfertschwenden, Germany). The vacuum bag (Clingvac 90; Curevac AB, Göteborg, Sweden) was 90 μm thick with transmission rates (cm^3/m^2, 24 h, 23°C): O_2 40, CO_2 150. In MAP the gas composition 80% O_2 and 20% CO_2 was used. Control samples (AP) were packaged under atmospheric air without giving any gas composition. The samples were stored in the dark under refrigeration conditions +4°C) for 9 days. Analyses of microorganisms and pH were carried out on the 1st, 3rd, 5th, 7th, and 9th day of storage. Samples for compositional analysis (protein, moisture, fat, and collagen) were obtained after 24 h after the treatment. Detection of biogenic amines was carried out on the 1st, 3rd, 5th, and 7th day of storage. The whole experiment was replicated three times.

Microbiological analysis. Samples of 10 g were taken at random for each sample and aseptically weighed into a sterile stomacher bag with 90 ml of sterile buffered peptone water 0.1% (w/v) (Ref. 611014; Liofilchem, Roseto degli Abruzzi, Italy) and homogenised for 1 min in a model 400 Stomacher (Seward Medical, London, UK). Serial decimal dilutions were done and total aerobic bacterial counts were enumerated by plating on Plate Count Agar (Ref. 610040; Liofilchem, Roseto degli Abruzzi Italy) at 30°C for 72 hours; *Escherichia coli* were enumerated by plating on Tryptone Bile X-Glucuronide Medium Agar

(Ref. 4021562; Biolife, Milano, Italy) at 37°C for 24 h; yeast and mould were enumerated by plating on Sabouraud CAF Agar (Ref. 610203, Liofilchem) at 30°C for 48 hours.

Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

Compositional analysis. The analysis was conducted using an AOAC-approved (Official Method 2007.04) near-infrared spectrophotometer (FOSS Food Scan™ 78800; Dedicated Analytical Solutions, Hillerod, Denmark). Compositional values are reported on a percent basis.

Detection of biogenic amines. A reversed-phase high-performance liquid chromatography (RP-HPLC) method was used for the quantitative analysis of the biogenic amines – tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine. Biogenic amines were extracted from a homogenised sample with 0.4 mol/l perchloric acid. The derivatisation of samples was carried out using the modified methodology of Ben-Gigirey *et al.* (2000). The extract was derivatised for 45 min by dansyl chloride (5-dimethylamino-naphthalene-1-sulfonylchloride) solution in acetone at 40°C. The samples were filtered through 0.45 μm membrane filter (Millipore Co., Bedford, USA), 10 μl was injected into a chromatographic system (Aligent 1200 Series, Waldbronn, Germany). The analysis was performed using LiChro column CART® 95 125-4.

Carrier phase – eluents: B – acetonitrile, A – ammonium acetate 0.1 mol/l. The analysis lasted 28 min changing the content of eluents during the first 19 min from 50% of B to 90% of B (from 50% of A to 10% of A, respectively), then leaving the content constant for 1 min – 90% of B. Later, to ensure the isolation of materials for another analysis, an eluent with the composition of 50% of B and 50% of A was added to the chamber for 8 minutes. The flow rate of 0.9 ml/min did not change during the analysis, column temperature 40°C. UV detection was observed at 254 nm. Biogenic amines were identified by comparing the retention time of each amine in the chamber with the retention time of the respective reference material. An internal standard method of calculating the peak area for the defined amount of reference material was used to perform the quantitative analysis. The limit of detection is between 0.02 and 0.1 μg/ml for different biogenic amines.

pH measurement. pH was measured on the surface of all samples according to the standard method for determination of meat pH (LST ISO 2917:2002). The average pH of the sample was determined. pH measurements were carried out using Professional pH Meter PP-15 (Sartorius GmbH, Göttingen, Germany).

Acceptability evaluation. A four-member trained panel was used to evaluate the acceptability of all samples. Before evaluation, samples were wrapped in aluminium foil individually and cooked in a steam

Table 1. Chemical composition of minced beef after 24 h of storage at +4°C

Samples	Fat (%)	Protein (%)	Moisture (%)	Collagen (%)	Non-collagenous protein content (%)	Sing.
DHQ+LA+LIN/V	25.55 ± 0.35	16.83 ± 0.38	56.65 ± 0.46	4.21 ± 0.34	12.62 ± 0.49	–
DHQ+LA+LIN/M	25.51 ± 0.47	16.84 ± 0.27	56.57 ± 0.58	4.21 ± 0.17	12.63 ± 0.34	–
DHQ+LA+LIN/A	25.58 ± 0.14	16.88 ± 0.40	56.55 ± 0.43	4.22 ± 0.38	12.66 ± 0.39	–
Sing.	–	–	–	–	–	–
DHQ+LA/V	25.49 ± 0.38	16.79 ± 0.41	56.72 ± 0.41	4.20 ± 0.36	12.59 ± 0.52	–
DHQ+LA/M	25.46 ± 0.31	16.81 ± 0.37	56.73 ± 0.29	4.20 ± 0.28	12.61 ± 0.43	–
DHQ+LA/A	25.45 ± 0.24	16.80 ± 0.28	56.67 ± 0.37	4.20 ± 0.24	12.60 ± 0.29	–
Sing.	–	–	–	–	–	–
C/V	25.25 ± 0.32	16.30 ± 0.33	57.40 ± 0.26	4.08 ± 0.34	12.22 ± 0.48	–
C/M	25.29 ± 0.37	16.37 ± 0.38	57.31 ± 0.33	4.10 ± 0.37	12.27 ± 0.29	–
C/A	25.34 ± 0.23	16.35 ± 0.30	57.34 ± 0.21	4.09 ± 0.31	12.26 ± 0.34	–
Sing.	–	–	–	–	–	–

DHQ – dihydroquercetin; LA – lactic acid; LIN – linalool; V – vacuum packaging; M – modified atmosphere packaging; A – aerobically packaging; C – untreated control group; Sign. – not significant.

cooker (MultiGourmet FS20; Braun, Kronberg, Germany) for 30 minutes. Each sample was served warm in dishes coded with 3-digit random numbers and presented in individual booths to each panellist for evaluation. Panellists were asked to evaluate flavour, texture, odour, and overall acceptability based on a standard 9-point hedonic scale, where 9 = like extremely and 1 = dislike extremely. Acceptability evaluation was accomplished on the 1st, 3rd, and 5th day of storage at +4°C.

Statistical analysis. Data were statistically analysed using the SPSS 20.0 software (SPSS Inc., Chicago, USA). Differences between dates were evaluated by the analysis of variance method (one-way ANOVA) with a significance level of $P \leq 0.05$ (Draper & Smith 1998). Multiple comparisons were estimated by Fisher's Least Significant Difference method and Dunnett's test was applied when the control group was present. Student's *t*-test was used to determine average values of indicators, standard deviations (SD) and linear correlations. The correlation was considered reliable when $P < 0.05$.

RESULTS AND DISCUSSION

The results obtained showed that the combination of packaging and lactic acid, dihydroquercetin and linalool mixes used on beef did not significantly modify the proximate composition of beef (Table 1).

Significant differences were observed between the pH values of control and treated samples throughout storage. However, there were no significant differences ($P > 0.05$) in pH between the different treatments and packaging (Figure 1).

Gutierrez *et al.* (2009) found that the antimicrobial activity of EOs against foodborne pathogens and spoilage bacteria was increased at acidic pH conditions (pH = 5). Previously, it was also observed that the inhibitory effect of plant extracts was greater at acidic pH values.

Results demonstrated that DHQ+LA+LIN and DHQ+LA effectively inhibited bacterial growth or reduced numbers of viable bacteria ($P \leq 0.05$). The numbers of total aerobic bacteria in control samples reached after 1 week of storage 7.23, 7.71, and 7.86 $\log_{10}$ CFU/g for VP, MAP, and AP packaging, respectively. DHQ+LA+LIN and DHQ+LA had a moderately inhibitory effect against these microorganisms. Regarding the total aerobic bacterial count (Figure 2), both DHQ+LA+LIN and DHQ+LA

evidently caused a highly significant decrease of microbial counts. However, there were no significant differences ($P > 0.05$) in the total aerobic bacterial count between the different packaging techniques.

In general, the antimicrobial activity of mixtures of lactic acid and bioactive components increased when the pH decreased. Previously, it was also observed that the inhibitory effect of plant extracts was greater at acidic pH values (Del Campo *et al.* 2000; Hsieh *et al.* 2001).

Results demonstrated that VP effectively inhibited yeasts and moulds in minced beef during 9 days of storage ($P \leq 0.05$). However, there were no significant differences ($P > 0.05$) in the total yeast and mould count between the different treatments. These results do not agree with Rubio *et al.* (2007), who found a progressive decrease in mould and yeast counts
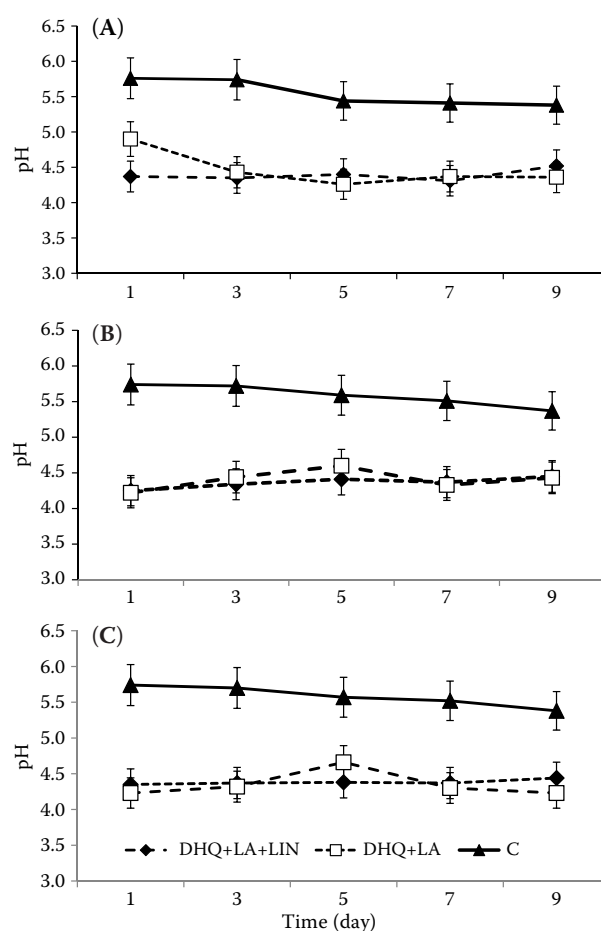

Figure 1. Variation of the pH mean values of minced beef during 9 days of storage at +4°C: (**A**) vacuum packaging (VP), (**B**) modified atmosphere packaging (MAP), and (**C**) vacuum packaging with lactic acid (LA)

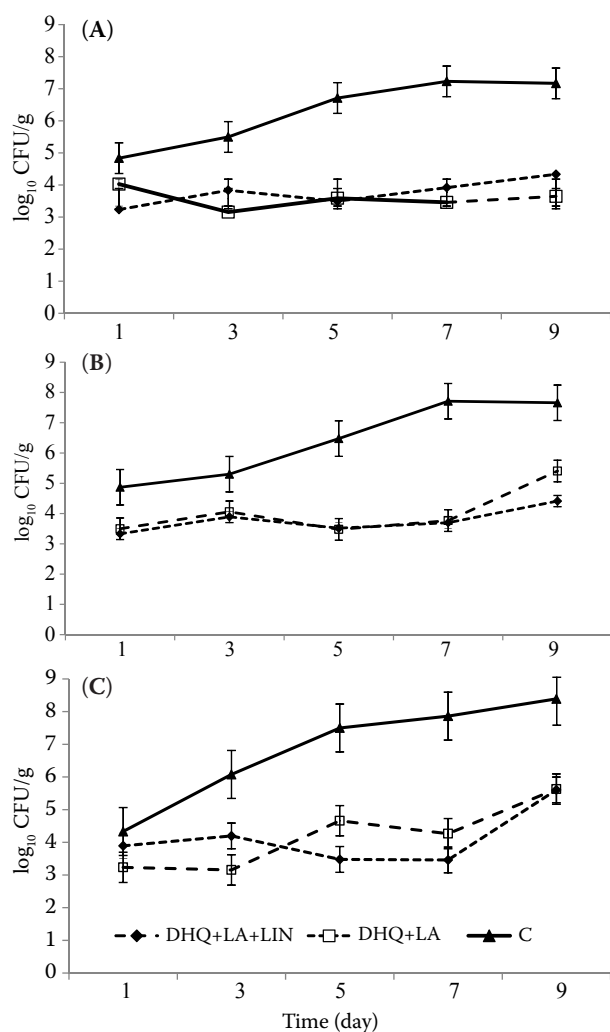DHQ – dihydroquercetin; LIN – linalool; V – vacuum packaging; C – untreated control group

Figure 2. Variation of the total aerobic bacterial count in minced beef during 9 days of storage at +4°C: (**A**) vacuum packaging (VP), (**B**) modified atmosphere packaging (MAP), and (**C**) atmosphere packaging (AP)

DHQ – dihydroquercetin; LIN – linalool; V – vacuum packaging; C – untreated control group

Figure 3. Variation of the total yeast and mould count in minced beef during 9 days of storage at +4°C: (**A**) vacuum packaging (VP), (**B**) modified atmosphere packaging (MAP), and (**C**) atmosphere packaging (AP)

DHQ – dihydroquercetin; LIN – linalool; V – vacuum packaging; C – untreated control group

during storage in *salchichón* packaged under MAP. Furthermore, the conditions within the package were not sufficient to slow down their growth.

Lactic acid, used in a mixture with LN and DHQ, was distinguished by a strong synergistic effect and statistically significantly reduced the *E. coli* count. In addition, the mixture of DHQ and LA was distinguished by a strong bactericidal activity against *E. coli.* Indeed, a reduction of 4.22 and 4.14 $\log_{10}$ CFU/g was recorded after 3 days compared with the control of storage in VP and MAP, respectively. However, the effect of DHQ+LA+LIN was much lower compared with that of DHQ+LA in VP. Regarding the *E. coli*
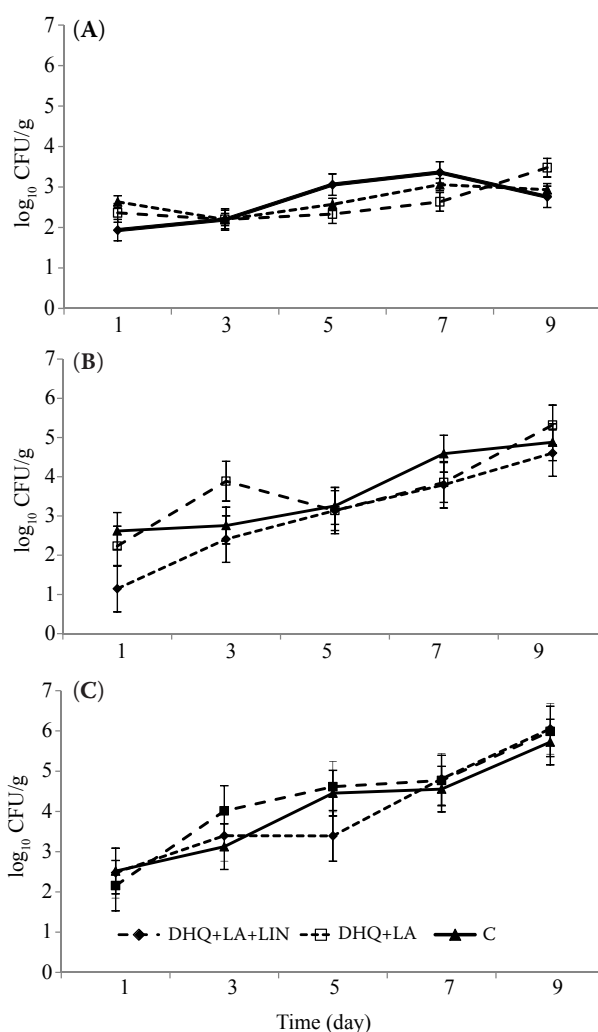
(Figure 4), both DHQ+LA+LIN and DHQ+LA caused a highly significant decrease of microbial counts most evidently. Nevertheless, there were no significant differences ($P > 0.05$) in the *E. coli* count between the different packaging techniques.

DIMITRIEVIĆ *et al.* (2007) noted that the antilisterial effect of essential oils (*Thymus vulgaris* and *Rosmarinus officinalis*) was noticeably increased using it with lactic acid. The same synergistic effect was reported by NAVEENA *et al.* (2006), who found that the combination of *Syzygium aromaticum* essential oil and lactic acid provided a decrease of psychrotrophic and coliform counts in buffalo meat.
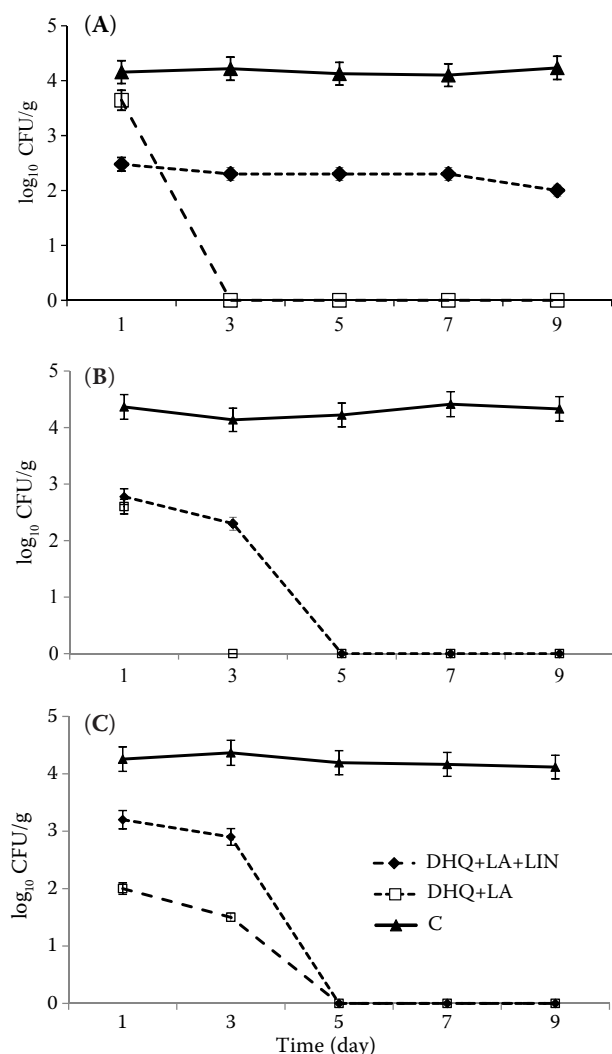
Figure 4. Variation of the *E. coli* count in minced beef during 9 days of storage at +4°C:(**A**) vacuum packaging (VP), (**B**) modified atmosphere packaging (MAP), and (**C**) atmosphere packaging (AP)

DHQ – dihydroquercetin; LIN – linalool; V – vacuum packaging; C – untreated control group

Figure 5. Variation of the total amount of biogenic amines in minced beef during 7 days of storage at +4°C: (**A**) vacuum packaging (VP), (**B**) modified atmosphere packaging (MAP), and (**C**) atmosphere packaging (AP)

DHQ – dihydroquercetin; LIN – linalool; V – vacuum packaging; C – untreated control group

According to Dimitrievič *et al.* (2007) it is difficult to understand the mechanism of the enhancing antimicrobial effect from the combined application of essential oils and organic acids. Studies on the antibacterial mechanism of phenolic compounds found in essential oils focused on their effects on the cell membrane, changing its structure and permeability. Lin *et al.* (2004) stated that damage to the cell membrane might explain the observed effects, since phenolics could cause a sublethal injury to cell membranes, causing disruption of the proton motive force due to a loss of H^+-ATPase. This could make bacteria more susceptible to an acidic environment.
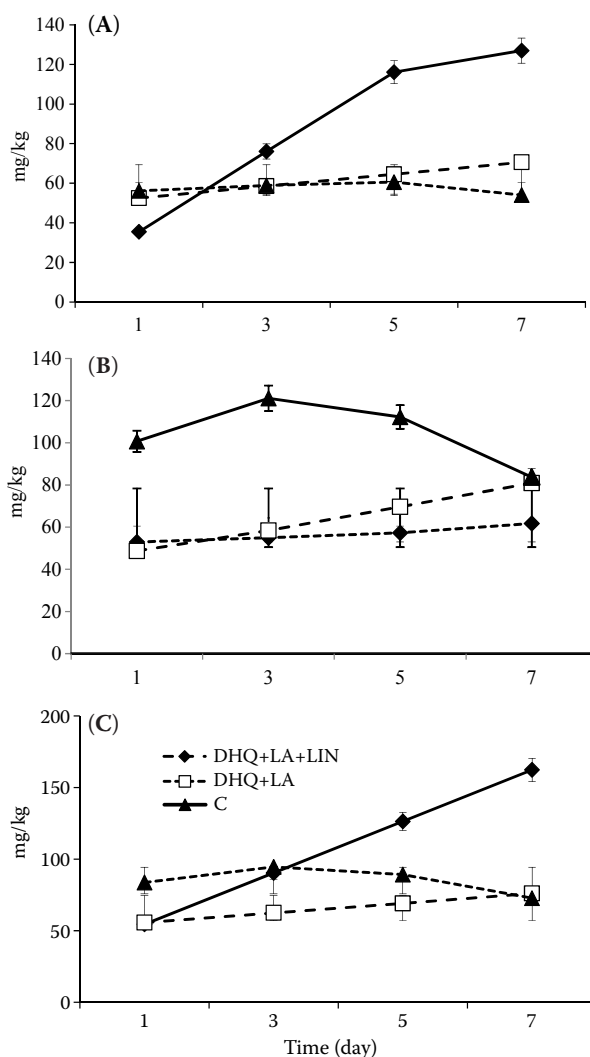
Moreover, at low pH the hydrophobicity of an essential oil increases, enabling it to more easily dissolve in the lipids of the cell membrane of target bacteria.

The strong positive correlation between pH and total aerobic bacterial count ($R = 0.736$, $P < 0.01$) and *E. coli* count ($R = 0.818$, $P < 0.01$) was observed during a 9-day period. We find a weak positive correlation between biogenic amine contents and total yeast and mould count ($R = 0.389$, $P < 0.01$). However, correlations between biogenic amine contents and total aerobic bacterial and *E. coli* counts were not observed. The capability to form biogenic amines is generally considered a strain specific characteristic

rather than a species property. It is thus difficult to find precise correlations between biogenic amine contents and total bacterial count (Halász *et al.* 1994; Suzzi & Gardini 2003; Standarová *et al.* 2008). Amine production has been recognised as a defence mechanism of microorganisms against an acidic environment (Suzzi & Gardini 2003; Karovičová & Kohajdová 2005). Tkachenko *et al.* (2001) suggested an interesting hypothesis on the physiological role of biogenic amines in microorganisms. Some strains, with amino acid decarboxylase activity, could overcome or reduce the effects of temperature, NaCl, and other biological and chemicophysical factors that induce stress responses in the cells, with the production of some biogenic amines (Karovičová & Kohajdová 2005; Galgano *et al.* 2009). We find a weak positive correlation between biogenic amine contents and pH ($R = 0.276$, $P < 0.01$). Hu *et al.* (2007) and Mah and Hwang (2009) suggested that lactic acid has a positive effect on reducing biogenic amines in meat products.

A significant difference ($P \leq 0.05$) was detected in the total amine content between the treated samples DHQ+LA+LIN and DHQ+LA in AP, while no differences were observed between the different packaging techniques (Figure 5). After 7 days of storage DHQ+LA solutions suppressed the formation of biogenic amines: putrescine (25.67 ± 1.37 to 15.98 ± 0.61 mg/kg) and histamine (32.96 ± 0.81 to 21.47 ± 0.54 mg/kg) in AP ($P \leq 0.05$). During the analysis tyramine, spermidine, and spermine were detected only in the control sample. Small quantities of tryptamine, phenylethylamine, putrescine, cadaverine, histamine were formed in all cases.

After the analysis of boiled samples a significant difference ($P \leq 0.05$) in flavour and overall acceptability was detected between the control and treated samples, while no significant differences were detected between the different treatments. After one day of storage samples treated with DHQ+LA showed the highest score of flavour (6.28 ± 0.17) and overall acceptability (6.08 ± 0.25), followed by DHQ+LA+LN treated samples (5.94 ± 0.11 and 5.87 ± 0.20, respectively), while control samples exhibited the least acceptable scores (4.42 ± 0.31 and 4.37 ± 0.24, respectively). Furthermore, after one day of storage the odour of samples treated with DHQ+LA+LN (5.86 ± 0.14) was significantly higher ($P \leq 0.05$) compared with the control (4.33 ± 0.33). The increased storage time decreased the sensory scores of flavour, texture, odour, and overall accept-

ability in all samples and after 5 days of storage the samples were unacceptable to use.

CONCLUSION

Both mixtures of DHQ+LA+LIN and DHQ+LA solutions showed a synergistic antibacterial effect in minced beef and showed the highest score of flavour and overall acceptability. But samples treated with DHQ+LA solutions showed the strongest synergistic antibacterial effect on *E. coli* count (in all different packaging techniques) and suppressed the formation of biogenic amines like putrescine and tyramine after 7 days of storage (AP) ($P \leq 0.05$). We identified the strong positive correlation between pH and total aerobic bacterial ($R = 0.736$, $P < 0.01$) and *E. coli* count ($R = 0.818$, $P < 0.01$).

These mixtures could be used in the food industry as a natural barrier to control the growth of pathogens and natural spoilage microflora in combination with VP. VP effectively inhibited the yeast and mould count in minced beef ($P \leq 0.05$).

Our results encourage further researches, focusing on the application of mixtures of lactic acid and bioactive components in low doses to control the growth of microorganisms mostly found in selected foods, particularly meat products.

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