PAPER

EFFECT OF LACTIC ACID AND BIOACTIVE COMPONENT MIXTURES ON THE QUALITY OF MINCED PORK MEAT

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ABSTRACT

The objective of this study was to investigate the effects of mixtures of lactic acid (LA), thymol (TH), linalool (LN) and dihydroquercetin (DHQ) on the quality of minced pork meat during 7 days of storage at +4 °C temperature. DHQ+LA+LN, DHQ+LA and LA exhibited the greatest antibacterial effect on the agar well diffusion assay and resulted in the best sensory evaluation. Samples treated with DHQ+LA had a statistically significant effect on the total bacterial count and showed the best antibacterial effect on the *E. coli* count. However, the reducing effect on the total amount of biogenic amines was not significant in all cases of treatment.

Keywords: dihydroquercetin, lactic acid, linalool, minced meat

1. INTRODUCTION

The production of safe and high quality meat and meat products along with recent consumer demand for all-natural and clean-label products is challenging. A significant level of meat product spoilage takes place every year at different levels of the production chain including preparation, storage, and distribution (DINESH and JAYASENA, 2013).
Many synthetic additives have been used over the years for preserving fresh meat and meat products and to extend the period of refrigerated storage (SOMOLINOS *et al*., 2010). Synthetic additives have been accused of having some carcinogenic and toxic properties. However, untreated products and natural foods may be more susceptible to the growth of food-borne pathogens than conventional food versions (JAY *et al*., 2005). The most important food-borne pathogenic bacteria that have survived and grow in these products include *Escherichia coli, Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus* spp. (OROOJALIAN *et al*., 2010). These bacteria cause a great proportion of food-borne outbreaks in different foods (WARRINER and NAMVAR, 2009). In this context, natural alternatives are attracting interest as food preservatives in order to ensure the safety of food (MARIUTTI *et al*., 2011).
One of the traditional ways of controlling microbial growth in these products, thus improving safety and delaying spoilage, is the addition of essential oils (EOs) (DINESH and JAYASENA, 2013). The composition and structure, as well as the functional groups of the oils, play an important role in determining their antimicrobial activity. Usually, compounds with phenolic groups are the most effective. EOs may be applied as part of a hurdle system to achieve preservative action (MASTROMATTEO *et al*., 2009). As an example, lower levels of EOs can be combined with existing and novel preservation technologies including low temperature and acidity, modified atmosphere packaging (MAP), high hydrostatic pressure, preservatives (e.g., nitrite, nisin, etc.) and low-dose irradiation. A series of preservative hurdles is established by these combined processes, which in turn improves the microbial stability and sensory quality of meat and meat products (AL-REZA, 2010; SKANDAMIS and NYCHAS, 2001; ZHOU *et al*., 2010). Although good antimicrobial activities were observed for many EOs, some limitations have also been identified in the application of EOs in meat and meat products. The interaction of some EOs with food ingredients and structure may decrease their effectiveness (SKANDAMIS and NYCHAS, 2000). Additionally, the markedly reduced activity of EOs may result in food systems such as meat and meat products when compared to *in vitro* results. This may be attributed to the presence of fats, carbohydrates, proteins, and salts in such systems. It is difficult to maintain consistent quality because the composition of an individual EO can vary due to several factors including the time of harvesting, variety, the part of the plant used, and method of extraction (HYLDGAARD *et al*., 2012). In addition, HYLDGAARD *et al*. (2012) reported that the antimicrobial potency of EO constituents depends on pH, temperature (RATTANACHAIKUNSOPON and PHUMKHACHORN, 2010), and the level of microbial contamination (SOMOLINOS *et al*., 2010). Further, 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 (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).
Weak organic acids are one of the several primary agents used to control microorganisms in both fermented and nonfermented 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 (MANI-LÓPEZ *et al*., 2012). Lactic acid has shown antimicrobial activities against many pathogenic organisms

because of it is abilities to reduce pH level, exert feedback inhibition and interfere with proton transfer across cell membranes (DAVIDSON *et al*., 2005).
Dihydroquercetin (also known as taxifolin) is a member of a group of flavonoids (VLADIMIROV et al., 2009). Satisfactorily pure dihydroquercetin may be extracted from Siberian larch *(Larix sibirica Ledeb).* It is also found in the açaí palm, in milk thistle seeds and in small quantities in red onion. Dihydroquercetin has a positive effect on human health, as it prevents the accumulation of free radicals (TROUILLAS *et al*., 2004), influences the physical properties of lipids in biological membranes, ameliorates cerebral ischemia-reperfusion injury (WANG *et al*., 2006) and activates the formation of collagen fibres (TARAHOVSKY *et al*., 2007). The application of dihydroquercetin is quite widely distributed in the production of different categories of products. In general, dihydroquercetin can be used as a natural antioxidant and antimicrobial activities additive in the food industry (WANG *et al*., 2011).
The objective of the study was to investigate the antimicrobial effects of bioactive components (lactic acid, linalool, dihydroquercetin) used in combination on microorganisms mostly found in pork minced meat and on the sensory properties and formation of biogenic amines.

2. MATERIALS AND METHODS

2.1. Preparation of bioactive component solutions for the antibacterial properties analyses

Powdered concentrate of DHQ (99.4%), extracted from Siberian larch *(Larix sibirica Ledeb)* and produced by the company Flavit Ltd, Pushtino (Russia) was used. DHQ was diluted into 35 °C distilled water to make 10 mL of 0.024% (w/v) DHQ aqueous solution.
LA (50.0%), TH (99.5%) and LN (97.0%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and kept at 4 °C. All of the solutions (each of them 10 mL) were made on the day of the research: 0.5% (w/v) LA aqueous solution, 0.003% (w/v) TH aqueous solution and 0.003% (w/v) LN aqueous solution.

2.2. Antimicrobial assay of bioactive components

The agar well diffusion method was used to determine the antimicrobial activity of LA (0.5%) and bioactive components (LN 0.03%, TH 0.03% and DHQ 0.024%). Reference strain cultures of conditionally pathogenic *Esherichia coli* ATCC 25922 and pathogenic bacteria such as *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 13076, *Bacillus cereus* ATCC 11778, and *Listeria monocytogenes* ATCC 19111 were used in this experiment. Bacteria cultures were kept in a "Viabank" (*Medical Wire & Equipment,* JK) system at minus 72 °C. During the tests, the examined cultures were pre-cultivated on the plate count agar (PCA, *Liofilchem*, Italy) slants for 18-24 h under the optimal temperature (30-37 °C). After cultivation, the bacterial culture was washed with a sterile physiological solution and cell suspensions were prepared according to the procedure of McFarland No 0.5 (approx. 1.5×10^8 cfu/mL). One millilitre of bacterial cell suspension was added to 100 mL of the melted PCA cooled to 45 °C. The prepared mixture of bacteria cell suspension and the medium was mixed and 10 mL was poured into each of 90 mm Petri dish. Fifty microlitres of the tested bioactive components was poured into wells of 8 mm diameter made in the hardened agar. The antimicrobial activity was assessed following 24 h of incubation at 30 ºC or 37 ºC by measuring the diameter of the inhibition zone around the wells (mm). The

strains were considered as exhibiting no antimicrobial activity if clear zones around the wells were not revealed.

2.3. Meat samples

Meat of pork carcasses from 1-year-old pig, after 48 h since postmortem were purchased from a local establishment in Kedainiai, Lithuania. The meat was trimmed of all exterior fat and connective tissue. The samples were transported to the laboratory while being kept at 4 °C and minced with a sterilized meat mincer to 3 mm in size. The minced meat samples were divided into 4 groups (4x0.5 kg) considering different treatments with bioactive substances. The minced meat samples were weighed and packed using a Multivac R230 model 542 packaging machine (Multivac, Wolfertschwenden, Germany). The samples were packaged under atmospheric air without the use of any gas composition. The samples were stored in the dark under refrigeration conditions (+ 4 °C) for 7 days. The samples were named as follows: (I) DHQ (0.024%) + LA (0.5%) + LN (0.003%), (II) DHQ (0.024%) + LA (0.5%), (III) untreated control group. Analyses of microorganisms, pH and biogenic amines were carried out on the 1st, 3rd, 5th and 7th day of storage. The whole experiment was replicated three times and the results are displayed as the mean values.

2.4. Microbial analysis

2.4.1 Detection of total aerobic bacterial count

Samples of 10 g were taken at random for each sample and aseptically weighed into a sterile stomacher bag with 90 mL of sterile 0.1% (w/v) Buffered peptone water (REF 611014, Liofilchem, Italy) and homogenized for 1 min in a model 400 Stomacher (Seward Medical, London, UK). 1.0 mL was seeded onto Plate count agar (REF 610040, Liofilchem, Italy) and incubated at 30 ºC for 72 hours.

2.4.2 Detection of *Escherichia coli*

Samples of 10 g were homogenized with 90 mL of sterile Buffered peptone water 0.1% (w/v). 0.1 mL of solution was plated using the pour plate method on Tryptone Bile X-Glucuronide Medium agar (REF 4021562, Biolife, Italy) and incubated at 37 ºC for 24 hours.

2.4.3 Detection of Salmonella

Samples of 25 g were homogenized with 225 mL of Buffered peptone water 0.1% (w/v) and incubated at 37 °C for 24 h. After incubation, 1.0 mL of the pre-enrichment culture was transferred into 9.0 mL of tetrathionate broth and incubated at 42 °C for 24 h. The enrichment culture was streaked onto XLT4 (Difco) agar plates and incubated for 24 h at 37 °C. Presumptive *Salmonella* colonies were confirmed by using API 20E (bioMérieux 20100). Agglutination tests were done with *Salmonella* polyvalent O and H antisera (Mast Diagnostics, UK).

2.4.4 Detection of Listeria monocytogenes

Samples of 25 g were homogenized with 225 mL of *Listeria* enrichment broth (Merck), incubated at 30 °C for 24 h. After incubation, 0.1 mL was plated in duplicate on PALCAM

Selective Listeria Agar (Merck). The presence of *L. monocytogenes* was determined after incubation of the plates at 37 °C for 24 h. Up to five colonies were selected for serological and biochemical confirmation using a *Listeria* latex test (Oxoid) and an API assay (BioMérieux sa, Marcy I' Etoile, France), respectively.

2.4.5 Detection of *Staphylococcus aureus*

Samples of 25 g were homogenized with 225 mL of buffered peptone water and seeded onto Baird-Parker RPF agar (Biolife, Milan, Italy) and incubated aerobically at 35 °C for 24 and 48 h.
Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g).

2.5. Determination of biogenic amines

A reversed-phase high-performance liquid chromatography 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 homogenized sample with 0.4 mol/l perchloric acid. The derivatization of samples was carried out using the modificated methodology of Ben-Gigirey *et al*. (2000). The extract was derivatised for 45 min by a dansyl chloride (5-dimethylaminonaphtalene-1-sulfonylchloride) solution in acetone at 40 °C. The samples were filtered through a 0.45 μm membrane filter (Millipore Co., Bedford, MA, USA) and 10 μl was injected into a chromatographic system (Aligent 1200 Series, Germany). An analysis was performed using a LiChro column CART® 95 125-4. Carrier phase – eluents: B – acetonitrile, A – ammonium acetate 0.1mol/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 (10% 99 of A); later, to ensure the isolation of materials for another analysis, eluent with a composition of 50% of B and 50% of A was added to the chamber for 8 minutes. A flow rate of 0.9 mL/min was maintained during analysis, with the column set at 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. The internal standard method of calculating the peak area for the defined amount of reference material was used to perform quantitative analysis. The limit of detection is between 0.02-0.1 μg/mL for different biogenic amines.

2.6. pH value

The pH of all samples was measured 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 a PP-15 pH-meter (Sartorius Professional meter for pH Measurement, Germany).

2.7. Acceptability evaluation

A ten-member panel was used to evaluate sensory taste, odour, and the overall acceptability attributes of the minced pork meat treated with LA and bioactive components. Before evaluation, the treated minced pork samples were wrapped in aluminium foil individually and cooked in a steam-cooker (MultiGourmet FS20, Braun, Germany) for 30 min. Each sample was served warm in dishes coded with 3-digit random

numbers and presented in individual booths to each panellist for evaluation. The panellists were required to rinse with water before tasting each sample. A 9-point hedonic scale was used to score the sensory attributes, where 1=dislike extremely, 9=like extremely, while the limit of acceptability was 5=neither like nor dislike. The sensory evaluation was accomplished at 2 day intervals up to the end of refrigerated storage at +4 °C.

2.8. Statistical analysis of the data

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

3. RESULTS AND DISCUSSION

The antimicrobial activity of the bioactive components was determined against common food-borne pathogenic bacteria (*Escherichia coli, Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Bacillus* spp.). The evaluation of antibacterial activity was done using the agar well diffusion method. The results suggest that the bioactive components exhibited different antimicrobial activity. LA (0.5%) and it is mixtures with other bioactive components (LN 0.03%, TH 0.03% and DHQ 0.024%) had an antimicrobial effect against all tested bacteria. The resulting inhibition zone diameters ranged from 10.5±0.2 mm to 21.5±0.2 mm. A significantly higher inhibitory effect on the *E. coli* reference strain was reported with the DHQ+LA mixture (the inhibition zone diameter was 14.3±0.2 mm) ($p \leq 0.05$). On the *S. aureus* reference strain a significantly higher inhibitory effect was reported with the LA solution (the inhibition zone diameter was 15.8±0.2) ($p \leq 0.05$). The DHQ+LA+LN mixture showed a higher antimicrobial activity on the *S. typhimurium* reference strain compared to the other bioactive components (the inhibition zone diameter was 13.0±0.0 mm) ($p \geq 0.05$). The LN and TH solution did not affect all the microbes examined except *B. cereus* (the inhibition zone diameter was 9.0±0.0 mm) ($p \geq 0.05$). Moreover, the DHQ solution (0.024%) did not inhibit the growth of all the bacteria tested (Table 1).

The acceptability evaluation scores of the minced pork meat treated with bioactive components during the 3 days of refrigerated storage at +4 °C are shown in Fig. 1. The odour of the minced pork meat treated with bioactive components DHQ+LA+LN, DHQ+LA and LN, assessed by the panellists, was significantly higher ($p \leq 0.05$) compared to the control. The taste and overall acceptability of the minced pork meat treated with DHQ+LA+LN were scored significantly higher ($p \leq 0.05$) than the control. The scores of taste, odour and the overall acceptability of the minced pork meat treated with DHQ+LA+TH as compared to the control were significantly lower ($p \leq 0.05$). Therefore, further studies have been carried out with DHQ+LA+TH, TH, LN and DHQ because these bioactive components have lower acceptability scores for minced pork meat and antimicrobial activity using the agar well diffusion method.

The bioactive components that exhibited the greatest antibacterial effect in the agar well diffusion assay and had the best acceptability evaluation (DHQ+LA+LN, DHQ+LA and LA) were further tested for their microbiological and chemical attributes.

Significant differences were observed between the pH values of the control meat and all treated samples after 5 and 7 days of storage ($p \leq 0.05$). Besides, there were significant differences in pH among LA and DHQ+LA+LN, DHQ+LA treated samples after 5 days of storage ($p \leq 0.05$) (Fig. 2).

Table 1. Antimicrobial activity of bioactive components against the reference strains.

Target Microorganisms	Zone of inhibition, mm Bioactive components						
	LA	**LN**	**TH**	**DHQ**	**DHQ+LA+LN**	**DHQ+LA+TH**	**DHQ+LA**
E. coli ATCC 25922	12.5±0.1	0.0±0.0	0.0±0.0	0.0±0.0	12.5±0.0	12.8±0.2	14.3±0.2*
S. aureus ATCC 25923	15.8±0.2*	0.0±0.0	0.0±0.0	0.0±0.0	15.5±0.1	10.5±0.2	12.5±0.2
S. typhimurium ATCC 13076	12.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	13.0±0.0	12.0±0.1	12.5±0.2
L. monocytogenes ATCC 19111	20.5±0.1	0.0±0.0	0.0±0.0	0.0±0.0	20.5±0.2	19.3±0.2	21.0±0.1
B. cereus ATCC 11778	15.5±0.1	9.00±0.0	9.0±0.0	0.0±0.0	15.5±0.1	15.5±0.1	21.5±0.2

* The mean difference is significant at the 0.05 level.
LA – lactic acid 0.5%; LN – linalool 0.03%; TH – thymol 0.03%; DHQ – dihydroquercetin 0.024%

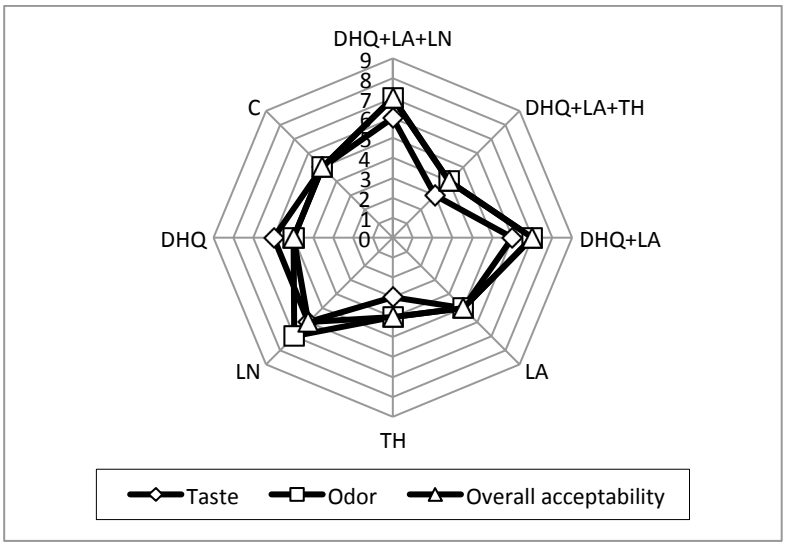

Figure 1. Acceptability evaluation of minced pork meat treated with bioactive components during 3 days of storage at +4 °C.

The results presented in this study showed the effect of DHQ and LA on the total aerobic bacterial count (Fig. 3). All combinations had effects on the total aerobic bacterial count compared to the control sample after 7 days of storage ($p \leq 0.05$).

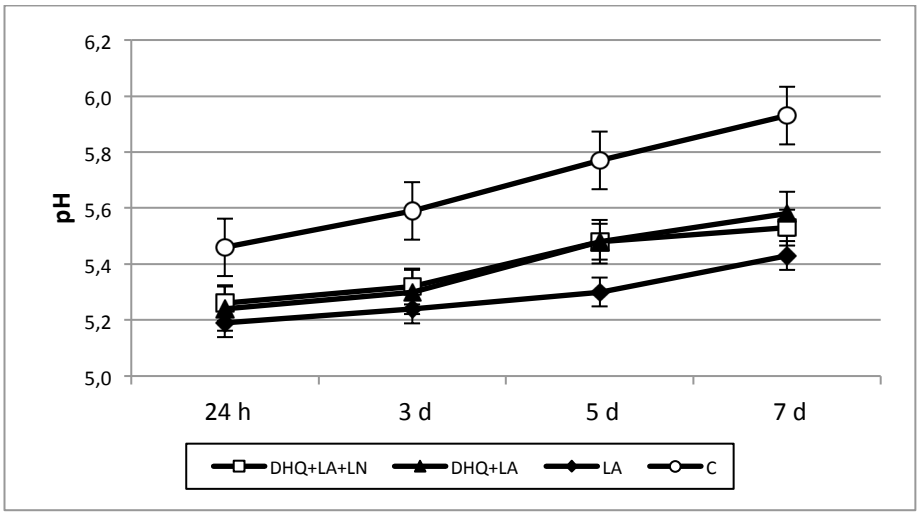

Figure 2. Variation of the mean pH values of minced pork meat during 7 days of storage at +4 °C.

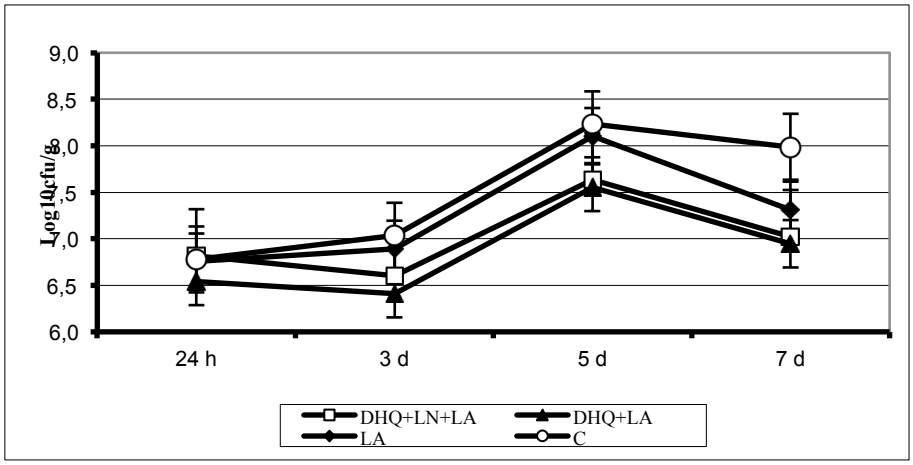

Figure 3. Variation of the total aerobic bacterial count in minced pork meat during 7 days of storage at +4 °C.

Salmonella spp., *L. monocytogenes* and *St. aureus* were not detected in any of the minced pork meat samples during the 7 days period. Therefore, we could not assess the antibacterial effect of the mixtures.

LA, used in a mixture with LN and DHQ, statistically significantly reduced the *E. coli* count. In addition, the mixture of LA and DHQ was distinguished by a strong bactericidal activity against *E. coli*. DIMITRIEVIĆ *et al.* (2007) noted that the anti-listerial effect of essential oils (*Thymus vulgaris* and *Rosmarinus officinalis*) was noticeably increased using it with LA. The same synergistic effect was reported by NAVEENA *et al*. (2006), who found that the combination of *Syzygium aromaticum* essential oil and LA provided a decrease in the psychrotrophic and coliform counts of buffalo meat.

Studies on the antibacterial mechanism of the phenolic compounds found in essential oils focused on their effects on the cellular membrane, changing it is structure and permeability. LIN *et al*. (2004) state that the damage to the cell membrane might explain the observed effects, since phenolics could cause sublethal injury to cell membranes, causing disruption of the proton motive force due to 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 membranes of target bacteria.

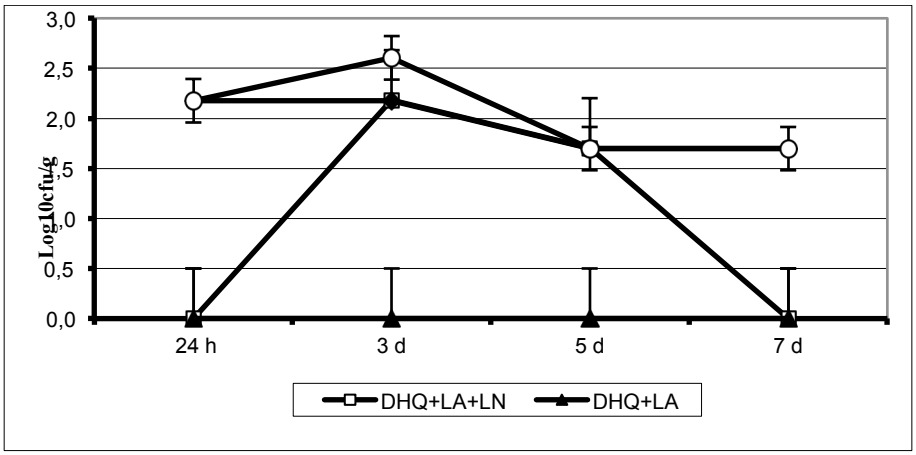

Figure 4. Variation of the *E. coli* count in minced pork meat during 7 days of storage at +4 °C.

The large correlation between the pH and total bacterial count was observed in samples treated with DHQ+LA+LN (R=0.648, p≤0.05) and DHQ+LA (R=0.692, p≤0.05) during the 7 day period (Table 2).

Table 2. Correlation coefficients R between the pH and average values of biogenic amines, *E. coli* counts and the total bacterial count during 7 days storage at +4 °C.

Samples		Correlation coefficients Parameters			
		pH	**TBC**	***E. coli***	**TBA**
DHQ+LA+LN	pH	1.00	0.648*	-0.070	-0.178
	TBC[1]	0.648*	1.00	0.078	-0.230
	E. coli	-0.070	0.078	1.00	0.149
	TBA[2]	-0.178	-0.230	0.149	1.00
DHQ+LA	pH	1.00	0.692*	-0.497*	-0.457
	TBC	0.692*	1.00	-0.474	-0.282
	E. coli	-0.497*	-0.474	1.00	0.484
	TBA	-0.457	-0.282	0.484	1.00
LA	pH	1.00	0.578	-0.033	-0.539*
	TBC	0.578	1.00	-0.539	-0.374
	E. coli	-0.033	-0.539	1.00	0.364
	TBA	-0.539*	-0.374	0.364	1.00
Control	pH	1.00	-0.348	-0.136	-0.326
	TBC	-0.348	1.00	-0.440	-0.336
	E. coli	-0.136	-0.440	1.00	-0.397
	TBA	-0.326	-0.336	-0.397	1.00

* The mean difference is significant at the 0.05 level.
1TBC - Total bacterial count; 2TBA - Total amount of biogenic amines.
LA – lactic acid 0.5%; LN – linalool 0.03%; TH – thymol 0.03%; DHQ – dihydroquercetin 0.024%

A medium negative correlation between the pH and *E. coli* count was found in samples treated with DHQ+LA (R=-0.497, p≤0.05). However, we did not find correlations between the biogenic amine contents and total bacterial count. 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 the biogenic amine contents and total bacterial count (STANDAROVÁ *et al*., 2008).
The formation of biogenic amines was intensive during the first 3 days of storage (Table 3).

Table 3. Variation of the biogenic amines (mg/kg) in minced pork meat during 7 days of storage at +4 °C.

Biogenic amines	Samples	Days of storage			
		24 h	3 d	5 d	7 d
Tryptamine	DHQ+LA+LN	14.39±0.86	13.71±2.41	6.47±0.52	3.47±0.39
	DHQ+LA	17.17±0.57	10.04±1.97	5.53±0.18	1.88±0.43
	LA	18.68±1.25	10.47±1.03	3.83±0.24	1.64±0.16
	Control	14.16±0.91	11.90±2.36	5.90±0.47	2.73±0.38
Phenylethylamine	DHQ+LA+LN	63.61±5.22	205.93±4.68	57.46±1.20	17.68±1.87
	DHQ+LA	57.85±4.72	148.36±7.42	6.23±0.73*	2.94±0.61*
	LA	78.27±2.18	159.28±8.13	38.42±1.54	16.23±1.13
	Control	113.65±4.56	168.64±5.61	37.38±0.93	16.66±1.58
Putrescine	DHQ+LA+LN	11.64±1.39	34.58±3.64	45.78±1.58	65.57±2.34
	DHQ+LA	16.52±1.94	29.43±2.18	38.54±1.12	43.18±3.50
	LA	17.60±2.40	23.09±3.05	35.67±1.39	44.61±1.92
	Control	16.52±1.28	27.89±4.24	36.85±0.91	42.10±2.48
Cadaverine	DHQ+LA+LN	23.05±3.72	40.92±3.29	51.99±4.38	77.71±4.66
	DHQ+LA	19.21±2.15	32.24±2.65	45.52±2.57	51.56±2.51
	LA	22.45±1.39	39.86±3.07	48.63±3.11	60.34±3.68
	Control	34.76±3.60	44.45±2.91	46.06±2.98	48.78±3.04
Histamine	DHQ+LA+LN	23.54±1.49	18.17±1.38	6.61±0.76	2.47±0.54
	DHQ+LA	21.88±2.08	16.49±1.60	4.18±0.35	1.82±0.21
	LA	19.84±1.67	14.33±0.92	3.07±0.64	1.26±0.35
	Control	24.32±0.68	13.01±1.77	5.65±0.51	1.87±0.72
Tyramine	DHQ+LA+LN	47.55±1.24	55.81±7.38	93.54±3.44	132.43±5.79
	DHQ+LA	52.48±0.92	67.05±5.06	87.48±2.76	115.97±4.17
	LA	34.37±2.48	41.88±8.17	84.07±2.95	145.41±6.82
	Control	38.64±1.61	49.35±6.98	66.65±1.73	108.24±3.48
Spermidine	DHQ+LA+LN	38.71±4.37	22.30±1.26	12.54±1.65	10.09±0.24
	DHQ+LA	48.96±1. 31	35.79±2.71	10.85±1.89	7.26±0.84
	LA	32.58±3.64	28.14±1.93	9.68±0.71	9.61±0.37
	Control	48.76±2.52	38.80±2.09	12.91±1.38	7.48±0.21
Spermine	DHQ+LA+LN	41.16±1.81	40.49±1.57	21.00±0.94	7.71±0.97
	DHQ+LA	54.03±3.77	48.06±1.30	18.04±2.48	5.43±0.60
	LA	51.38±2.26	42.18±2.61	17.68±1.57	5.89±2.34
	Control	48.19±3.49	48.67±1.55	18.85±1.90	3.97±0.91
Total biogenic amines	DHQ+LA+LN	263.65±6.58	431.91±5.46	295.39±4.82	317.13±5.07
	DHQ+LA	288.10±4.61	387.46±7.14	216.37±5.66	230.04±4.60
	LA	275.17±5.14	359.23±6.62	241.05±4.92	284.99±4.75
	Control	339.00±4.62	402.71±5.57	230.25±3.24	231.83±5.68

* The mean difference is significant at the 0.05 level
LA – lactic acid 0.5%; LN – linalool 0.03%; TH – thymol 0.03%; DHQ – dihydroquercetin 0.024%

The total amount of biogenic amines decreased from the 3rd to 5th days of the experiment in all cases of treatments. A significant lower amounts of phenylethylamine was found between DHQ+LA and all cases of samples between 5 and 7 days of storage ($p \leq 0.05$). After 5 days of storage, the control meat sample was fully unacceptable and the degration of amines began, with a noticeable smell of ammonia. In the cases of the other treatments, the BA increased after 5 days of storage.
Moreover, in a lower pH environment, bacteria are strongly encouraged to produce the amino acid decarboxylase as a part of their defence mechanism against acidity (KAROVIČOVÁ and KOHAJDOVÁ, 2005; TEODOROVIC *et al*., 1994). Therefore, the acidic conditions caused here by the addition of organic acids to minced meat may have not only reduced but also encouraged the production of BA. Besides this, the same raw material can lead to different amine levels depending on the presence of decarboxylating microorganisms, either derived from environmental contamination and the conditions supporting their growth and activity (STADNIK and DOLATOWSKI, 2010). The type and amount of BA detected in the minced pork meat was far below the level that can cause a health risk during the 7 days of storage.

4. CONCLUSIONS

The mixture of 0.024% DHQ and 0.5% LA solutions exhibited the greatest antibacterial effect on minced pork meat. A significant negative correlation between the pH and *E. coli* count was only found in samples treated with DHQ+LA ($R=-0.497$, $p \leq 0.05$). However, the DHQ+LA and DHQ+LA+LN mixtures could be used by the food industry as a natural barrier to control the growth of pathogens and natural spoilage microflora, while reducing the formation of biogenic amines in minced pork, thus providing a balance between sensory acceptability and antimicrobial efficacy. Our results encourage further research, focused on the application of LA and bioactive component mixtures in low doses to control the growth of the microorganisms mostly found in selected foods, particularly meat products.

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