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EFFECT OF ADDING OREGANO ESSENTIAL OIL, GARLIC AND TOMATO PREPARATIONS SEPARATELY AND IN COMBINATION ON THE STABILITY OF VACUUM-PACKED MINCED PORK DURING STORAGE*

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Abstract

This paper investigates the effect of adding oregano essential oil (0.02% v/w), freeze-dried garlic (1%), tomato concentrate (15%) and a combination of all three (in the same concentrations) on the shelf life of minced pork meat. Vacuum-packed samples with additives and a control were stored at 6±1°C for 16 days. Sensory, microbiological and biochemical changes were analysed over the whole storage period. The beneficial effect of oregano essential oil was generally to inhibit lipid oxidation, although its effect on bacterial growth was very weak. Adding tomato concentrate, combined with other additives, slowed the rate of microbiological and sensory changes, but had a marked effect on changes in proteins (SDS-PAGE analysis) and the proportion of meat pigments. The addition of freeze-dried garlic did not cause a substantial reduction of detectable bacteria count. Adding a combination of all three additives resulted in a product with a distinctly longer shelf life.

Key words: minced pork, vacuum storage, oregano essential oil, garlic, tomato concentrate

Two recent concurrent trends observed in developed countries are: the pressure to extend the shelf life of food products and make them more convenient for the consumer; and a growing interest in the nutritional and health-giving qualities of food products. The former is largely due to changes in lifestyle and shopping habits (the trend towards less frequent shopping trips), as well as increasing distances between producers and consumers; the latter is connected with the desire to avoid disease and extend life expectancy as well as a rising awareness of the influence of diet on human health. In general, food products considered "healthy" are characterized by a lower level of potentially harmful substances and/or a higher level of those de-

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fined as health-promoting (Jiménez-Colmenero et al., 2001). Not only do consumers tend to be suspicious of conventional food preservatives but chemical preservatives are excluded from natural or organic products, which are gaining in popularity (Sebranek and Bacus, 2007). Therefore, taking current nutritional trends and consumer expectations into account, research is ongoing to find natural alternatives for preserving meat and also to modify the composition of meat products. Essential oils are among the substances with potential as preservatives which have been examined (Busatta et al., 2008; Govaris et al., 2010; Hayouni et al., 2008; Skandamis and Nychas, 2001; Skandamis et al., 2002). There have been studies on the use of garlic, horseradish, tomato, paprika, green tea extract, fermented mustard leaf and pine bark extracts as preservatives and additives to prevent lipid oxidation (Aguirrezábal et al., 2000; Ahn et al., 2007; Lee et al., 2010; Østerlie and Lerfall, 2005; Salejda et al., 2011; Szczawińska et al., 2008; Yin and Cheng, 2003). Meat products have also been enriched with such substances as citrus fibre or defatted melon seed kernel flour in order to modify their composition (Igyor et al., 2008; Viuda-Martos et al., 2010 a, b). Many of these products or substances, for example lycopene in tomato or allicin, ajoene and other substances in garlic, are also characterized by high biological activity. They are thought to have anticarcinogenic properties and be able to reduce the risk of cardiovascular disease (Omoni and Aluko, 2005; Qi and Wang, 2003; Rao and Agarwal, 1999). Hence, such additives may be perceived by consumers as being beneficial to health.

The aim of the study was to investigate the effect of adding oregano essential oil, garlic and tomato preparations separately and in combination on microbial stability and changes in protein and fat fractions of minced vacuum-packed pork during cold storage.

Material and methods

Sample preparation

The experimental material consisted of pork meat (shoulder) collected directly from a slaughterhouse one day after slaughtering, then minced using a MADO Primus MEW 613 meat grinder (4 mm in diameter) and finally divided into five batches. The first batch was mixed with essential oil of oregano (0.02% v/w), the second with freeze-dried garlic (1%); the third with tomato concentrate (15%); the fourth with a combination of all three additives (essential oil of oregano 0.02% v/w, freeze-dried garlic 1%, tomato concentrate 15%); and the fifth was a control sample (without additives). Additives were applied at concentrations established on the basis of earlier sensory evaluation and were the highest values accepted by a sensory panel and 22 untrained volunteers. Next, the samples, divided into 0.4 kg portions (two for each day of analysis), were vacuum-packed in high-barrier Opalen HB 65 bags using a Vac-Star 1000 packaging machine and stored at 6±1°C for 16 days without exposure to light. Analyses were conducted on the fresh raw material and then every four days during the 16-day storage period.

Freeze-dried garlic was obtained as follows: the garlic was peeled, pureed in a kitchen blender, frozen at –20°C and finally lyophilized with heating plates at 30°C. After drying, the material was ground and passed through a sieve (1×1mm).

Analysis

Commercially available tomato concentrate and natural, 100% oregano essential oil were used, the latter being subjected to GC/MS analysis.

The composition of essential oil of oregano was determined by diluting in hexane and injecting (1 µl) into a gas chromatograph (Varian 450-GC) connected to a mass spectrometer (Varian 320 MS). Separations of components were carried out on a VF-5ms column with a helium flow rate of 0.5 cm^3/min and a split mode of 1:100. The following heating programme was applied: 50°C for 1 min; increased to 250°C at a rate of 4°C/min; temperature held for 10 min. The injector temperature was set at 250°C. The range of mass analysed by the detector was 40–400 m/z at a scan speed of 0.8 sec/scan. Kováts Retention Indexes were calculated on the basis of the alkane series (C10-C40) (Van Den Dool and Kratz, 1963).

The sensory evaluation was conducted by a 7-member sensory panel, trained and proven in sensory sensitivity (Polish Standard, 1996), who assessed the odour of raw meat as well as the odour and taste of meat following thermal processing. Samples were cooked in water for 15 minutes in the form of 50 g meatballs and served in random sequence on stoneware plates at a temperature of about 45°C, each sample being evaluated three times in the laboratory. White bread and water were provided after each sample to cleanse the palate. The characteristics of the evaluated samples were compared with those on a previously elaborated table. Evaluation was performed on a 4-point scale, 5 being the best quality and 2 the poorest and unacceptable quality. The acceptability threshold was established at 3 points.

The Total Viable Count (TVC) was determined on plate count agar (PCA) incubated for 72 h at 30°C (Polish Standard, 1994).

Total anaerobic count was determined on plate count agar (PCA) incubated in containers from which air was removed and replaced by nitrogen. Colonies were counted after 48-hours' incubation at 35°C (Babji and Murthy, 2000).

Lactic Acid Bacteria were determined on de Man, Rogosa, and Sharpe (MRS) medium after 72-hours' incubation at 30°C (Polish Standard, 2002).

Brochothrix thermosphacta was enumerated on Streptomycin Thallous Acetate Actidione (STAA) Agar Base after 48 h incubation at 25°C (Hayes, 1995).

Enterobacteriaceae were determined using Violet Red Bile Glucose agar (VRBG), incubated at 37°C for 24 hours (Polish Standard, 2001). Growth media were obtained from Biocorp (Poland).

pH measurements were carried out using an HI 9025 (Hanna Instruments) pH-meter.

Fat content was determined by Soxhlet's method in accordance with the Polish Standard (2000).

Acid number, expressed as mg of free fatty acids per g of fat, was determined according to the Polish Standard (2005). The calculations took into account corrections

to the results necessitated by the fact that acid-containing plant raw materials were introduced into the samples.

The 2-thiobarbituric acid (TBA) assay was carried out according to the extraction method described by Krełowska-Kułas (1993). TBA value was expressed as mg malondialdehyde (MDA) per kg of sample.

Myoglobin was determined by the spectrophotometric differentiation method (Warriss, 1979; Krzywicki, 1982). Absorbance of clear filtered supernatant was measured at 525, 545, 565 and 572 nm on a Lambda Bio+ (Perkin Elmer) spectrophotometer. Total meat pigments and the proportion of myoglobin, oxymyoglobin and metmyoglobin were calculated according to Krzywicki (1982).

Samples of meat were homogenized with deionized water (1:20) and mixed with the denaturing buffer solution (1:1; pH 6.8: 0.125 M Tris, 4% SDS, 20% glycerol, 2% 2-mercaptoethanol, 0.03 mM bromophenol blue as tracking dye), incubated in a boiling water bath (90 seconds) and then centrifuged (2000 g, 10 minutes). Electrophoretic separation (SDS-PAGE) was carried out according to the Laemmli method (Laemmli, 1970) using a Mighty Small II SE 260 vertical unit (Hoefer, Inc.). 12.5% acrylamide was used in separating gel. Separation was run at a constant current of 40mA for approximately 1.5 h. Coomassie Brilliant Blue R-250 solution was used for gel staining. After destaining, the gel was scanned and analysed with Image Master TotalLab software (Amersham Pharmacia Biotech, Uppsala, Sweden). Molecular weights of separated pork meat proteins were estimated by interpolation from a standard molecular weight calibration curve (30-200 kDa and 14-66 kDa, i.e. SDS6H2 and SDS7, Sigma Chemical Co., St. Luis, MO, USA).

Experiments were performed in triplicate and results were expressed as mean ± SD. The significance of differences between means was determined by one-way ANOVA at a confidence level of $P<0.05$, using CSS Statistica software (Stat Soft, Tulsa, OK, USA).

Results

Chemical analysis

The composition of essential oil of oregano is given in Table 1. While carvacrol was the dominant component, gamma-terpinene, para-cymene and thymol were also detected in substantial amounts. The results of the analysis did not differ significantly from those reported in the literature (Govaris et al., 2010). The composition of tomato concentrate is given in Table 2. Total protein content was determined by Kjeldahl method, total sugar content by Lane-Eynon method and total carotenoids according to AOAC (1995).

Sensory analysis

Table 3 illustrates the results of sensory evaluation. All samples were awarded the highest score up to the 4th day; thereafter, sensory quality decreased by varying amounts. Up to the 8th day of the experiment, all samples were still acceptable (scoring at least 3 points). However, by the 12th day acceptability was limited to sam-

ples containing oregano, garlic and a combination of all three additives; the sensory quality of samples with only tomato concentrate added was, by this time, no longer acceptable even though this additive had a beneficial effect in inhibiting the growth of *Enterobacteriaceae* family bacteria, TVC and anaerobes (Figures 1, 2, and 4). By the last day of the experiment the only samples acceptable to the panel were those to which a combination of all three substances had been added. Moreover, these samples received distinctly higher scores for sensory quality than the remaining samples over the entire storage period.

Table 1. Major components of oregano essential oil

Constituent	% of total content
Beta-pinene	1.4
Alpha-terpinene	0.5
Para-cymene	3.6
Limonene	0.7
1,8-cineole	1.9
Gamma-terpinene	4.8
Linalool	2.5
Camphor	1.0
Borneol	1.5
Terpinen-4-ol	0.9
Alpha-terpineol	0.7
Thymol	4.4
Carvacrol	68.5
E-caryophyllene	3.2
Alpha-humulene	0.8

Table 2. Major components of tomato concentrate

Compound/Attribute	Value
Dry matter (%)	31.4±0.83
Total sugar (%)	15.3±0.61
Total protein (%)	4.4±0.07
Ash (%)	2.2±0.03
pH	4.2±0.00
Acidity (% as citric acid)	1.95±0.05
Volatile acidity (% as acetic acid)	0.03±0.002
Total carotenoids (mg/100 g)	45.5±2.73

Table 3. Changes in sensory evaluation of vacuum-packed minced pork during storage

Sensory parameter	Sample	Day of storage				
		0	4	8	12	16
1	2	3	4	5	6	7
Raw smell	Control	5.0±0.00 a	5.0±0.00 a	3.8±0.22 a	2.0±0.11 a	2.0±0.00 a
	Oregano	5.0±0.00 a	5.0±0.00 a	4.4±0.24 ab	3.5±0.16 b	2.3±0.24 a
	Garlic	5.0±0.00 a	5.0±0.00 a	4.5±0.24 bc	3.4±0.28 b	2.5±0.29 a
	Tomato	5.0±0.00 a	5.0±0.00 a	4.0±0.27 ac	2.8±0.29 c	2.0±0.00 a
	Mix	5.0±0.00 a	5.0±0.00 a	4.8±0.27 b	4.2±0.24 d	3.1±0.20 b

Table 3 – contd.

1	2	3	4	5	6	7
Cooked smell	Control	5.0±0.00 a	5.0±0.00 a	3.5±0.45 a	2.1±0.17a	2.0±0.00 a
	Oregano	5.0±0.00 a	5.0±0.00 a	4.5±0.23 bc	3.8±0.21 b	3.0±0.08 b
	Garlic	5.0±0.00 a	5.0±0.00 a	4.5±0.24 bc	3.7±0.29 b	2.9±0.22 b
	Tomato	5.0±0.00 a	5.0±0.00 a	4.0±0.51 ac	3.0±0.23 c	2.0±0.00 a
	Mix	5.0±0.00 a	5.0±0.00 a	4.8±0.20 b	4.1±0.15 b	3.4±0.20 b
Cooked taste	Control	5.0±0.00 a	5.0±0.00 a	3.4±0.43 a	2.1±0.13 a	2.0±0.00 a
	Oregano	5.0±0.00 a	5.0±0.00 a	4.4±0.26 b	3.8±0.25 b	2.8±0.31 b
	Garlic	5.0±0.00 a	5.0±0.00 a	4.4±0.29 b	3.8±0.30 b	2.8±0.33 b
	Tomato	5.0±0.00 a	5.0±0.00 a	4.2±0.22 b	2.5±0.05 a	2.0±0.00 a
	Mix	5.0±0.00 a	5.0±0.00 a	4.8±0.20 b	3.9±0.22 b	3.2±0.26 b

Values in a column with different letters within the same group are significantly different ($P<0.05$).

Microbiological analysis

After four days of storage, there was a sharp increase in the microorganisms examined (Figures 1–4), particularly in *Enterobacteriaceae* and lactic acid bacteria. However, the sharp growth of TVC and anaerobic microorganisms was limited mainly to the samples without tomato concentrates. The results presented in Figures 1–4 show that adding essential oil of oregano had a very slight inhibitory effect on the growth of the microorganisms examined. Adding tomato concentrate had a marked inhibitory effect on the growth of *Enterobacteriaceae*; however, the effect was much weaker in the case of the remaining microorganism groups.

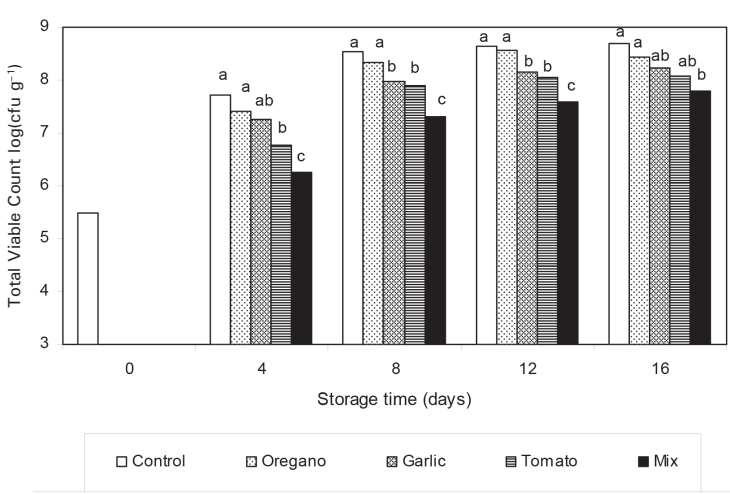

Figure 1. Changes in total viable count (TVC) in vacuum-packed minced pork during storage at 6±1°C

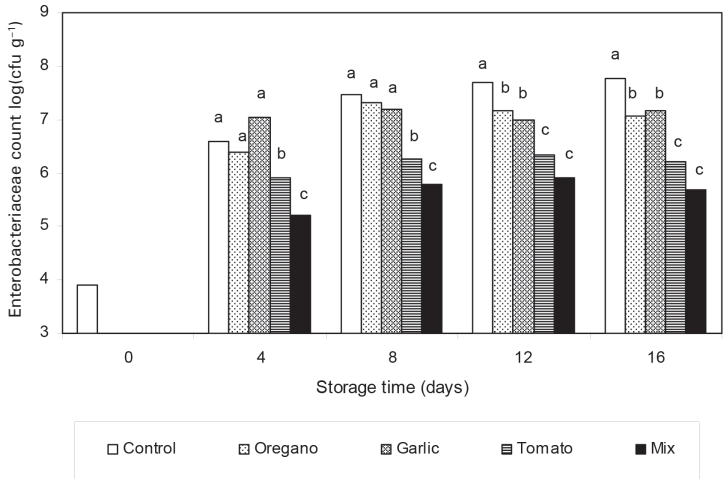

Figure 2. Changes in *Enterobacteriaceae* count in vacuum-packed pork during storage at 6±1°C

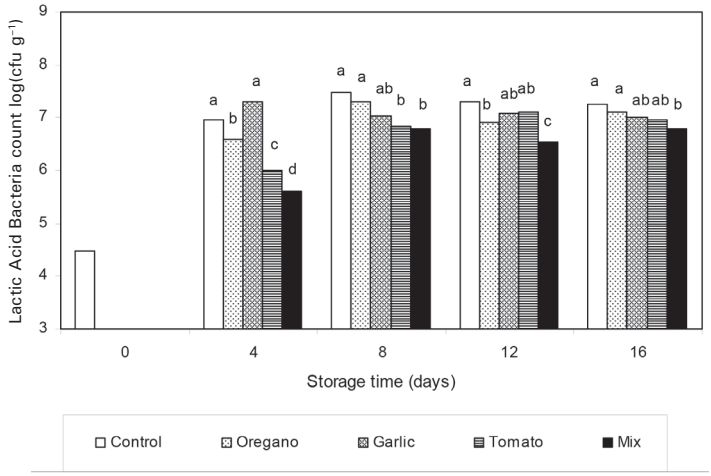

Figure 3. Changes in lactic acid bacteria (LAB) count in vacuum-packed minced pork during storage at 6±1°C

Although the additives did not have a clear beneficial effect on the shelf life of meat when applied separately, they did reduce the rate of bacterial growth in the examined groups of microorganisms when combined in a single sample. By the 16th day of storage, TVC in meat with all additives combined was 6.3×10^7, while the count for the remaining microorganism groups was still below 7.0×10^6 cfu/g on the final day of storage. *Enterobacteriaceae* levels remained constant at 10^5 cfu/g throughout the whole storage period.

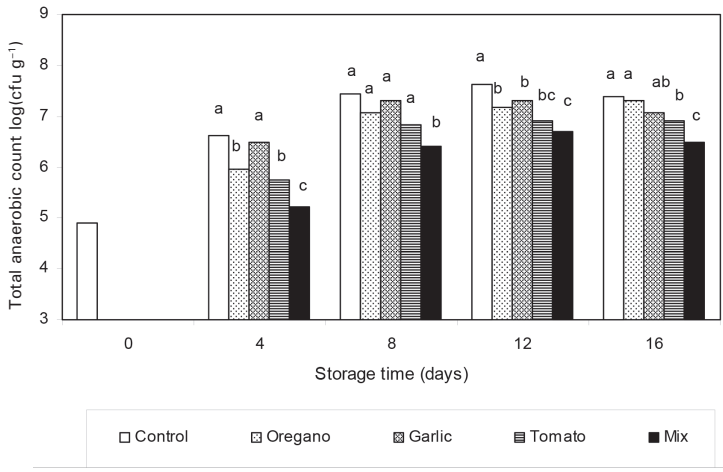

Figure 4. Changes in total anaerobic count in vacuum-packed minced pork during storage at 6±1°C

The population of *Brochothrix thermosphacta* in fresh meat was 3.0×10^4 cfu/g (data not shown). Levels of these bacteria remained virtually constant in the control sample but fell in the remaining samples. After 16 days, the count in samples with tomato concentrate and with all three additives combined was 4.2×10^3 and 8.2×10^2 cfu/g, respectively. The population in samples with garlic and oregano was approximately 1×10^4 cfu/g at the end of the storage period.

pH measurements

The addition of tomato concentrate resulted in a decline in pH value in fresh meat (Table 4). After storage, the pH of control samples and those with essential oil of oregano fell by approx. 0.5 unit, while the changes were slightly less noticeable in samples with garlic. The pH value of samples containing tomato concentrate alone continued to decline markedly up to the end of the storage period, while that of samples containing the combination of additives remained higher throughout.

Table 4. pH value of fresh and stored meat

Sample	pH	
	fresh meat	after storage
Control	5.81±0.030 a	5.27±0.010 a
Oregano	5.81±0.028 a	5.25±0.008 a
Garlic	5.78±0.005 a	5.43±0.008 b
Tomato	5.25±0.000 b	4.78±0.069 c
Mix	5.30±0.029 b	5.03±0.024 d

Values in a column with different letters within the same group are significantly different ($P<0.05$).

Changes in fat fraction

After storage, visible changes of a hydrolytic and oxidative nature were observed in the fatty fraction of meat (Table 5). The highest increase in acid number was de-

termined in samples containing tomato concentrate (both alone and mixed with the other additives). In the remaining samples there was an almost twofold increase in acid number compared with fresh meat. The addition of essential oil of oregano or lyophilised garlic had no clear effect on this value.

Table 5. Changes in fat fraction given as acid value and thiobarbituric acid value (TBARS)

Sample	Acid value (mg/g)	TBARS (mg MDA/kg)
Control 0 day	4.14±0.038 a	1.74±0.052 a
Control 16 days	7.28±0.124 b	3.28±0.018 b
Oregano 16 days	7.46±0.014 b	1.76±0.046 a
Garlic 16 days	7.51±0.051 b	2.87±0.021 bc
Tomato 16 days	10.91±0.153 c	2.49±0.038 c
Mix 16 days	10.61±0.113 c	2.17±0.035 ac

Values in a column with different letters within the same group are significantly different ($P<0.05$).

Meat pigments

The addition of tomato concentrate, whether alone or combined with other additives had a strong negative effect on the proportions of meat pigments (Figure 5). In samples containing tomato concentrate, oxymyoglobin content was lower and metmyoglobin content higher than in both control samples and samples with oregano. The addition of garlic had a similar, although somewhat weaker effect on the proportions of pigments. Essential oil of oregano, in the dose applied, had no significant effect on inhibiting oxidation of the meat pigment examined.

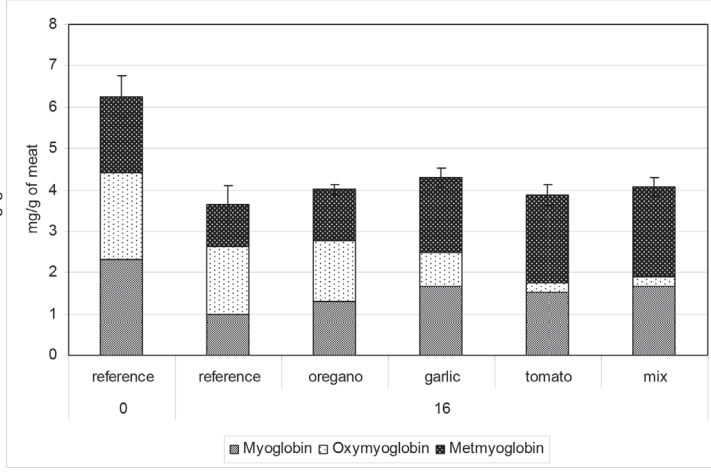

Figure 5. Meat pigments content in fresh minced pork (0) and in vacuum-packed minced pork after 16 days of storage at 6±1°C (16)

Electrophoresis

Table 6 gives the results of electrophoresis of stored minced pork. The identification of separate bands was based on sources in the literature (Barbieri and Rivaldi, 2008; Benito et al., 2003; Lametsch et al., 2004; Livisay et al., 1996).

Table 6. Effect of adding selected additives on changes in relative content (%) of protein fraction in fresh minced meat and after 16 days of vacuum-packed storage under refrigerated conditions. The most intensive bands have been presented

Molecular weight (kDa)	Protein	Fresh meat	Stored control meat	Stored meat with oregano	Stored meat with garlic	Stored meat with tomato concentrate	Stored meat with mix
255.8		4.27	5.83	7.2	6.18	3.95	5.72
223.3		2.24	0.99	1.43	1.7	0.28	0.45
191.0	MHC*	21.23	21.34	22.48	21.43	17.29	18.26
133.9		3.09	4.25	3.87	4	14.49	14.12
102.8		1.46	1.32	1.07	1.34	0.54	0.47
94.0		5.68	5.23	5.47	5.07	3.87	4.26
80.9		0.18	0.5	0.45	0.41	0.56	0.52
72.6		0.37	0.31	0.31	0.25	0.92	0.73
64.8		0.28	0.41	0.37	0.67	0.71	0.58
61.2		1.51	1.68	1.57	1.6	2.43	2.09
48.9		2.36	2.26	2.32	2.72	1.31	1.17
43.2	actin	24.82	23.28	26.58	23.71	28.69	27.58
36.3	tropomyosin	5.68	2.9	1.38	1.95	1.43	1.18
35.4	troponin T	3.16	1.44	0.69	2.22	0.69	1.26
34.8		1.45	2.36	0.15	1.75		
33.8	tropomyosin	6.76	7.49	5.91	7.35	5.57	6.09
32.2		0.49	0.39	0.44	0.42	0.3	0.2
31.2			0.16	0.16	0.14	0.1	0.12
29.9		1.02	0.92	0.99	1.01	0.35	0.36
28.8		2.96	3.37	3.73	3.48	3.69	3.56
26.3		0.44	0.28	0.51	0.51	0.59	0.49
24.3		1.35	2.64	2.51	2.46	1.87	2
22.4		0.89	1.37	1.39	1.49	1.21	0.98
20.4		0.6	0.43	0.41	0.56	0.15	0.15
20.0		1.58	1.59	1.46	1.38	1.43	1.69
19.5		2.26	1.76	2.13	1.67	0.13	0.18
17.1		0.1	0.16	0.08	0.14	0.1	0.13
15.9		0.24	0.22	0.31	0.25	0.22	0.25
14.9		0.52	0.41	0.46	0.42	0.14	0.21
14.0					0.07	0.23	0.11
13.3		1.31	3.36	2.95	2.38	5.39	3.88

*MHC – myosin heavy chain.

Protein fractions of tomato concentrate and garlic were also separated. Electrophoregrams of stored meat revealed no traces of these additives (as protein bands).

Discussion

Essential oil of oregano is known for its relatively strong antimicrobial properties (Gutierrez et al., 2009; Oussalah et al., 2006; Teixeira et al., 2013). However, in

studies confirming its efficacy on stored meat, essential oil of oregano was frequently applied at many times the concentration used in the present work (Skandamis and Nychas, 2001; Skandamis et al., 2002). One possible explanation for the very weak antimicrobial effect of this additive observed in the present study may be the fairly high initial microbial population of pork meat. Its fat content (9.24%) may have been another factor impairing the efficacy of the essential oil applied (Burt, 2004). Oxidative changes, evaluated on the basis of changes in TBA values, demonstrated the beneficial effect of adding this compound. Essential oil of oregano contained mainly carvacrol and considerably less thymol as well as small amounts of other constituents which may have antioxidant activity (Table 1). According to Yanishlieva et al. (1999), both carvacrol and thymol showed antioxidant properties in purified triacylglycerols of lard. The essential oil applied, however, had no significant effect on the pH value of samples (Table 4) as well as the proportions of muscle pigments (Figure 5).

Kumar and Berwal (1998), who noted the inhibitory effect of fresh garlic on *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Listeria monocytogenes* in broth, suggested that garlic could be used to preserve processed food and extend its shelf life. It was also observed that the addition of the garlic-derived organosulfur compound diallyl sulfide had a significant effect on reducing aerobic plate counts and retarding the growth of five pathogenic bacteria during the storage of ground beef (Yin and Cheng, 2003). Our results show that the addition of freeze-dried garlic did not cause a substantial reduction of detectable bacteria count in any of the groups investigated, although samples containing garlic showed very slightly lower levels mainly of Total Viable Count. The dose applied may have been too low to have an antimicrobial effect. It is also possible that the lyophilisation process itself impaired the effectiveness of this additive, for example, as a result of the partial loss of essential oil. In a study on the antimicrobial activity of various products obtained from garlic, Rahman et al. (2006) found that the greatest efficacy was in fresh garlic, followed by freeze-dried garlic. Ratti et al. (2007) found that during air drying and freeze drying of garlic, processing temperature is a factor in allicin loss. According to Marques et al. (2008), non-heated garlic powder investigated *in vitro* strongly inhibited the growth of *Salmonella enterica*, whereas garlic heated to 120°C did not have an inhibitory effect on the bacterium. On the other hand, after storage, the pH of samples with garlic decreased to a lesser degree than in the control sample. This demonstrates that despite having very little effect on microbiological growth, garlic could have a slight inhibitory effect on the metabolic activity of microorganisms.

Compared with the control sample, an initial small increase in *Enterobacteriaceae* and LAB populations in samples with garlic may have been due to the growth of bacteria introduced into the samples with the garlic itself (Figures 2 and 3). Levels of TVC, *Enterobacteriaceae* and LAB in lyophilised garlic were 1.6×10^3, 3.6×10^2 and 1.4×10^3 cfu/g, respectively. This concurs with the findings of Shim and Kyung (1999), who found similar levels of microorganisms in freshly peeled garlic. When analysing the natural microflora of pre-peeled garlic, they isolated *Pseudomonas*, *Enterobacter*, *Cryptococcus neoformans* and numerous lactic acid bacteria, 26 of which were extremely resistant to garlic. Moreover, compared to tomato concentrate,

changes in the proportion of muscle pigments due to this compound were slightly lower, however still negative (Figure 5). In samples containing garlic only, this may be considered a serious defect of the product obtained. However, the effect of additives on colour stability and the proportions of myoglobin forms depends on the type of meat and its storage conditions (Krala, 2001).

The inhibitory effect of tomato concentrate on bacterial growth was somewhat clearer, the decline in pH value in samples with this additive no doubt being a contributory factor (Table 4). According to Østerlie and Lerfall (2005), who analysed the effect of lycopene from tomato products on the storage quality of minced meat, this substance had no effect on the microbiological stability of meat.

It has also been found that adding tomato concentrate had a slight beneficial effect on the TBARS indicator (Table 5). As Østerlie and Lerfall (2005) reported, samples of meat farces with added lycopene or tomato paste were more stable, showing a smaller increase in peroxide value than the control sample. These authors concluded that such changes could be attributed to the antioxidative properties of lycopene and a low pH value.

In samples containing tomato concentrate, unfavourable changes in the pigment proportions in meat (Figure 5) may be of less significance for potential consumers due to the beneficial effect of tomato-derived carotenoids on colour (Østerlie and Lerfall, 2005).

Adding tomato concentrate caused also visible changes in the relative content of protein fraction (Table 6), i.a., the MW 34.8 kDA band disappeared in the samples containing this additive.

In stored samples compared with fresh meat, there was a slight fall in the proportion of individual proteins within molecular weight bands 36.3 kDa and 35.4 kDa, accompanied by a rise in the proportion of bands of molecular weight 28.8 kDa, 24.3 kDa, and 22.4 kDa as well as in the fraction of low-molecular proteins (below 14 kDa). On the other hand, bands of very low intensity (about 31.2 kDa) emerged in samples of stored meat. Similar changes, manifested by a decline of troponin T/tropomyosin fraction accompanied by an appearance and rise in the fractions of molecular weight around 30 kDa, were observed in other studies on changes in meat during storage (Lametsch et al., 2004; Livisay et al., 1996; Okumura et al., 2003).

The additives applied did have no distinct effect on meat protein fractions except in the case of tomato concentrate, for which decreasing intensities were observed in myosin heavy chain band (191 kDa), bands of molecular weight approximately 102.8 kDa, 94 kDa, and 48.9 kDa, and 19.5 kDa. On the other hand, there was an increase in the intensity of bands of molecular weight 133.9 kDa, 61.2 kDa, and 13.3 kDa (low-molecular peptides) as well as in the region of the band corresponding to actin (43.2 kDa). This may suggest the emergence of a product of proteolysis of a molecular weight very close to actin. Benito et al. (2003) also observed increased intensity of the 44 kDa band as a result of proteolytic activity during the maturation of pork loin. It is probable that the decline in the pH value of meat associated with the addition of tomato concentrate creates a favourable environment for the proteolytic activity of cathepsin.

Using a combination of all three additives resulted in acceptable sensory quality of products being extended up to the end of their storage period. These additives are widely used as traditional ingredients in meat dishes. The potential for consumer acceptance of products containing all three preservative additives is suggested by the results of a study by de Barcellos et al. (2010), which found that consumer acceptance of new beef processing technologies increased when they were perceived as traditional and familiar.

In conclusion, compared with additive-free minced pork meat, the application of a combination of all three additives resulted in a product with an extended shelf life. Applying additives separately also affected the rate of sensory and microbiological changes in stored products, but only slightly extended shelf life. The main benefit of adding essential oil of oregano was to lower the values of lipid oxidation indicator, but it had little effect on microbial growth (less than 1 log cycle compared with the control sample). Whether added alone or in combination with other additives, tomato concentrate had an inhibitory effect on *Enterobacteriaceae* growth, but also brought about changes in electrophoretic protein profiles and had an adverse effect on the proportion of meat pigments.

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