PAPER

THE EFFECT OF PLANT EXTRACTS ON PORK QUALITY DURING STORAGE

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ABSTRACT

This study investigated the effects of the natural antioxidants contained in onion, garlic and marjoram on the oxidant and microbial stability of raw minced pork during refrigerated storage. The most antioxidant active plant extract was marjoram. The highest inhibition of secondary oxidation products was observed in the samples with garlic, next was onion and finally, marjoram. GC analysis showed that the lowest rates of fatty acids degradation were recorded in the samples with garlic. The highest bacteriostatic effect was noted in meat samples with fresh onion added. These results suggest that the addition of these plants enhances the quality of the raw minced pork meat during refrigerated storage.

Keywords: antimicrobial, antioxidant, meat, extract, plant, storage

1. INTRODUCTION

Consumer concerns on the quality and safety of meat have greatly increased during the past decades. Lipid and microbial stabilities are important parameters that influence the quality and acceptance of meat, particularly minced meat. Minced meat has its cell structure disrupted, leading to increased exposure of lipids to oxygen, enzymes, heme pigments, and metal ions. Oxidative products, which are the results of lipid oxidation and/or microbiological changes in the meat, negatively affect the quality and safety of the product. For consumers this means the undesirable rancidity of meat. The heme pigment content, in conjunction with catalase activity, determines the lipid peroxidation potential in raw meat. In cooked meat, the major determinant for lipid peroxidation is the content of PUFA (MIN and AHN, 2005). The application of suitable ingredients possessing both antimicrobial and antioxidant properties can be useful for extending meat's shelf life and thus preventing economic losses. In recent years, naturally occurring compounds exhibiting antioxidant and antimicrobial properties have been preferred for use in meat products because of their potential health benefits and safety, especially when compared with synthetic derivatives, which were routinely used in processed foods. Studies associated with natural antioxidants for the inhibition of microbial spoilage and lipid oxidation have been extensively conducted (FALOWO *et al*., 2014; KARRE *et al*., 2013; SHAH *et al*., 2014). Onion (*Allium cepa* L.), garlic (*Allium sativum* L.) and marjoram (*Origanum majorana* L.) possess both antioxidant and antimicrobial activity (BUSATTA *et al*., 2008; ROBY *et al*., 2013; SALLAM *et al*., 2004; YE *et al*., 2013). Onion and garlic bulbs, as well as dried marjoram, are three major meat additives widely used in food preparation. Park and Chin (2010) used onion extracts to control the development of lipid oxidation in fresh pork patties. EL-ALIM *et al*. (1999) investigated the use of ground marjoram as an antioxidant in raw ground chicken. CAO *et al*. (2013) proved the antimicrobial and antioxidant properties of onion and garlic for extending the shelf life of stewed pork during refrigerated storage. Moreover, PARK *et al*. (2008) demonstrated the antimicrobial and antioxidant activity of garlic and onion powder in fresh pork belly and loin during storage. Fresh garlic blended with paprika was also successfully used as an antioxidant in dry sausages (AGUIRREZÁBAL *et al*., 2000). However, due to the fact that the rate and extent of lipid oxidation are influenced by a number of factors, which include iron content, distribution of unsaturated fatty acids, pH, the form of natural additives (fresh, dried or extract) and various processing technologies, further research is still needed, particularly with reference to the meat system.

The aim of the present study was to evaluate the effect of natural antioxidants contained in onion, garlic and marjoram on the oxidant and microbial stability of refrigerated minced pork blade meat during refrigerated storage.

2. MATERIALS AND METHODS

2.1. Samples preparation

Samples of dried marjoram leaves, fresh garlic and onion bulbs were purchased in a local market. All the products were produced in Poland. Three pork blades (each from different animals) were obtained from a local market (where the process of cutting and deboning is conducted locally) and transported to the laboratory under chilled conditions in iceboxes. Blade muscles containing approximately 4% intramuscular fat were used. The blades were chopped and minced separately in a meat grinder, after the removal of excessive fat and connective tissue. Dried marjoram leaves and freshly crushed garlic and onion bulbs were

mixed with the meat. The following four samples were prepared from each blade: 1 control (meat without any addition) and 3 treatments, namely, with garlic 0.1% (m/m), onion 0.5% (m/m) and marjoram 0.5% (m/m). The mixing process for each sample was conducted separately for each blade. The concentrations of the additives were established during preliminary studies and were accepted by a sensory panel (data not presented). Meat samples were placed in plastic foam meat trays, wrapped with polyethylene and kept at 4°C for 9 days. A strict sanitation procedure was followed during the preparation of the meat samples to avoid microbial contamination.

2.2. Antioxidant activity of plant extract

The antioxidant activities of marjoram leaves, garlic and onion were determined by methods, which reflected the various mechanisms of antioxidant action, such as the scavenging of free radicals or chelation of transition metal ions (methods such as DPPH and TEAC or FRAP, respectively). Each method has its advantages and limitations, thus a combination of these methods was implemented for assessing antioxidant activity to get more insight into the antioxidative potential of the extracts studied.
To investigate the antioxidant activity of the plant material, extracts were prepared in methanol. For the extraction, 2 g of dried marjoram leaves, onion or garlic were mixed with 30 mL of pure ethanol. The procedure was conducted in the dark at room temperature. After 30 min of extraction, each extract was put through a filter (type 388) and the solutions of the corresponding concentrations were prepared in methanol for antioxidant activity measurements.

2.3. DPPH method

The antioxidant activity of marjoram leaves, garlic and onion were evaluated by the DPPH method according to the procedure described by Sánchez-Moreno *et al*. (1998) with some modifications. Briefly, 10 μL of the plant extract samples was added to 990 μL DPPH in methanol (giving a final concentration 0.1 mM) and mixed in a vortex. The reaction mixtures were incubated in the dark at room temperature for 30 min, and the decrease in absorbance caused by the plant extract was measured at 515 nm using a Cary 1E spectrophotometer (Varian, Berlose, Australia). For each sample, three separate determinations were conducted. The corresponding solvent blank readings were also recorded (methanol). The DPPH· radical scavenging activities of the plant extracts were expressed in TEAC(DPPH) values - Trolox Equivalents Antioxidant Capacity (mM of Trolox/g of dry weight). TEAC(DPPH) values were calculated as the ratio of the slope of the linear plot for the scavenging of DPPH· radicals by the plant extract tested to the slope of the plot for DPPH· radicals scavenging by the water-soluble vitamin E analogue Trolox, used as an antioxidant standard.

2.4. TEAC assay

The TEAC (Trolox Equivalent Antioxidant Capacity) assay is based on the inhibition of the absorbance of the blue-green coloured ABTS·⁺ radical cation by antioxidants (Miller *et al.* 1993). In the study, the ABTS·⁺ radical cation was generated with potassium disulphate according to the modifications of Re *et al*. (1999). Briefly, ABTS was dissolved in water to a 7 mM concentration and mixed with 2.45 mM potassium disulphate at a ratio of 2:1 (stoichiometrical reaction). To produce ABTS·⁺ radical cation, the mixture was allowed to stand in the dark at room temperature for 12-16 h. The radical was stable in this form for more than two days (i.e. stored in the dark at room temperature). The ABTS·⁺ solution was

diluted with PBS (phosphate buffer saline), pH 7.4 to an absorbance of 0.7 (±0.02). 10 μL of plant extract was added to 990 μL $ABTS^{\cdot+}$ solution in PBS, pH 7.4 and the decrease in the absorbance of the mixture after 6 min of incubation in the dark was monitored spectrophotometrically at 734 nm. The corresponding solvent blank readings were run (ABTS in PBS, pH 7.4 with methanol). All determinations were conducted in triplicate, and the results were expressed as TEAC values. The TEAC value represents the ratio of the angle of the plot for the scavenging of $ABTS^{\cdot+}$ by the particular extract under investigation to the slope of the plot for $ABTS^{\cdot+}$ scavenging by Trolox, used as an antioxidant standard (MILLER *et al.*, 1993). The TEAC value is expressed in milimolar concentrations (mM), according to the definition of the TEAC value introduced by MILLER *et al*. (1993). The TEAC value is defined as the concentration of Trolox solution with an equivalent antioxidant potential to a 1 mM concentration of the compound under investigation (MILLER *et al*., 1993).

2.5. FRAP assay

The FRAP (Ferric Reducing Antioxidant Power) assay directly measures the ability of antioxidants to reduce a ferric tripyridyltriazine complex (Fe+3-TPTZ) to a ferrous complex (Fe+2-TPTZ) at a low pH, with an intense blue color and an absorption of 593 nm (BENZIE and STRAIN 1996). The FRAP solution was prepared by mixing an acetate buffer (300 mM, pH 3.6), 10mM TPTZ (2,4,6-tripyridyl-s-triazine) and $FeCl_3 \bullet 6H_2O$ (20 mM in 40 mM HCl) at a ratio of 10:1:1. The mixture was allowed to stand in the dark at 30°C for 30 min directly before use. Then, 50 μL of an increasing concentration of plant extract was added to the FRAP solution in a 1mL total volume and allowed to react for 10 min in the dark. Readings were taken at 593 nm and results were expressed as mM Trolox equivalent (TE) per g of dry weight. All determinations were conducted in triplicate.

2.6. Total polyphenol content of plant extracts

The Total Polyphenol Content (TPC) of the plant materials was determined using the Folin- Ciocalteu reagent (FCR) as described by Singleton and Rossi (1995). An aliquot of 20 μl of plant extract (onion, garlic or marjoram), prepared in the same way as for antioxidant activity measurements, was added to 100 μl of FCR, mixed and incubated for 3 min at room temperature in a dark place. Then, 300 μl of sodium carbonate (20% m/v) was added and filled to 2 mL with distilled water. After 2h of incubation in the dark at room temperature the absorbance was read at 765 nm against the blank sample (solvent instead of extract). The results were presented as mg Gallic Acid Equivalent (GAE) per g of dry weight (DW). All determinations were conducted in triplicate.

2.7. Total flavonoid content

The total flavonoid content was determined by using the aluminum chloride colorimetric method as described by MEDAA *et al*. (2005). The basis of this assay is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, it also forms acid labile complexes with the ortho-dihydroxyl groups in the A or B ring of flavonoids. 100 μL of plant extracts were mixed with aluminium chloride in methanol (2% m/v) to a final volume of 1 mL. The mixtures were incubated for 15 min in the dark at room temperature. Then, the absorbance was monitored at 410 nm. The results were expressed in mg of quercetin per g of dry weight.

2.8. Measurement of lipid oxidation

The extent of lipid oxidation was monitored by the formation of Thiobarbituric Acid-Reactive Substances (TBARS). The TBARS index was determined, in triplicate samples, by the extraction method of SØRENSEN and JØRGENSEN (1996) with some modification. For extraction, 10 g meat was homogenized (15,000 rpm, 30 s, 20°C) together with 30 mL of a 7.5% aqueous solution of trichloroacetic acid (TCA). After filtration and centrifugation, (5 min, 5000 rpm), a 5.0 mL extract was mixed with 5.0 mL of 0.02 mol l^{-1} aqueous thiobarbituric acid (TBA) in a stoppered test tube. The samples were incubated at 100C for 35 min in a water-bath and subsequently cooled for 10 min in cold water. Absorbance was measured at 532nm by a Carry 1E UV/VIS spectrophotometer against a blank containing 5mL distilled water and 5mL TBA reagent. The results, expressed as milligrams malondialdehyde per kg meat, were calculated from the standard curve of TEP (1,1,3,3-tetraethyoxypropane) standards. The antioxidant potential, which is expressed in terms of the percentage of antioxidant activity (%AOA), were calculated using the following equation (WIJEWICKREME and KITTS 1998):

$$\%AOA = ((X \text{ value of the control} - X \text{ value of the test sample}) \times 100) / X \text{ value of the control}$$

where X is the TBARS value.

2.9. Fatty acids composition analysis

The meat samples were minced in a blender. Lipids were extracted from the samples with chloroform:methanol (2:1 v/v) according to FOLCH *et al*. (1957). The extracts were dried in a vacuum on a rotary evaporator and under a nitrogen flow. The fatty acid content was determined as Fatty Acid Methyl Ester (FAME) derivatives in a HP 5980 series II gas chromatographer (Hewlett Packard, Oalo Alto, USA) using the AOCS Official Method Ce 1k-07. The GC system consisted of an Innowax capillary column (30 m x 0.2 mm x 0.2 mm) and FID detector. Hydrogen was used as the carrier gas, at a flow rate of 1.5 mL/min. The temperature of both the injector and detector was set at 240°C. The column temperature was maintained at 220°C isotherm. Peak identification was based on the fatty acid ester standards, and fatty acid compositions were estimated from the chromatogram peak areas and were expressed as mg/1 g meat sample.

2.10. Microbial analysis

An amount of 10 g of meat was collected from prepared samples and introduced, under sterile conditions, to a flask containing 90 mL of 0.1% sterile peptone diluents (Bacteriological Peptone, Oxoid, England). The samples were homogenized using an Ultra-Turrax T25 homogeniser (IKA, Germany), producing an initial suspension of 1:10.
The microbiological analyses were conducted in line with ISO reference methods (according to the Polish Standard of PN-ISO). Bacterial counts were given in CFU g^{-1}. The total bacterial count was determined on a Standard Plate Count Agar (CM 463, Oxoid, England). Incubation was run at 30°C for 72 h. For counts of the *Enterobacteriaceae* rods, a 1 mL sample was inoculated into 15 mL of a molten selective VRBG medium (P-0256, BTL, Poland). After setting, a 10 mL overlay of molten medium was added and incubation was conducted at 37°C for 24-48 h. The counts of *Pseudomonas* were determined on a solid Pseudomonas Agar medium (CM 0559, Oxoid, England) supplemented with Pseudomonas CFC Selective Agar Suplement (SR 0103, Oxoid, England) after incubation

at 30°C for 48 h. De Man, Rogosa and Sharpe (MRS) Agar (CM 0361, Oxoid, England) was used for determining lactic acid bacteria counts. MRS agar was also overlayed with a molten medium and incubation was conducted at 30°C for 48-72 h. An oxidase test was used to confirm lactic acid bacteria (MBO 266, Oxoid, England). Dichloran Rose Bengal Chloramphenicol DRBC Agar (CM 0727B, Oxoid, England) was used in the determination of moulds and yeasts. Incubation was conducted at 20°C for 5 days. Total Viable aerobic bacteria Counts (TVC) were conducted as an indicator of microbial spoilage in the pork meat samples. The samples (10 g) were homogenized with 90 mL with sterile peptone water (1g/l) using an Ultra-Turrax T25 homogenizer (IKA, Germany). Serial decimal dilution was performed and plated onto a Standard Plate Count Agar (CM 463, Oxoid, Basingstoke, England). Incubation was performed at 30°C for 72 h. Bacterial counts were enumerated and expressed as $\log_{10}$ cfu/g.

2.11. Statistical analysis

All tests were run in triplicate. The statistical package Statistic 13.1 was used for analysis of covariance (ANCOVA). The influence of various plant additions on oxidative and microbial raw meat stability was assessed using analysis of covariance. This analysis procedure is applied when looking at group effects on a continuous outcome (TBARS value, fatty acid content and microbial count) when another continuous explanatory variable (storage time) may also have an effect on the outcome. Comparison of the treatment means was based on Tukey's Honest Significant Difference (HSD) test. This test is the most useful for multiple comparisons and avoids Type II errors. In addition, Dunnett's T-test was performed for a comparison of the treatments to the control. To compare the rates (slope of regression equation) of fatty acids' degradation, a linear regression analysis between the storage time and the fatty acid content was calculated. To compare the regression coefficients (slope) of the control with the other treatments, the null hypothesis H0: $B_c=B_t$, (where B_c is the regression coefficient for the control and B_t is the regression coefficient of the sample with additives) was tested. The comparisons between the coefficients were performed introducing two dummy variables as predictors to regression analysis. The first dummy variable was coded "0" for the control and "1" for the meat sample with plant additive, and the second, which was the product of the first dummy variable and storage time. The significant differences between the regression coefficients were based on the result of the *T*-test ($P \leq 0.05$) for the second dummy variable. Bacterial counts were converted to their equivalent log cfu for data uniformity. Differences were considered significant at the $P \leq 0.05$ level.

3. RESULTS AND DISCUSSIONS

3.1. Antioxidant activity and total polyphenol content of the plant extracts

All results of the antioxidant activity and phenolic content (total polyphenol content TPC and total flavonoid content TFC) measurements are presented in Table 1. According to radical scavenging methods like DPPH and ABTS and ion chelating methods such as FRAP, the most antioxidant active plant extract was marjoram. There were no statistically significant differences between the antioxidant activities of garlic and onion. GORINSTEIN *et al*. (2008) showed higher antioxidant activity of garlic in comparison to onion in the DPPH method but significantly lower antioxidant activity of garlic than onion in the ABTS and FRAP methods. MARIUTTI *et al*. (2008) reported that marjoram is the most antioxidant active among the tested extracts in the DPPH and ABTS methods, which

is in accordance with the results presented in this paper. However, the authors showed that garlic is more antioxidant active than onion as a DPPH• and ABTS•+ radical scavenger

Table 1. Phenolic content and the antioxidant activities of marjoram, garlic and onion.

	Plant extract		
	Marjoram	**Garlic**	**Onion**
Phenolic content	*Mean±SD*	*Mean±SD*	*Mean±SD*
TPC (mg GAE/g)	24.48 ± 2.66 [a]	0.79 ± 0.23 [b]	2.74 ± 0.65 [b]
TFC(mg QE/g)	15.72 ± 1.26 [a]	0.22 ± 0.00 [b]	0.27 ± 0.01 [b]
Antioxidant activity			
TEAC(DPPH)(mM TE/g)	175.11 ± 11.43 [a]	3.05 ± 0.46[b]	3.77 ± 0.51[b]
TEAC(ABTS)(mM TE/g)	261.84 ± 15.04 [a]	7.68 ± 0.25[b]	19.09 ± 1.15[b]
FRAP(mM TE/g)	186.9 ± 10.60[a]	4.16 ± 0.06[b]	3.86 ± 0.19[b]

[(a-b)]Means with the same superscript within the same row are not different ($P > 0.05$).

In this study, the TPC and TFC values correspond to the antioxidant activity (Table 1). r-Pearson coefficients for the linear correlations between the antioxidant activity and TPC or TFC values are equal to 0.99. The higher the TPC (and TFC) value, the higher the antioxidant activity is. In literature, data on the TPC values of onion, garlic and marjoram vary. Generally, it was shown that onion possesses a higher TPC value compared to garlic (GORINSTEIN *et al*. 2008; NUUTILA, *et al*. 2002). It is also hard to compare the TPC values of marjoram to reference results since various modifications to the methods and units were implemented by the authors.

3.2. Measurement of lipid oxidation

The lipid oxidation in the fresh minced pork meat subjected to refrigerated storage was determined using the TBARS method. This method is widely used to estimate the degree of lipid oxidation because of its relatively simple measurement and good correlation with the sensory quality of foods (MOTTRAM 1998). TBARS are formed through the second stage of lipid oxidation, during which peroxides are oxidized to aldehydes and ketones such as malondialdehyde (MDA). Fig. 1 presents the TBARS values of the samples under study. The results showed that both the storage period and the type of additive affected the TBARS values (with $P < 0.05$). The TBARS values of all the tested samples (control and treatment) increase constantly with the time of storage (Fig. 1). This increase was the most pronounced for the control sample, whose TBARS values rose from 0.11 mg MDA/kg meat on the first day of analysis to 0.352 mg MDA/kg meat at the end of storage (9th day). The TBARS values in the minced meat were influenced by onion, garlic and marjoram, resulting in significantly lower values than those without treatment. As shown in Fig. 1, the most effective treatments in inhibiting the lipid oxidation were the onion and garlic ones. There were no statistically significant differences between these two samples ($P >0.05$). Samples treated with onion and garlic reached TBARS values of 0.22 and 0.186 mg MDA/kg respectively on the last day of storage, compared to the control samples which exhibited a TBARS value equal to 3.5 MDA/kg. Significant differences in the TBARS were found between the treated samples and the control from day 3 to the end of storage. YIN and CHENG (1998) found that garlic had a stronger antioxidant effect than onion in their

liposome model system. In this study, due to sensory acceptability, different levels of additives were tested (0.15 m/m for garlic and 0.5% m/m for onion), which may have caused the lack of difference between the onion and garlic inhibition of lipid oxidation. A statistically significant protective effect for lipid oxidation in meat was also noted for samples with the addition of marjoram. The TBARS value for the treatment with marjoram at the end of storage was 0.28 mg MDA/kg and was significantly lower than the control one. However, this effect was less pronounced when compared to the samples with garlic and onion. In conclusion, the highest inhibition of secondary oxidation products was observed in the samples with garlic (47%). In the samples with onion and marjoram, the percentage of inhibition was 37% and 25% respectively. These results were consistent with those of previous studies in which fresh onion and garlic bulbs or their extracts showed antioxidant effects in the meat matrix (CAO *et al*., 2013; KIM *et al*., 2010; PARK *et al*., 2008; SALLAM *et al*., 2004).

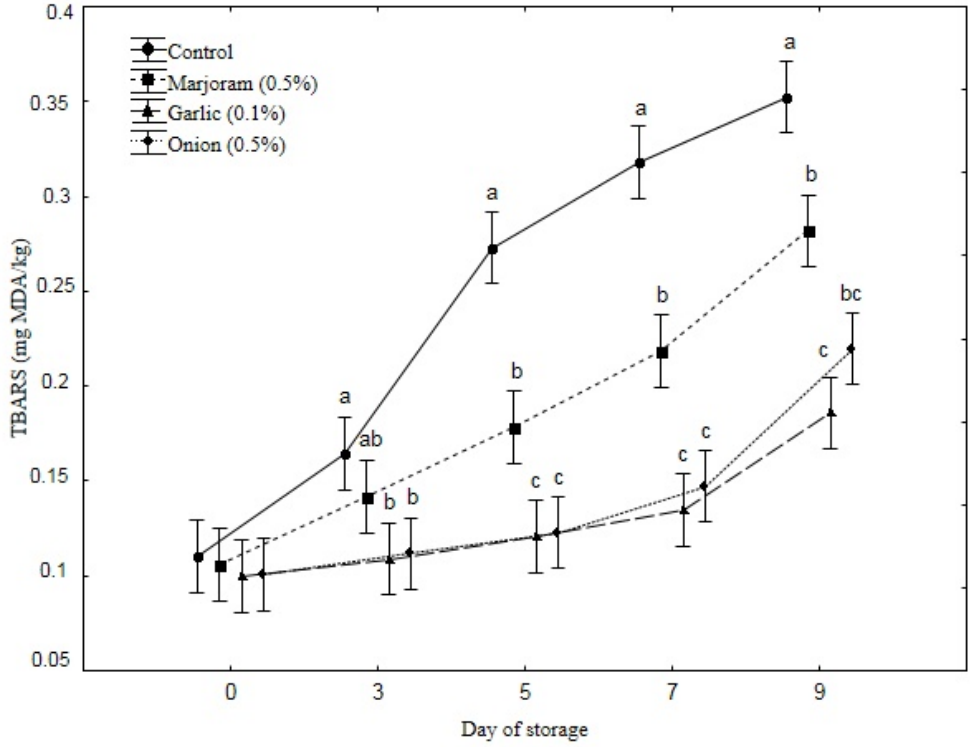

Figure 1. Changes in the TBARS values of fresh minced pork with different treatments during refrigerated storage at 4°C.

3.3. Fatty acid composition analysis

The fatty acid composition of minced pork muscle fat is shown in Table 2. Both the type of additive and storage time influenced the fatty acid composition. The predominant fatty acids in all meat samples were oleic (C18:1n-9), palmitic (C16:0), stearic (18:0) and linoleic (C18:2n-6) acids, in order of the increasing concentration in the samples. The amount of these acids accounted for more than 91% of the total fatty acid contents in all samples. PARK *et al*. (2008) obtained similar results in fresh pork belly and loin. MUFAs were the major part followed by SFAs. Generally, a decrease in the fatty acid content in the

intramuscular lipid was noted during storage. This effect was expected because, due to cellular structure damage and exposure to the oxygen, minced muscle is very prone to lipid and protein oxidation (MIN and AHN, 2005). Therefore, to evaluate whether the fatty acid contents were equal in the groups being studied (type of additive) analysis of covariance was performed. This analysis allows for comparisons of the effects of additives and control the influence of storage time simultaneously. Comparison of the additives showed that a stronger inhibition of lipid oxidation was exhibited by garlic (Table 2). Statistically significantly higher contents of stearic (56.82 mg/g) palmitileic (15.28 mg/g), linoleic (41.1 mg/g), cis-10-heptadanoic (1.62 mg/g) and arachidonic acids (5.14 mg/g) were observed in this treatment when compared to the control. The lowest content of fatty acids was noted in the samples with the addition of fresh onion, which makes it the least effective in the retardation of lipid oxidation. The marjoram additive was the most effective in inhibiting the decomposition of linoleic (but only until the 7th day of storage) and cis-10-heptadanoic acids. Furthermore, to compare the effect of the added natural ingredients on the change rates of fatty acids, linear regression analysis was performed.

Table 2. Effect of onion, garlic and marjoram on the fatty acid profile of fresh minced pork during storage at 4°C (mg/g).

Fatty acid	Treatment				
	Control	Onion	Garlic	Marjoram	SE
C14:0	4.90^{a}	4.62^{a}	6.08^{a}	5.01^{a}	0.39
C16:0	84.31^{ab}	79.95^{a}	106.26^{b}	89.22^{ab}	6.86
C18:0	41.41^{a}	39.89^{a}	56.82^{b}*	47.02^{ab}	3.40
C16:1	12.42^{a}	11.56^{a}	15.28^{b}*	12.64^{a}	0.62
C17:1	1.26^{a}	1.18^{a}	1.62^{b}*	1.85^{c}*	0.05
C18:1	176.59^{ab}	166.92^{a}	198.84^{b}	159.50^{a}	12.06
C20:1	3.35^{a}	3.33^{a}	3.20^{a}	2.74^{a}	0.15
C18:2	35.53^{ab}	33.27^{a}	41.10^{bc}*	42.75^{c}*	1.79
C20:2	1.52^{a}	1.52^{a}	1.47^{a}	1.52^{a}	0.08
C18:3	4.91^{a}	4.56^{a}	2.38^{b}	2.52^{b}	0.21
C20:4	3.33^{a}	3.31^{a}	5.14^{b}*	3.92^{a}*	0.17
SFA	130.63^{ab}	124.46^{a}	169.15^{b}*	141.26^{ab}	10.62
MUFA	193.62^{ab}	182.99^{a}	218.95^{b}	176.73^{a}	8.94
PUFA	45.29^{ab}	42.66^{a}	50.08^{ab}	50.70^{b}	3.19

$^{a-c}$Means with the same superscript within the same row are not different ($P > 0.05$). (*) Paired comparison (control compared with other natural additives) significantly greater than control at the $P < 0.05$ level using Dunnett's-*T* test.

Results showing the regression coefficients from the linear models of the decay of fatty acids are presented in Table 3. The linear model was statistically significant in almost all cases ($P \leq 0.05$). Only for saturated fatty acids in the meat samples with garlic and marjoram, was the linear model not significant ($P > 0.05$). In the samples with garlic, the level of saturated fatty acids was stable during storage. However, in the samples with marjoram the level of saturated fatty acids increased till the 7th day of storage and then rapidly decreased, reaching the lowest values of all the meat samples. The values of the determination coefficients ranged from 74% to 96%.

Table 3. Effect of onion, garlic and marjoram on the fatty acids content changes of fresh minced pork during storage at 4°C (expressed as slope (mg/g/day)).

Fatty acid	Treatment			
	Control	Onion	Garlic	Marjoram
C14:0	-0.568	-0.472	-0.057^{N}	-0.340^{N}
C16:0	-0.500	-7.801	-1.282^{N}	-6.130^{N}
C18:0	-4.357	-3.713	-0.831^{N}	-3.040^{N}
C16:1	-1.391	-1.298	-0.762*	-1.865
C17:1	-0.123	-0.098	-0.076*	-0.173
C18:1	-19.89	-18.97	-9.381*	-23.94
C20:1	-0.401	-0.422	-0.144*	-0.346
C18:2	-3.299	-3.169	-1.613*	-6.089*
C20:2	-0.162	-0.148	-0.068*	-0.133
C18:3	-0.652	-0531	-0.214*	-0.256
C20:4	-0.282	-0.200	-0.165*	-0.621*
SFA	-14.42	-11.99	-2.165*	-9.512
MUFA	-21.81	-20.79	-10.36*	-26.32
PUFA	-4.396	-4.051	-2.060*	-7.101*

$^{(N)}$Regression coefficients are not significant ($P > 0.05$). (*) Paired comparison (control compared with other natural additives) were significantly different to the control at $P < 0.05$.

All the slopes have a negative value indicating the inverse relationship between the storage time and fatty acid content. The lowest rate of fatty acid degradation was recorded in the samples with the fresh garlic addition. This dependency was noted for all fatty acids, which confirms the highest inhibiting effect of garlic on lipid degradation. In addition, discriminant analysis corroborates these outcomes. As shown in Fig. 2, the first discriminant function extracts the sample of meat with garlic, while the second extracts the meat samples with marjoram. The meat samples with onion exhibit the same fatty acid composition during storage as the meat samples without any addition (the points are located in the same place of the graph). The LDA model allows meat samples enriched with garlic and marjoram to be distinguished with 100% accuracy. The highest decomposition rate was noted in the meat samples with marjoram, namely, arachidonic and linoleic acid. The contents of these acids decreased from 6.24 to 1.63 and from 60.2 to 9.2 mg/g respectively. The level of linoleic acid in the samples with marjoram was significantly the lowest among the tested samples at the end of storage.

3.4. Microbial changes

The bacteria selected in this study such as *Pseudomonas* and *Enterobacteriaceae* belong to the genera of bacteria constituting microflora typical for spoilage processes in meat during storage. To assess the effect of the addition of the plants on bacterial growth in meat samples covariance analysis was performed.
Throughout the storage period, the inhibition effect of the plant additives on bacterial growth was observed. Only for lactic acid bacteria was this effect not statistically significant.

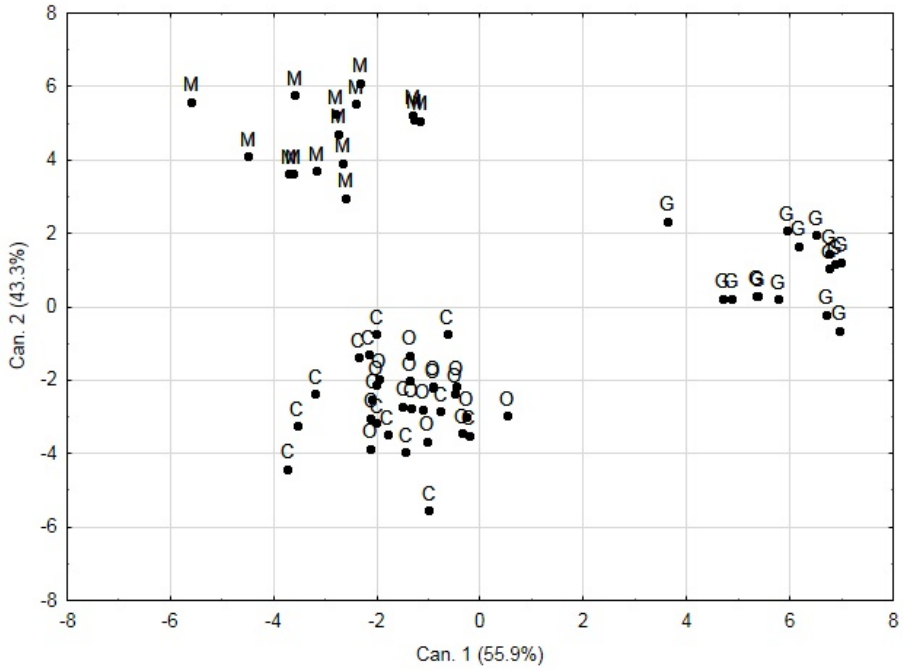

Figure 2. Discrimination of minced meat samples based on the fatty acid composition during cold storage (4°C); C-control, G-meat with garlic (0,1%), M- meat with marjoram (0.5%) and O- meat with onion (0.5%).

As shown in Table 4, the highest numbers of all the tested bacteria were noted in the control samples. The results showed that the addition of onion and marjoram to fresh meat exerted a statistically significant bacteriostatic effect against TVC, *Enterobacteriaceae* and *Pseudomonas* bacteria. The highest inhibitory effect against *Enterobacteriaceae* and *Pseudomonas* bacteria was noted in the meat samples with the addition of fresh onion. The results of our study supported the findings of other authors. GROHS *et al*., (2000) reported that spice mixtures might delay bacteria growth in fresh pork and beef. PARK *et al*., (2008) showed that the addition of garlic and onion powder to pork loin and belly inhibited the growth of the total amount of bacteria and *Enterobacteriaceae*. SALLAM *et al*., (2004) noted that fresh garlic and garlic powder, through their antioxidant and antimicrobial effects, are potentially useful in preserving meat products. However, MURRAY (1997) claimed that only fresh garlic preparations provide the full range of beneficial compounds.

Table 4. Effect of onion, garlic and marjoram additions on the microbial changes (cfu/g) of fresh minced pork stored at 4°C.

Type of bacteria	Treatment			
	Control	Onion	Garlic	Marjoram
TVC	8.02^{a}	7.54^{c*}	7.92^{ab}	7.8^{b*}
Enterobacteriaceae	6.16^{a}	5.65^{b*}	6.09^{a}	5.70^{b*}
Pseudomonas	8.00^{a}	7.52^{c*}	7.84^{ba}	7.74^{bc*}
Lactic acid bacteria	2.84	2.64	2.71	2.65

[a-c]Means with the same superscript within the same row are not different ($P > 0.05$).
(*) Paired comparison (control compared with other natural additives) is significant at the $P < 0.05$ level using Dunnett's *T*- test.

4. CONCLUSIONS

Due to the natural potential health benefits and safety of the plant species when compared to synthetic derivatives, natural species have gained appreciable interest among the research and industry community. The highest antioxidant activity is demonstrated by fresh garlic, whereas fresh onion in minced pork meat reveals the highest antibacterial activity. The synergistic behaviour of these plants would require further research. Although marjoram was characterised by the highest antioxidant activity, its protective effect on lipids in meat was less significant than other treatments. This result confirms the necessity to examine the plant antioxidant effect in particular food products. Our data indicated that the addition of fresh garlic and onion, as well as dried marjoram, enhances raw minced meat safety and shelf life. In addition to their economical and health promoting benefits, the application of plants, as natural and safe bio-preservatives, could be highly recommended for the improvement in the quality of ground pork.

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