The Effect of Rosemary and Other Natural Food Additives on the Quality of Minced Pork Meat

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Key words: *minced meat, bacteria cultures, antioxidant activity, fatty acids, peroxides.*

Summary. *The demand for high-quality safe meat products has been increasing. In the study, the effects of chosen antioxidants (rosemary, coenzyme Q10, taurine, and creatine) on minced pork meat quality were evaluated. The pork was obtained from Lithuanian producer X without any added water or spices. The study assessed the effects of selected antioxidants in combination with bacterial cultures (Staphylococcus xylosus and Pediococcus pentosaceus) on microbial, physical, chemical and sensory indicators as well. Testing was done on day 1 to day 5 of the study at a 2–4°C temperature, under aerobic conditions. Samples with mixtures of rosemary and other natural additives reduced the pH, amount of yeast and mould, and amount of biogenic amines, acids, and peroxides compared with control. The visual quality of the meat was also improved; therefore, rosemary, in addition to other natural additives, could be used in preparation of new meat products.*

Introduction

Meat and meat products occupy one of the most important places in nutrition in terms of their high-quality proteins, essential amino acids, vitamins, minerals and other nutrients (Zhang et al., 2010). Therefore, more and more consumers and scientists have recently been discussing the quality and healthiness of meat products. Producers are being urged by consumers to create more natural, safer and higher-quality meat products. Consumers want products to be made of high-quality raw materials using fewer artificial preservatives that do not cause allergies or increase the sensitivity of the body (Mariutti et al., 2011). Due to synthetic antioxidants that cause toxic effects, there is an increased demand for natural antioxidants by consumers and the meat industry (Fernandes et al., 2018). Currently, there is a high interest in natural antioxidants as many plants (and their extracts) that have antioxidant activity and at the same time health benefits are usually used as spices (Fernandes et al., 2017; Poojary et al., 2017; Putnik et al., 2017). Anti-oxidative properties of spices are particularly interesting because of their impact on oxidative stress suppression which could lead to the prevention of inflammatory, cardiovascular, neurodegenerative diseases and cancer (Srinivasan et al., 2016). Natural antioxidants in products postpone oxidative lipid degradation, improve food nutritional value, quality and replace synthetic antioxidants (Fadda et al., 2010). They also have a positive impact on health by protecting biologically important cellular structures, such as membrane lipids and proteins, DNA, from reactive oxygen attacks (Su et al., 2007). Phenolic compounds are major components of most plant extracts that determine the anti-oxidative properties (Munekata et al., 2017). They act as free radical inhibitors and as chelating agents for metal ions, e.g., iron and copper (Chan et al., 2014). Natural food additives are also useful in attempting to reduce the amount of biogenic amines (Lee et al., 2018; Wang et al., 2019), which are toxic nitrogenous compounds and are often used as indicators of food spoilage (Fiddes et al., 2014; Li et al., 2014). Biogenic amines can also be converted into nitrosamines, which are known to be carcinogens (National Toxicology Program, Department of Health and Human Services, 2016), in the presence of nitrite and nitrate. (De Mey et al., 2017). Therefore, eliminating biogenic amines as much as possible seems to be an important challenge.

Another alternative that can ensure effectiveness of the technological process and safety meat products, are bacterial cultures which usually contain lactic acid bacteria. Lactic acid bacteria are naturally found in many food products during fermentation. Studies have shown that using bacterial cultures improved meat product colour and taste properties (Bourdichon et al., 2012). In addition to being non-toxic, bacteria can be digested with proteases and thus have no effect on the gut microbiota (Zendo, 2013).

In minced pork meat production, bacterial cultures are usually not used, but in order to ensure the quality of the product during the marketing period without preservatives, the use of bacterial cultures is being looked into.

The aim of this research was to evaluate the effect of added antioxidants and bacterial culture mixtures in minced pork meat on microbiological and physical-chemical factors in order to select the most appropriate supplement.

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Materials and Methods. Pork tenderloin and notch were purchased from the Lithuanian manufacturer X without added water or spices. At the beginning of the technological process, the meat was minced using a sterile 3 mm sieve. The minced meat was divided into 0.5 kg batches and kept under aerobic conditions at +4°C temperature. Chemical substances and their selected concentration relied on a literature review of previous studies and analyses. During the study, the effect on microbiological, physicochemical and organoleptic characteristics of selected antioxidants with bacterial cultures (*S. xylosus, P. pentosaceus*) in minced pork was evaluated. The tests were carried out on day 1 to day 5, under aerobic conditions, at 2–4°C temperature.

Combinations of compounds used in study were as follows: group 1 samples – rosemary extract 0.05% + taurine 0.01% + bacterial cultures; group 2 samples – rosemary extract 0.05% + creatine 0.01% + bacterial cultures;group 3 samples – rosemary extract 0.05 % + coenzyme Q10 0.05 % + bacterial cultures; group 4 samples – rosemary extract 0.05 % + bacterial cultures; group 5 samples – coenzyme Q10 0.05% + bacterial cultures; group 6 samples – creatine 0.01 % + coenzyme Q10 0.05% + bacterial cultures; and group 7 samples – control without tested chemicals and bacterial cultures.

The microbiological and physical-chemical analysis of the samples were performed after 24, 72 and 120 h of storage.

pH measurement

The pH of the sample was measured according to the standard method for determination of meat pH: EN ISO 2917:2002 (EN ISO, 2002). pH measurements were carried out using a PP-15 pH-meter (Sartorius Professional meter for pH Measurement, Germany).

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% (*m/V*) (REF 611014, Liofilchem, Italy) and homogenized for 1 min in a model 400 Stomacher (Seward Medical, London, UK). The total number of beta-glucuronidase-positive *Escherichia coli* (*E. coli*) with a positive beta-glucuronidase reaction was determined using a pour plate technique on the tryptone, bile, and glucuronide medium, incubating for 24 h at 44 °C in accordance with LST ISO 16649-2:2002 (LST ISO, 2002). The number of colonies of yeasts and moulds were determined on a Dichloran Rose agar (DRBC; REF 17147, Sigma-Aldrich, Italy) with chloramphenicol selective supernatant, incubating for 120 h at 25 °C in accordance with LST ISO 21527-1:2008 (LST ISO, 2008). The total count of mesophilic bacteria was determined on plate count agar (PCA, Sigma-Aldrich, Merck) after incubation at 30 °C for 72 h in accordance with LST EN ISO 4833-1:2013 (LST ISO, 2013). After incubation, colonies were counted according to LST ISO 7218:2007 (LST ISO, 2007). The microbiological data were transformed into the logarithm of the number of colony forming units (CFU/g).

Biogenic amines content

A reversed-phase high-performance liquid chromatography (RP-HPLC) method was used for the quantitative analysis of biogenic amines: tryptamine, putrescine, cadaverine, histamine, tyramine, and spermine. The whole cured samples were cut into small pieces and mashed mechanically using a homogenizer (Moulinex Masterchef 20, Nieune, France). Biogenic amine content was extracted from the homogenized sample with 0.4 mol/L perchloric acid. The derivatization of samples was carried out using the modified methodology of Ben-Gigirey et al. (2000). The extract was derivatized for 45 min with 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, Waldbronn, Germany). The analysis was performed using LiChro column CART® 95 125-4 (Merck, Darmstadt, Germany).

Free radical scavenging activity (DPPH assay)

The method used by Takao et al. (1994) was adopted with suitable modifications from Kumarasamy et al. (2007). DPPH (2, 2-dephenyl-1-picrylhydrazyl) (8.0 mg) was dissolved in MeOH (100.0 mL) to obtain a concentration of 80 μg/mL. Serial dilutions were carried out with the stock solution (1mg/mL) of the samples extract. Solutions (2.0 mL each) were then mixed with DPPH (2.0 mL) and left to stand for 30 min for any reaction to occur, and the absorbance was measured at 515 nm.

Acid value

Acid value of the extracted lipids was determined according to EN ISO 660:2009 (EN ISO, 2009) procedure.

Peroxide value

Peroxide value of the studied lipids was determined according to EN ISO 3960:2010 (EN ISO, 2010) iodometric method and was presented as meqv O_2/kg lipids (Latimer GWJr).

Fatty acids content

The amount of fatty acids was determined by the method of gas chromatography using a flame ionization detector. For the analysis of fatty acids, the samples were prepared according to the standard EN ISO 12966-2:2011 (EN ISO, 2011). Fatty acids were methylated using anhydrous KOH methanol solu-

tion. Chromatographic analysis of fatty acid methyl esters was performed using gas chromatograph Shimadzu GC – 17A, using BPX – 70, 120 m column, following the methodology determined in EN ISO 15304:2003/AC:2005 2 (EN ISO, 2005). The fatty acid methyl esters (FAME) were identified by comparison of each retention time with the Supelco 37 Component FAME mix (catalog No-47885-U).

Colour determination

Meat surface colour was measured using a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan). Parameters measured in the reflection mode were L^*, a^* and b^* (corresponding to brightness, redness and yellow coordinates according to the CIE scale) (C.I.E. 1978).

Statistical Analysis of the Data

Data were analyzed using the SPSS 20.0 software (SPSS Inc., Chicago, Illinois, USA). Differences between data were evaluated by tGhe analysis of the variance method (one-way ANOVA) with a significant level of $p < 0.05$. Multiple comparisons were estimated by the Fisher's least significant difference method, and the Dunnet test was applied when the control group was present. The Student *t* test was used to determine average values of indicators, standard deviations and linear correlations. The correlation was considered reliable when $p < 0.05$.

Results and Discussion

The initial pH of minced pork was 5.35 ± 0.02. In all groups of examined samples, the pH was evenly reduced (Table 1). The results showed a statistically significant difference between control and samples with bacterial cultures after 72 and 120 h ($p < 0.05$). The lower pH values in inoculated samples could be related to the fact that the inoculation of the bacterial cultures resulted in a stronger acidification during the storage process.

The number of *E. coli*, which depends on the quality of the raw material and hygiene conditions during the process, was similar after 24 h in all tested samples: from 0.64 ± 0.25 to 1.78 ± 0.24 $\log_{10}$ CFU/g (Table 2). After 3 days, a decrease in *E. coli* was observed in all samples with bacterial cultures compared with control ($p < 0.05$). This outcome is in agreement with those reported previously by other authors (Lorenzo et al., 2014; Dominguez et al., 2016) who observed lower *E. coli* counts in inoculated samples compared with a control batch. The decrease on *E. coli* counts could be explained by the pH decrease and the growth of lactic acid bacteria (Lorenzo et al., 2014).

The total number of mesophilic aerobic bacteria found in Group 3 (5.23 ± 0.31 $\log_{10}$ CFU/g) and Group 4 (5.14 ± 0.50 $\log_{10}$ CFU/g) samples of minced pork, containing rosemary extract, showed a statistically significant reduction in the total bacterial count compared with the rest of the samples ($p < 0.05$) after 24 h. After 72 h, the total number of bacteria increased significantly in all the samples compared with the samples after 24 h, and there were no statistically significant differences compared with the control group ($p > 0.05$).

A slight increase in the number of yeasts and moulds was observed from 24 h during storage. There were no significant differences between control and samples with added antioxidants and bacterial cultures ($p > 0.05$).

Biogenic amine accumulation (expressed as mg/kg) is shown in Table 3. Generally, tyramine, cadaverine and putrescine are the main amines found in meat products (Dominguez et al., 2016).

In our study, the main biogenic amine in the samples was spermine (between 14 and 20 mg/kg), followed by cadaverine (about 11 mg/kg), spermidine (about 10 mg/kg) and tyramine (between 6 and 8 mg/kg). The total amount of biogenic amines after 24 h was statistically higher in Group 6 (98.55 ± 3.67 mg/kg) and control (101.66 ± 4.63 mg/kg) samples ($p < 0.05$). Bio-

Table 1. Effects of antioxidants and bacterial cultures on acidity (pH) during storage in minced pork

Samples	t (storage)/hours		
	After 24	After72	After120
Group 1	5.46 ± 0.09[a]	4.97 ± 0.02[a]	5.36 ± 0.07[a]
Group 2	5.51 ± 0.02[a]	5.08 ± 0.05[a]	5.30 ± 0.02[a]
Group 3	5.49 ± 0.08[a]	5.05 ± 0.09[a]	5.21 ± 0.03[a]
Group 4	5.50 ± 0.05[a]	5.09 ± 0.05[a]	5.23 ± 0.08[a]
Group 5	5.45 ± 0.06[a]	5.18 ± 0.05[a]	5.47 ± 0.06[a]
Group 6	5.42 ± 0.05[a]	5.11 ± 0.06[a]	5.35 ± 0.10[a]
Control	5.39 ± 0.02[a]	5.48 ± 0.05[b]	5.77 ± 0.06[b]

Results are expressed as mean value ± standard deviation. Different letters in superscript indicate significant differences between the samples in the same row. Group 1 samples – rosemary extract 0.05 % + taurine 0.01 % + bacterial cultures; Group 2 samples – rosemary extract 0.05 % + creatine 0.01 % + bacterial cultures; Group 3 samples – rosemary extract 0.05 % + coenzyme Q10 0.05 % + bacterial cultures; Group 4 samples – rosemary extract 0.05 % + bacterial cultures; Group 5 samples – coenzyme Q10 0.05 % + bacterial cultures; Group 6 samples – creatine 0.01 % + coenzyme Q10 0.05 % + bacterial cultures; and Group 7 samples – control without tested chemicals and bacterial cultures.
a, b – Means in the same column with different letters are significantly different, $P < 0.05$

Table 2. Effects of antioxidants and bacterial cultures on microbiological profile (log_{10} CFU/g) during storage in minced pork

Microbiological profile	Samples	t (storage)/hours		
		After 24	After 72	After 120
Escherichia coli	Group 1	1.05 ± 0.32^{a}	0.04 ± 0.01^{a}	0.00 ± 0.00^{a}
	Group 2	0.64 ± 0.25^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Group 3	0.77 ± 0.18^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Group 4	0.82 ± 0.27^{b}	00.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Group 5	1.65 ± 0.19^{b}	0.21 ± 0.02^{b}	0.00 ± 0.00^{a}
	Group 6	1.75 ± 0.11^{b}	0.14 ± 0.03^{b}	0.00 ± 0.00^{a}
	Control	1.78 ± 0.24^{b}	1.35 ± 0.29^{c}	0.66 ± 0.14^{b}
Total mesophilic aerobic bacteria	Group 1	6.32 ± 0.44^{a}	6.50 ± 0.32^{a}	7.63 ± 0.44^{a}
	Group 2	6.41 ± 0.69^{a}	6.31 ± 0.24^{a}	7.55 ± 0.30^{a}
	Group 3	5.23 ± 0.31^{b}	6.17 ± 0.37^{a}	7.10 ± 0.25^{a}
	Group 4	5.14 ± 0.50^{b}	6.10 ± 0.41^{a}	7.13 ± 0.72^{a}
	Group 5	6.54 ± 0.67^{a}	6.41 ± 0.62^{a}	7.55 ± 0.41^{a}
	Group 6	6.76 ± 0.36^{a}	6.89 ± 0.57^{a}	7.82 ± 0.40^{a}
	Control	6.78 ± 0.51^{a}	6.53 ± 0.74^{a}	8.03 ± 0.62^{a}
Sum of yeasts and moulds	Group 1	1.11 ± 0.29^{a}	2.06 ± 0.29^{a}	2.52 ± 0.29^{a}
	Group 2	1.85 ± 0.12^{a}	2.19± 0.34^{a}	2.36 ± 0.31^{a}
	Group 3	1.10 ± 0.21^{a}	1.63 ± 0.26^{a}	2.36 ± 0.31^{a}
	Group 4	1.23 ± 0.29^{a}	1.87 ± 0.31^{a}	2.06 ± 0.17^{a}
	Group 5	1.05 ± 0.21^{a}	1.94 ± 0.35^{a}	2.25 ± 0.25^{a}
	Group 6	1.95 ± 0.34^{a}	2.11 ± 0.21^{a}	2.15 ± 0.41^{a}
	Control	1.13 ± 0.29^{a}	2.21 ± 0.29^{a}	2.75 ± 0.23^{a}

Results are expressed as mean value ± standard deviation. Different letters in superscript indicate significant differences between the samples in the same row. Sample abbreviations are given in Table 1.
a, b – Means in the same column with different letters are significantly different, $p < 0.05$.

Table 3. Effects of antioxidants and bacterial cultures on total biogenic amine content (mg/kg) and biogenic amine index during storage in minced pork

Biogenic amines	Samples	t(storage)/hours		
		After 24	After 72	After 120
Total biogenic amine content	Group 1	53.82 ± 6.51^{a}	95.72 ± 3.24^{a}	187.30 ± 5.18^{ab}
	Group 2	60.05 ± 5.38^{a}	97.27 ± 4.48^{a}	176.02 ± 7.60^{a}
	Group 3	32.23 ± 3.59^{c}	69.34 ± 8.61^{b}	109.49 ± 6.31^{b}
	Group 4	20.34 ± 2.55^{c}	51.04 ± 5.79^{b}	114.81 ± 5.17^{b}
	Group 5	51.81 ± 1.38^{a}	94.85 ± 9.72^{b}	158.49 ± 8.28^{b}
	Group 6	98.55 ± 3.67^{b}	159.27 ± 4.33^{a}	180.32 ± 9.07^{a}
	Control	101.66 ± 4.63^{b}	194.57 ± 7.41^{a}	201.72 ± 7.65^{a}
Biogenic amine index	Group 1	46.62 ± 2.51^{a}	85.93 ± 6.82^{a}	163.52 ± 8.36^{a}
	Group 2	50.95 ± 4.26^{a}	80.87 ± 5.37^{a}	161.21 ± 7.22^{a}
	Group 3	27.93 ± 2.39^{b}	58.23 ± 6.05^{ab}	97.39 ± 7.31^{ab}
	Group 4	17.96 ± 3.15^{b}	47.03 ± 5.39^{a}	100.05 ± 8.25^{a}
	Group 5	45.95 ± 2.62^{a}	79.75 ± 4.38^{a}	150.47 ± 5.11^{a}
	Group 6	85.58 ± 6.33^{c}	106.35 ± 4.36^{a}	173.86 ± 9.50^{a}
	Control	86.16 ± 3.08^{c}	170.91 ± 3.25^{a}	184.61 ± 8.74^{a}

Results are expressed as mean value ± standard deviation. Different letters in superscript indicate significant difference between the samples in the same row. Sample abbreviations are given in Table 1.
a, b, c – Means in the same column with different letters are significantly different, $p < 0.05$.

genic amines increased in all samples during storage, but a significantly smaller amount was found in Group 3 sample (109.49 ± 6.31 mg/kg) and IV (114.81 ± 5.17 mg/kg) after 120 h ($p < 0.05$). Biogenic amines form from the enzymatic decarboxylation of amino acids by microbial enzymes (Li et al., 2014). Therefore, the higher the amount of spoilage microorganisms, the higher the amount of biogenic amines.

The same trend of increase can be seen in the control group when taking into account the biogenic amine index as well. The biogenic amine index (BAI) takes histamine, putrescine, cadaverine, and tyramine into account and is the sum of these four biogenic amines (Özogul and Özogul, 2019). Cadaverine and putrescine are precursors to N-nitrosopyrrolidine, a carcinogenic nitrosamine, as well as other nitrosamines (Drabik-Markiewicz et al., 2011). High levels of tyramine and histamine have many adverse effects on human health, such as high blood pressure caused by tyramine, and allergy-like reactions due to histamine (Latorre-Moratalla et al., 2017). Because of these effects to human health, it is ideal to have a low biogenic amine index. After 120 h, a statistically lower BAI, compared with the rest of the samples, was only found in samples 3 and 4 ($p < 0.05$). Taking into account both the results of the total amount of biogenic amines and the BAI, it seems that samples 3 and 4 with rosemary extract and bacterial cultures were the best at reducing biogenic amines and are a valuable additive to prevent the accumulation of high levels of toxic biogenic amines.

Research has shown that rosemary extract has high antioxidant activity and can be used in the meat industry. In the tested minced pork, the highest DPPH free radical binding was in samples with a mixture of rosemary extract and coenzyme Q10 (42.26 ± 0.03%) (Table 4). The study showed that the number of acids and the number of peroxides were effectively reduced compared with control samples. This ensures the reduction in oxidation of lipids during the production and storage of minced meat. In a sample of minced pork with rosemary extract (Group 4), a statistically significant ($p < 0.05$) low number of acids (1.77 ± 0.01 mg KOH/g) and statistically significant ($p < 0.05$) low acidity based on oleic acid (0.89 ± 0.01%) were found. Antioxidants have a positive effect on the reduction of acid and peroxides during minced pork storage.

Interest in the composition of fatty acids in meat has increased due to the need to find ways to produce healthier meat and meat products. That is to produce meat and its products with a higher content of polyunsaturated fatty acids compared with saturated fatty acids, and the adjusted ratio between Omega-6 and Omega-3 fatty acids (Wood et al., 2004). The supplementation of minced pork with antioxidants and bacterial cultures additives in the samples tested was different (Table 5).

Table 4. Effects of antioxidants and bacterial cultures on antioxidant activity during storage in minced pork

Indicators	Samples	t (storage)/hours		
		After 24	After 72	After 120
DPPH (%)	Group 1	38.51 ± 6.82 ab	24.86 ± 2.47 ab	53.52 ± 8.36 ab
	Group 2	40.15 ± 3.71 ab	26.80 ± 4.21 b	56.21 ± 7.22 ab
	Group 3	42.26 ± 4.36 a	38.11 ± 6.33 b	43.86 ± 9.50 a
	Group 4	40.21 ± 3.25 a	27.13 ± 3.06 ab	44.61 ± 8.74 a
	Group 5	32.07 ± 4.38 b	26.15 ± 2.62 a	30.47 ± 5.11 b
	Group 6	39.18 ± 6.05 ab	31.35 ± 2.31 a	37.39 ± 7.31 ab
	Control	32.03 ± 5.39 a	29.15 ± 3.16 a	37.05 ± 8.25 a
Acid value (mgKOH-kg)	Group 1	0.68 ± 0.21 a	2.52 ± 0.34a	2.81 ± 0.21a
	Group 2	1.23 ± 0.17 a	3.09 ± 0.26 a	3.20 ± 0.16 a
	Group 3	0.94 ± 0.25 a	1.96 ± 0.20 a	2.14 ± 0.17 a
	Group 4	1.11 ± 0.16 b	1.68 ± 0.31 b	2.32 ± 0.25 b
	Group 5	2.02 ± 0.32 b	2.81 ± 0.15 b	3.21 ± 0.13 b
	Group 6	1.45 ± 0.19 b	2.73 ± 0.28 b	2.92 ± 0.15 b
	Control	1.95 ± 0.22 b	2.91 ± 0.34 ab	3.22 ± 0.37 ab
Peroxide value (meqvO2-kg)	Group 1	0.73 ± 0.04 ab	0.82 ± 0.02 ab	1.52 ± 0.06 ab
	Group 2	0.84 ± 0.03 ab	0.91 ± 0.03 b	1.21 ± 0.02 ab
	Group 3	0.67 ± 0.03 a	0.69 ± 0.02 b	0.86 ± 0.05 a
	Group 4	0.51 ± 0.01 a	0.62 ± 0.01 ab	0.91 ± 0.04 a
	Group 5	0.91 ± 0.03 b	0.97 ± 0.01 a	1.47 ± 0.11 b
	Group 6	0.56 ± 0.02 ab	0.65 ± 0.02 a	1.39 ± 0.03 ab
	Control	0.73 ± 0.03 a	0.88 ± 0.03 a	1.05 ± 0.05 a

Results are expressed as mean value ± standard deviation. Different letters in superscript indicate significant differences between the samples in the same row. Sample abbreviations are given in Table 1.
a, b – Means in the same column with different letters are significantly different, $p < 0.05$.

Table 5. Effects of antioxidants and bacterial cultures on fatty acid content during storage (after 72 hours) in minced pork

	Samples						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Control
Saturated fatty acids	39.59 ± 0.03 [a]	35.59 ± 0.03 [b]	40.63 ± 0.06 [b]	43.54 ± 0.05 [a]	37.25 ± 0.08 [b]	40.00 ± 0.03 [ab]	40.09 ± 0.24 [b]
Monounsaturated fatty acids	45.63 ± 0.45 [a]	52.60 ± 0.04 [a]	48.57 ± 0.40 [a]	43.93 ± 0.03 [b]	44.21 ± 0.10 [b]	44.08 ± 0.00 [a]	42.58 ± 0.15 [ab]
Polyunsaturated fatty acids	11.30 ± 0.03 [a]	9.71 ± 0.11 [ab]	7.21 ± 0.03 [b]	11.47 ± 0.85 [a]	13.74 ± 0.05 [a]	11.99 ± 0.14 [b]	11.01 ± 0.16 [a]
Trans isomers	2.84 ± 0.06 [a]	1.24 ± 0.28 [b]	2.35 ± 0.03 [a]	2.65 ± 0.02 [a]	2.75 ± 0.18 [ab]	2.88 ± 0.16 [a]	2.90 ± 0.25 [b]
Omega-3 fatty acids	0.55 ± 0.03 [b]	0.65 ± 0.01 [b]	0.76 ± 0.52 [b]	0.43 ± 0.01 [a]	0.25 ± 0.21 [b]	0.23 ± 0.17 [b]	0.55 ± 0.23 [b]
Omega-6 fatty acids	9.91 ± 0.01 [b]	8.62 ± 0.03 [b]	6.09 ± 0.40 [a]	10.58 ± 0.08 [a]	12.71 ± 0.14 [a]	11.25 ± 0.18 [a]	9.57 ± 0.45 [a]
Ratio omega-6 / omega-3 fatty acids	17.89 ± 0.63 [a]	13.19 ± 0.02 [a]	7.98 ± 1.18 [a]	24.72 ± 0.12 [ab]	51.04 ± 0.09 [b]	49.57 ± 0.26 [b]	17.50 ± 0.38 [b]

Results are expressed as mean value ± standard deviation. Different letters in superscript indicate significant differences between the samples in the same row. Sample abbreviations are given in Table 1.
a, b – Means in the same column with different letters are significantly different, $p < 0.05$.

Most of the saturated fatty acids were found in Group 4 (43.54 ± 0.05) and the least in Group 2 (35.5 ± 0.03). The highest amount of monounsaturated fatty acids was found in sample 2 of minced pork (52.60 ± 0.04). Most polyunsaturated fatty acids were found in samples with coenzyme Q10. Fatty acid trans-isomers were detected in very small amounts in all tested samples. Omega-3 fatty acids were detected in similar amounts in minced pork from 0.23 ± 0.17 up to 0.76 ± 0.52. From this study, we can conclude that the largest ratio was in Group 2 with rosemary extract and creatine.

Color is a very important factor in the quality of meat. Consumer perception of the product is often influenced by the color of the product. Color gives meat not only an aesthetic appearance, but also is related to qualities. Minced pork redness (a^*) increased ($p < 0.05$) in all samples after day 1 (Table 6).

Muscle color refers to the amount of protein myoglobin and its form in the muscle. Due to the effect of oxygen, myoglobin (purple) turns into oximyoglobin, which gives the meat a reddish-red color. At the end of the minced pork storage, we found a statistically significant dif-

Table 6. Influence of antioxidants on colour change during storage in minced pork

Colour	Samples	t (storage)/hours		
		After 24	After 72	After 120
L *	Group 1	44.43± 2.21ab	41.73 ± 2.11ab	39.68 ± 2.14ab
	Group 2	42.80 ± 1.09b	41.93 ± 1.41a	40.03 ± 1.19b
	Group 3	45.60 ± 1.67b	42.24 ± 2.11a	32.87 ± 1.71b
	Group 4	44.84 ± 1.48ab	39.71 ± 1.38ab	36.10 ± 1.12ab
	Group 5	45.60 ± 1.67a	42.24 ± 2.11a	40.20 ± 1.89a
	Group 6	42.76 ± 1.48b	34.61 ± 1.17a	31.46 ± 1.17a
	Control	39.11 ± 2.14b	35.63 ± 1.18a	33.65 ± 1.85b
a *	Group 1	12.77 ± 1.39b	15.18 ± 1.31a	14.15 ± 0.31ab
	Group 2	10.21 ± 1.21b	11.82 ± 1.01a	10.80 ± 1.88a
	Group 3	11.08 ± 0.96b	13.80 ± 0.92a	12.25 ± 1.92b
	Group 4	12.81 ± 1.85b	15.20 ± 0.88a	13.74 ± 1.18ab
	Group 5	12.23 ± 1.45ab	13.81 ± 0.58ab	11.63 ± 1.10ab
	Group 6	10.02 ± 0.96ab	13.70 ± 0.92ab	12.45 ± 0.83ab
	Control	11.08 ± 0.96b	12.80 ± 0.92a	12.70 ± 0.92a
b *	Group 1	11.94 ± 1.68a	10.98 ± 1.52b	9.47 ± 1.49b
	Group 2	12.23 ± 1.45b	11.81 ± 0.53a	10.82 ± 0.39a
	Group 3	10.25 ± 2.11a	9.31 ± 0.32a	8.96 ± 0.67b
	Group 4	11.27 ± 1.57b	9.92 ± 1.61a	9.92 ± 1.61a
	Group 5	14.01 ± 1.32ab	10.98 ± 1.52ab	8.74 ± 0.74ab
	Group 6	12.26 ± 1.52b	11.25 ± 1.06ab	10.23 ± 1.06b
	Control	11.94 ± 1.68a	10.98 ± 1.51b	9.34 ± 1.87a

Results are expressed as mean value ± standard deviation. Different letters in superscript indicate significant differences between the samples in the same row. Sample abbreviations are given in Table 1.
a, b – Means in the same column with different letters are significantly different, $p < 0.05$.

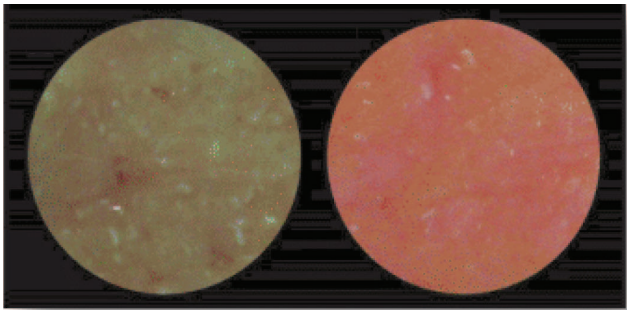

Fig. 1. The effect of rosemary extract on minced pork after 72 hours

ference between the samples with the added antioxidants and the control ($p < 0.05$). The effect of rosemary extract on minced pork (Fig. 1) was also noticeable as the extract had an effect on red color stability.

The yellow color (b^*) of minced pork decreased rapidly, but there was no statistically significant difference between control and samples with added bacterial cultures ($p > 0.05$).

Conclusion

The results of the study confirmed that rosemary extract has antioxidant activity and can be used in the development of new food products. Rosemary paired with coenzyme Q10 reduced pH the most out of the studied samples, and yeasts and moulds were best reduced in a sample with rosemary extract. The additives also reduced the amount of acids and peroxides, as well as the amount of biogenic amines. The effect of rosemary extract on minced pork color was also noticeable as it affected the pink color stability. Because minced pork gets pink, which is the preferred indicator, consumers appreciate it as the product looks more attractive.

The effect of each antioxidant is different when used in different meat matrices, e.g., the antioxidant effect of coenzyme Q10 in minced pork is significantly higher in mixtures with other antioxidants, and may not work, e.g., in marinated thighs. Therefore, not only the chosen concentration but also the complex components of the meat product influence the effect of the antioxidant on the safety and quality of the meat product. To conclude, rosemary extract, with not only strong antibacterial but also antioxidative properties, can be used as an antioxidant.

Acknowledgments

We thank the Lithuanian Science Foundation for financial support for this research.

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Received 5 April 2020
Accepted 28 April 2020