

## ROSEMARY, THYME AND OREGANO ESSENTIAL OILS INFLUENCE ON PHYSICOCHEMICAL PROPERTIES AND MICROBIOLOGICAL STABILITY OF MINCED MEAT

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### ABSTRACT

In this paper we have analysed the capacity of essential oils from aromatic plants in order to slow or stop down the physicochemical degradation and microbiological infestation of raw minced meat processes. Three essential oils: thyme, rosemary and oregano were chosen for the experimental study due to their acknowledged antiseptic effect and their flavour acceptance by consumers. The physicochemical and microbiological properties for a mixture of pork and beef meat were analysed throughout four days. Water content, total fat, total protein, easily hydrolysable nitrogen, pH and water activity were determined. The experimental measurements were performed considering the following concentrations: 0.5, 1.0 and 1.5% for the essential oil incorporated into meat. The evaluation of microbiological stability was determined as well. The results indicated that essential oils have a significant influence on the physicochemical properties correlated with microbiological stability when prolonging the shelf - life of minced meat.

**Keywords:** Essential oils, microbiological stability, minced meat, physicochemical analysis, shelf – life

### INTRODUCTION

Alteration of meat consists in a set of processes that affect the substrate, leading to formation of low molecular weight compounds in conjunction with the development of microbial associations. The consequences are the modification on sensory properties, on nutritional quality and advanced alteration conducting to a negative impact of the product on consumer's health. Different oils from aromatic plants can have preserving effect on meat. Antimicrobial effect of essential oils is acknowledged due to their hydrophobicity, being capable of damaging the cell wall of microorganisms, disturbing the metabolism of phospholipids from cytoplasmic membrane, damaging cell membrane proteins and increasing the permeability of cell wall, thus leading to loss of the liquid constituents of the cell (Burt 2004; Bakkali et al., 2008; Bassolé and Juliani 2012). The antimicrobial activity of essential oils may be related to more than one component. It is considered that the strong character of antibacterial essential oils against food borne pathogens is due to their rich content in phenolic compounds such as: carvacrol, eugenol (2-methoxy-4- (2-propenyl) phenol) and thymol (Faleiro et al., 2003; Burt 2004; Erkan et al., 2011a; Faleiro 2011; Proestos et al., 2013a). Phenolic compounds from plants contain the highest antioxidant potential, being able to decrease the local concentration of oxygen, form chelates with transition metals, inhibit the initialization of oxidative processes chain by free radicals. In addition they are capable for regenerating endogenous  $\alpha$ -tocopherol in lipoprotein layer from tissues (Kähkönen et al., 1999; Frankel and Meyer 2000; Rocha-Guzmán et al., 2007; Su et al., 2014). The preserving effect of oils from aromatic plants such as rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.) and oregano (*Origanum vulgare* L.) was analysed in this paper. The synergic action of thymol and carvacrol in the essential oils of thyme and oregano inhibits the growth of several bacteria strains, e.g. *Escherichia coli*, *Bacillus cereus*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas Aeruginosa* (Helander et al., 1998; Burt 2004; Cox and Markham 2007; Roldán et al., 2010; Erkan et al., 2011b; Bassolé and Juliani 2012; Erkan 2012). Compounds such as carvacrol and thymol get the major antioxidant capacity from their essential oils (Kulisic et al., 2004; Erkan and Bilen 2010; Chatli and Joseph 2014). Pork and beef meat added with 3% oregano essential oil showed lower levels of oxidation after 12 days of refrigerated storage (Fasseas et al., 2008). Oregano essential oil (0.05%, 0.5% and 1%) could delay the growth of microorganisms and decrease the final counts of spoilage microorganisms (Skandamis and Nychas 2001; Zhang et al., 2010; Zhou et al., 2010). The effect of these oil compounds on food has become

much more important since their antimutagenic, anti-inflammatory anti-carcinogenic, anti-haemolytic and immuno-stimulating effect was demonstrated (Barnes et al., 2005; Bakkali et al., 2008; Chao et al., 2008; Dung et al., 2009; Ocaña-Fuentes 2012; Ozkan and Erdoğan 2011; Proestos et al., 2013b; Ramchoun et al., 2015; Sá et al., 2014; Sivasothy et al., 2012; Wojdylo et al., 2007).

The purpose of our study is to evaluate the possibility of extending shelf - life of minced meat with rosemary, thyme and oregano essential oils additions, under safety conditions by maintaining nutritional value and sensory properties without bringing any chemical or microbiological threat to consumer's health.

### MATERIAL AND METHODS

#### Samples

The matrix chosen was purchased from a local market from Romania, consisted in a minced meat (equal mixture of beef and pork), intended to immediate consumption and stored at 4°C. The whole amount was mixed and homogenized, and then the batch of minced meat was divided into ten portions. A portion was kept as a control sample and each of the other portions were further mixed with an appropriate volume of rosemary, thyme and oregano essential oil (final concentrations 0; 1 and 1.5% v/w). The samples were placed into 50 plastic containers, closed and stored at 4°C. All physicochemical and microbiological determinations were performed at equal time intervals over a period of 4 days, in compliance with the Romanian standards.

#### Reagents

All the reagents used were purchased from Sigma - Aldrich and they were of pro analysis purity: 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), ethanol, L(+)-ascorbic acid, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, quarter-strength Ringer's solution, peptone, meat extract, yeast extract, D(+) glucose, dipotassium hydrogen phosphat, sodium acetate trihydrate, triamonium citrate, magnesium sulphate heptahydrate, manganous sulphate tetrahydrate, malt extract, peptone, maltose, dextrin, glycerol, monopotassium phosphate, ammonium chloride, petroleum ether, H<sub>2</sub>SO<sub>4</sub>, NaOH, Tashiro indicator, MgO and paraffin oil.

## Oils Analysis

Ten grams of rosemary, thyme and oregano essential oils were sterilized at 121°C for 15 min (Autoclave Raypa). The spectrophotometric assay was used for the analysis of the following properties of essential oils: antioxidant activity with 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging considering the method described by **Brand-Williams et al. (1995)** (UV VIS - NIR Spectrometer - Shimadzu, 3600, Japan) and total polyphenol content with Folin-Ciocalteu method (spectrophotometer Shimadzu UV-2550, Japan) by **Fujita et al. (2012)**.

## Microbiological Analysis

Samples (10g) of minced meat were aseptically weighed, added to sterile quarter-strength Ringer's solution (90 ml) and homogenized in a stomacher (Lab Blender - BagMixer 400 P, INTERSCIENCE, France) for 60 s at room temperature. Decimal dilutions in quarter-strength Ringer's solution were prepared and duplicate 1 ml samples of appropriate dilutions were poured or spread on the following media made from basic ingredients considering the method described by **Tofan et al. (2002)**: MRS for lactic acid bacteria incubated at 35°C for 72 h and MMA for yeasts and moulds incubated at 30°C for 72 h. The number of colony forming units was obtained by reading at Colony Star (Funke Gerber, Germany) and the data (growth counts) were transformed to log<sub>10</sub> values.

## pH and Water Activity

The pH value was recorded by a pH meter (HQ11d pH by HACH), the glass electrode being immersed in the homogenate of minced meat at the end of microbiological analysis, according to SR ISO 2917:2007. The water activity (a<sub>w</sub>) value was determined by a water activity meter (AquaLab LITE by Decagon, USA).

## Physicochemical Analysis

The water content was determined by using gravimetric method according to SR ISO 1442:2010 and Soxhlet method was applied to establish the total fat content (Soxhlet Extractors - SER 148, VELP SCIENTIFICA, Italy), considering **SR ISO 1443:2008**. We determined total proteins by using Kjeldahl analysis method (**SR ISO 937:2007**), with the following steps: digestion (mineralization) of organic substances in acidic medium (Kjeldahl Digestion Units - DK 6 VELP Scientifica, Italy), distillation and capturing nitrogen in a solution of 0.1N H<sub>2</sub>SO<sub>4</sub> (Kjeldahl Distillation Units - UDK 129, VELP Scientifica, Italy), titration of 0.1N H<sub>2</sub>SO<sub>4</sub> excess with 0.05N NaOH solution in the presence of Tashiro indicator. The determination of easily hydrolysable nitrogen (according to **SR 9065-7:2007**) was performed based on the following operations: distillation in aqueous solution (in the presence of MgO and paraffin oil) distillate capture in measured volume of 0.1N H<sub>2</sub>SO<sub>4</sub> solution and final titration of the excess acid with 0.1N NaOH solution.

## RESULTS AND DISCUSSION

### The properties of essential oils

The DPPH method was used to evaluate the antioxidant capacity of basil, rosemary and thyme essential oils in comparison with known synthetic antioxidant L(+)-ascorbic acid. The disappearance of the DPPH radical based on the absorbance at 518 nm wavelength can be monitored by decreasing of optical density. Based on their antioxidant capacity, spice essential oils can be sorted in descending order: thyme (*Thymus vulgaris* L.) > oregano (*Origanum vulgare* L.) > rosemary (*Rosmarinus officinalis* L.). The DPPH radical scavenging activities of all the essential oils (oregano, rosemary and thyme) increased with the increasing of concentration.

Antioxidant capacities in concentrations series of oregano, rosemary and thyme essential oil and ascorbic acid were used to calculate the effective relative concentration EC<sub>50</sub>. The concentration of essential oils that caused 50% neutralization of DPPH radicals (EC<sub>50</sub> values), were calculated from the plot of inhibition percentage against concentration. A higher DPPH radical scavenging activity was associated with a lower EC<sub>50</sub> value. EC<sub>50</sub> values of scavenging DPPH radicals for rosemary, oregano and thyme essential oils were, 9.423 ± 0.06 µg/mL, 7.258 ± 0.02 µg/mL and 3.067 ± 0.09 µg/mL, respectively, while ascorbic acid registered an EC<sub>50</sub> of 161.578 ± 0.03 µg/mL.

The results are consistent with previous reported data on antioxidant activity of essential oils of oregano (**Lagouri et al. 1993; Özcan 1999; Proestos et al. 2013b**), rosemary (**Hussain et al. 2010; Kadri et al. 2011**) and thyme (**Kähkönen et al. 1999; Juki and Milo 2005**) respectively. EC<sub>50</sub> values generally vary considerably among studies, fact that can be explained by different chemical compositions of essential oils of oregano, rosemary and thyme, due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants.

Thyme essential oil analyses by gas chromatography coupled with mass spectrometry (GC-MS) have revealed a significant quantitative differences

between the oils (due to genotypic and environmental differences, namely, climate, location, temperature, fertility, diseases and pest exposure) within species (**Wojdylo et al. 2007**) for seven compounds: the alcohols  $\alpha$ -terpineol and linalool, the two isomeric phenols carvacrol and thymol, their precursor  $\alpha$ -terpinene, *p*-cymene and  $\alpha$ -pinene. The concentration of these components varied greatly among the oils examined, in particular that of carvacrol (from 2.8 to 20.6%), *p*-cymene (from 4.1 to 27.6%) and  $\alpha$ -pinene (from 0.8 to 25.2%). Cosentino and others (1999) observed that the sum of the two phenols and their precursors constituted the bulk of each essential oil are: 53.6%, 74.9%, 86.9% and 77.3% of the total oil, respectively. Strong antioxidant activity is attributed to carvacrol and thymol (**Sacchetti et al. 2005**). Oregano oil has a high content of phenolic monoterpenes (35.0% thymol and carvacrol 32.0%) and monoterpenic hydrocarbons (10.5 %  $\gamma$ -terpinene, 9.1 % *p*-cymene, 3.6%  $\alpha$ -terpinene) with a strong antioxidant (**Kulisic et al. 2004; Rocha-Guzmán et al. 2007**) and antimicrobial character (**Burt 2004**), more than 48 compounds being chromatographically identified (**Liolios et al. 2009**). Rosemary contains a number of phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid and betulinic acid (**Keokamnerd et al. 2008**), the high levels of phenolic compounds leading to its great antioxidant activity. The antioxidant activity of rosemary extract and its constituents, carnosic acid, carnosol and rosmarinic acid is stronger than  $\alpha$ -tocopherol, butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) (**Özcan 1999; Almeida-Doria and Regitano-D'arce 2000**).

Total phenol content was calculated from the standard curve of gallic acid ( $y=0.27x-0.022$ ,  $r^2=0.9929$ ) and expressed as µg/mL samples and its values are: 33.12 µg/mL for rosemary oil, 27.58 µg/mL for thyme oil and 23.78 µg/mL for oregano oil.

### The essential oils influence on microbiological stability

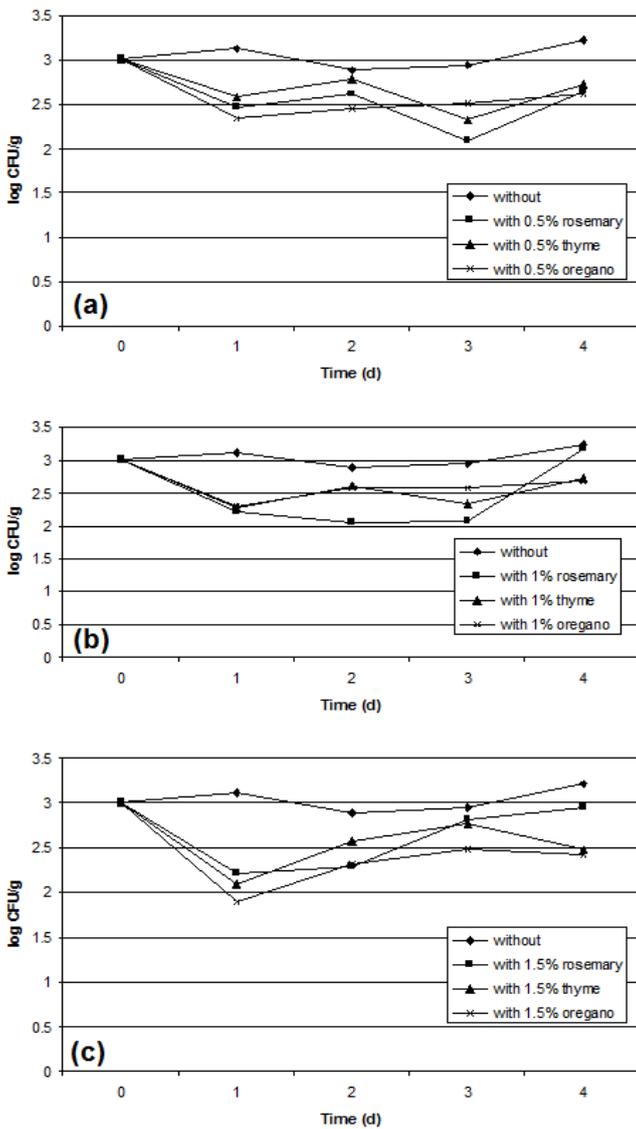
Plant extracts, essential oils and edible film coating treatments are proven to extend the shelf life of seafood by the use of natural sources. The potential effects of these treatments are delayed lipid oxidation, inhibited microbial growth and enhanced sensorial properties. Due to their antimicrobial and antioxidant properties, plant extracts and essential oils are promising their use instead of synthetic chemicals (**Aymerich et al. 2008; Karre et al. 2013; Erkan et al. 2015**). Contaminating bacteria are those specific to meat microbiota, such as Gram positive bacteria (*Micrococcus*, *Corynebacterium*) and Gram negative (*Acinetobacter*, *Flavobacterium*) bacteria prevail (**Banu et al. 2006**).

Lactic bacteria are the most resistant Gram-positive bacteria, they can be prevalent in the whole microbiota, without being affected by storage conditions (microaerophilic conditions). They are resistant to salt (4-7%) and sodium nitrite, being capable of growing at low temperatures in vacuum sealed packaging. Although lactic acid bacteria of *Lactobacillus*, *Streptococcus* and *Leuconostoc* species, are competitive with other spoilage bacteria which they inhibit by antagonism, still their presence is undesirable because they cause meat colour change (turning green) and their presence in high concentrations indicates failure to comply with hygiene conditions, before and after processing (**Banu et al. 2006**).

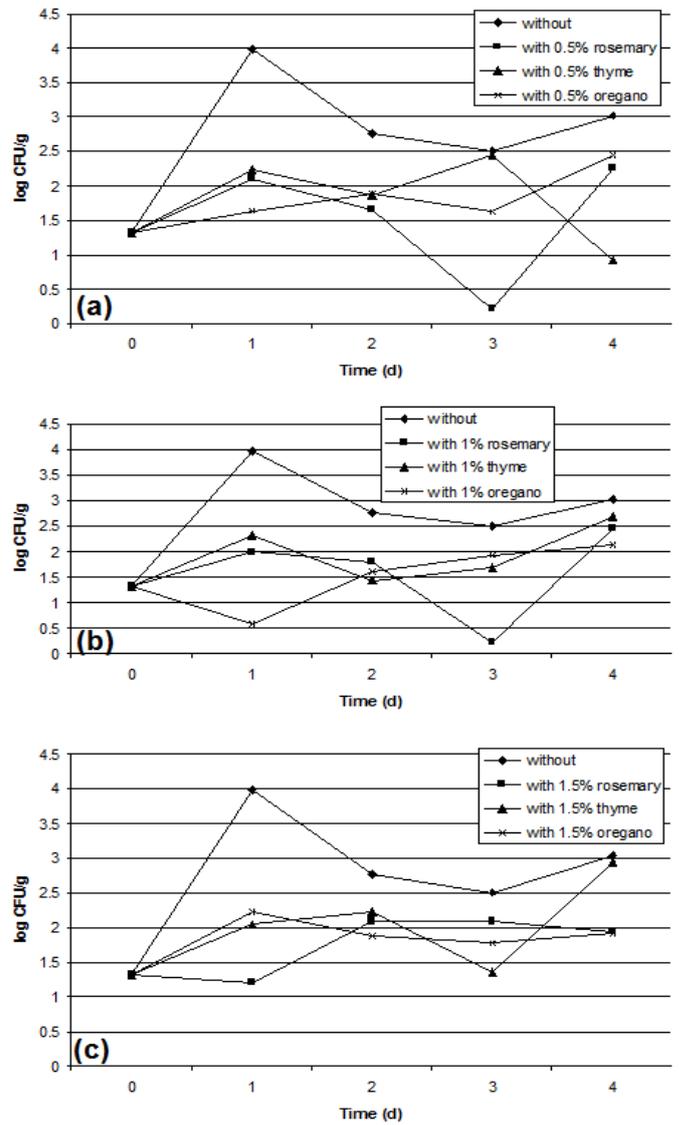
Gram positive bacteria are more sensitive to antimicrobial compounds from essential oils than Gram-negative bacteria. Fungi appear to be more sensitive than Gram negative bacteria, although there are very sensitive Gram-negative bacteria (**Aymerich et al. 2008; Nicolau 2006**).

The contribution of lactic acid bacteria to final flora depends on types and levels of essential oil (Figure 1). It is necessary to mention that the rate of growth and final counts were affected by the addition of essential oil (Figures 1 and 2). The control samples showed the highest total viable counts of lactic acid bacteria and yeast and mould counts respectively, as compared to others containing rosemary, oregano and thyme essential oils with different concentrations. The relatively high initial counts of control samples may be attributed to the grinding process that introduced the microorganisms inside the meat and leads to the increase of total viable counts of meat. Lactic acid bacteria, yeasts and mould counts were gradually increased during cold storage in all samples with different ratios depending on the concentration of oil. Similar studies on the antimicrobial activity of essential oils were performed by **Salem et al. (2010)** and **Erkan and Bilen (2010)**.

**Hać-Szymańczuk et al. (2011)**, have suggested that rosemary preparations did not exhibit either antibacterial properties against aerobic mesophilic or psychrophilic bacteria. The essential rosemary oil was observed to inhibit the growth of coliform bacteria and enterococci, whereas the dried spice examined was found to increase the counts of aerobic mesophilic bacteria, coliforms and enterococci.



**Figure 1** Changes on total viable count of lactic acid bacteria in minced meat without or with rosemary, thyme and oregano essential oils addition (concentration of: (a) 0.5%, (b) 1% and (c) 1.5%)



**Figure 2** - Changes of yeast and mold counts in minced meat without or with rosemary, thyme and oregano essential oils addition (concentration of: (a) 0.5%, (b) 1% and (c) 1.5%)

**Water Activity**

The water activity value of minced meat ranged between 0.83 - 0.92, during storage for 5 days at 4°C, Figure 3. The addition of essential oils increased the water activity of minced meat because the water vapour transfer generally occurs through the hydrophilic portion of the film; thus, water vapour permeability depends on the hydrophilic-hydrophobic ratio of the film components from the surface of meat. Water vapour permeability increases with polarity, unsaturation and branching degree of the lipids, depending also on water absorption properties of the polar part of the film and due to the formation of chemical compounds of degradation by lipid oxidation and protein hydrolysis during storage. Phenolic compounds containing alcohol groups in their chemical structure seem to be a good barrier as compared to aldehyde compounds (e.g., cinnamaldehyde, citral) as hydroxyl group has less affinity for water than for carbonyl groups. All the values obtained for minced meat with rosemary, thyme and oregano essential oil additions (concentration of: 0.5%, 1% and 1.5%) stored at 4°C for 4 days were between 0.88 and 0.97 (Kingchaiyaphum and Rachtanapun 2012; El Adab et al. 2015).

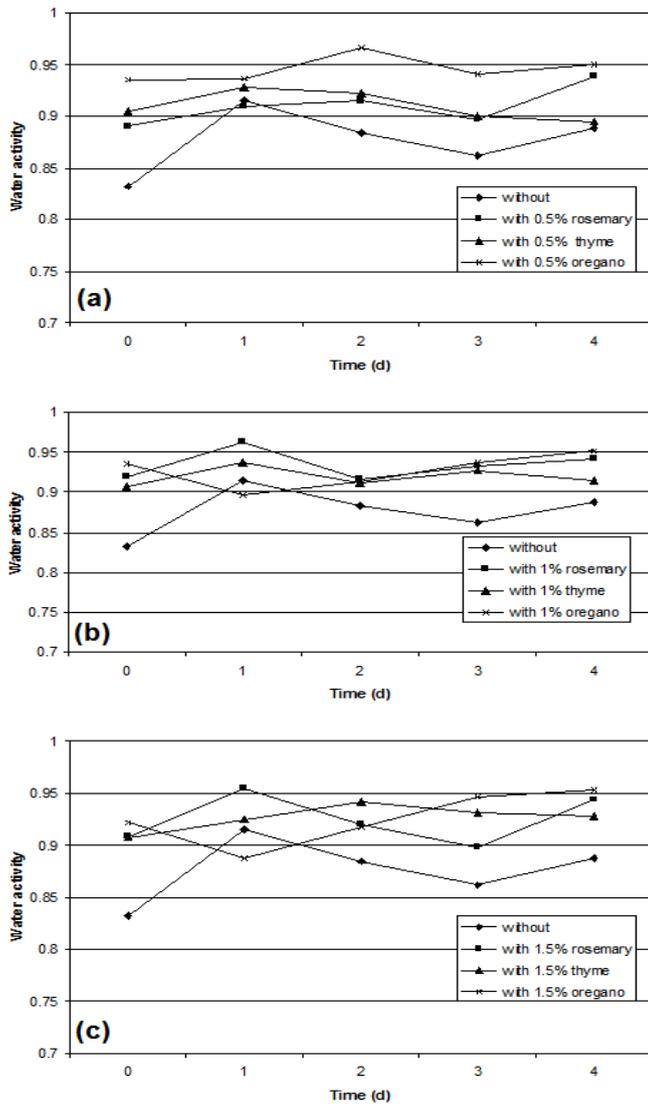


Figure 3 Water activity ( $a_w$ ) in minced meat without or with rosemary, thyme and oregano essential oils (concentration of: (a) 0.5%, (b) 1% and (c) 1.5%) stored at 4°C for 4 days

**pH and physicochemical properties of minced meat**

Figure 4 illustrate the maximum range in which the values for the five groups of parameters studied differ in terms of their stability. If the values of moisture, total protein and total fat do not vary significantly in the case of easily hydrolysable nitrogen and pH, an increase in the minimum, maximum and average values can be notice.

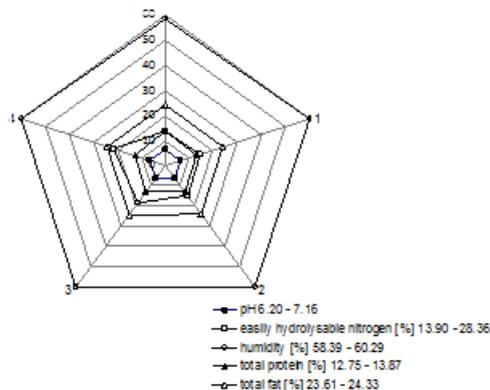


Figure 4 Graphical representation of descriptive statistics for the values of the following parameters: pH, easily hydrolysable nitrogen, humidity, total protein, total fat, on days 0-4, regardless of the essential oil used

This observation was supported by the data resulted from statistical processing: standard deviation values for moisture, total protein and total fat do not exhibit

clear upward trend, while the standard deviation for the easily hydrolysable nitrogen shows a clear upward trend of about 8 times between day 1 and day 4, and about 7 times for the pH between the same days as in Table 1.

**Table 1** Variation of standard deviation values for the following parameters: pH, easily hydrolysable nitrogen, humidity, total protein, total fat, on days 0-4, regardless of the essential oil used

Day	Parameters Analysis				
	pH	Easily hydrolysable nitrogen	Humidity	Total protein	Total fat
0	0.00	0.00	0.00	0.00	0.00
1	0.03	0.43	0.30	0.08	0.31
2	0.03	0.93	0.31	0.02	0.14
3	0.14	2.62	0.24	0.06	0.12
4	0.20	3.26	0.10	0.06	0.10

This fact suggests that there are differences in growth between the analysed samples. It is considered that some independent factors are causing these differences to a greater or lesser extent. In our case the growth it influenced by the amount and type of oil used. Similar results have been reported by **Erkan and Bilen (2010)** for bay leaf, thyme, rosemary, black seed, sage, grape seed, flaxseed and lemon essential oil on chub mackerel.

Figures 5 and 6 shows a similar situation both in the evolution of easily hydrolysable nitrogen parameter and in that of pH parameter. Thus, for both, the factor time (days) was the strongest determinant of the parameters evolution and significant differences between measurements every day were recorded. The analysis of individual differences between days regarding the percentage of easily hydrolysable nitrogen by slope of growth revealed that there are significant differences between days 2 - 3, and 3 - 4, suggesting an acceleration from day 2 in increasing the amount of easily hydrolysable nitrogen in the sample. The average difference between the percentages of easily hydrolysable nitrogen was 3.24% on days 2 and 3 and 2.70% between days 3 - 4. For pH, significant difference between two consecutive days were registered only on days 3 - 4, with a mean difference of 0.30 pH value units. Both in the case of easily hydrolysable nitrogen and in that of pH, the amount of oil used was significant to determine differences in the evolution of parameters.

The type of oil used did not influence significantly the evolution in time of easily hydrolysable nitrogen and pH indicators.

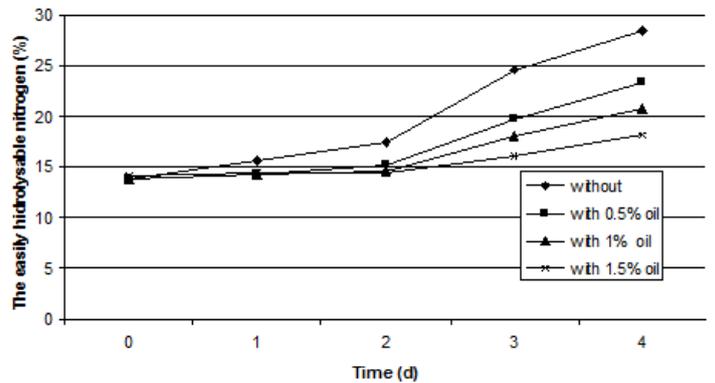


Figure 5 Variation of easily hydrolysable nitrogen percentage on days 0-4, for 0.5%, 1.0% and 1.5% concentration of oil added

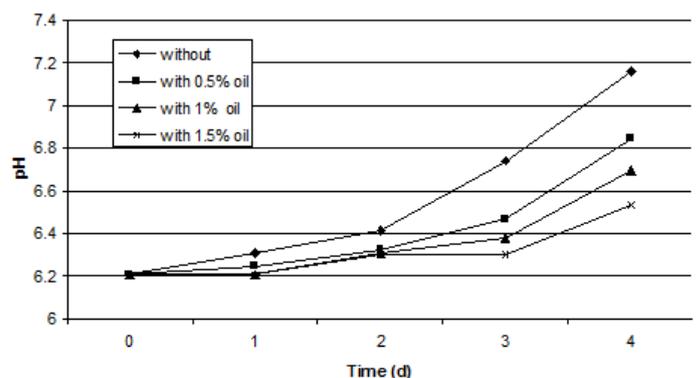


Figure 6 pH variation, on days 0-4, 0.5%, 1.0% and 1.5% concentrations of oil

Figure 7 illustrate the evolution of easily hydrolysable nitrogen percentage on 0-4 days, for the control sample and for each type of oil and the amount of oil incorporated. The lines representing the highest oil concentration used are located at the bottom of the graph, and thyme oil of 1.5% concentration corresponds to the lowest percentage of final easily hydrolysable nitrogen, the slope that increases the percentage of easily hydrolysable nitrogen in days 2-4 being the lowest. Figure 7 confirms the statistical results presented in Table 1, according to which the growth rate of easily hydrolysable nitrogen in all samples is accelerating from day 2, except for that of thyme oil in concentration of 1.5% which seems to adjourn the process to the 3rd day.

The pH evolution from Figure 8 confirms the results mentioned above, higher concentrations of the oil resulting in a less steep increase slope of pH value. Also, it can be seen that all three oils at maximum concentration of 1.5% incorporated adjourn the acceleration from day 2 to 3rd day that it was found to control sample. The differences between day 2 and day 4 remain considerable. Recent studies demonstrated that evaluation of essential oils in different products with refrigeration and frozen methods (Erkan 2012; Erkan et al. 2011a) or with modified atmosphere packaging (Erkan et al. 2011b) can be applied as effective conservation methods in order to extend the shelf-life of processed food without the use of chemical preservatives.

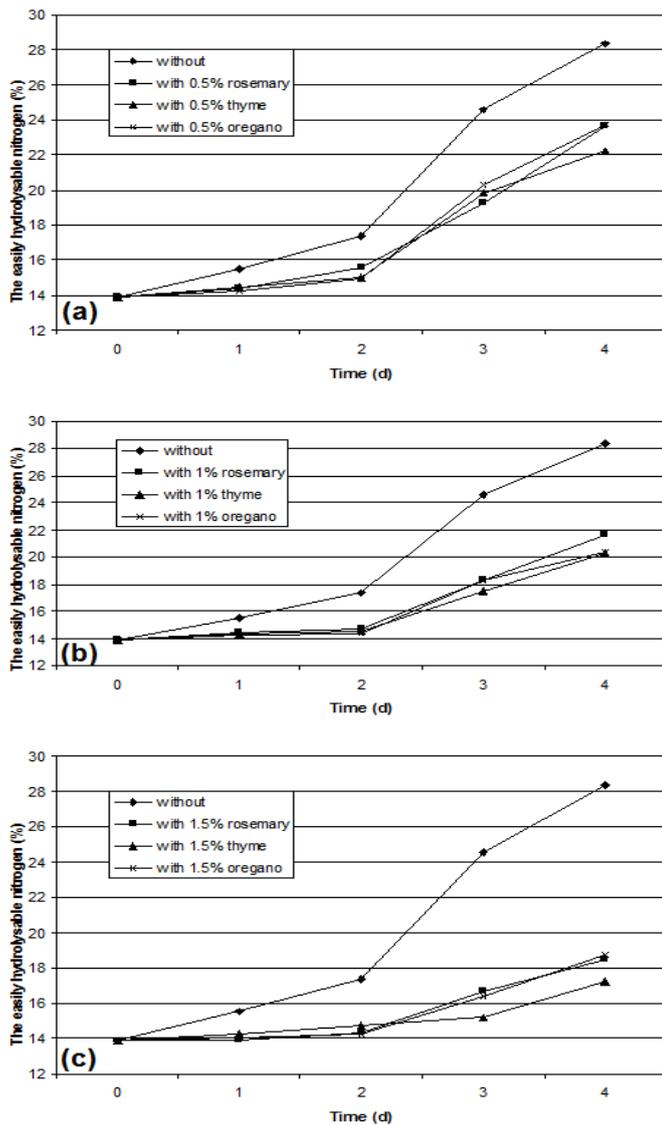


Figure 7 The evolution of the easily hydrolysable nitrogen percentage on days 0-4, depending on the type and amount of oil used (concentration of: (a) 0.5%, (b) 1% and (c) 1.5%)

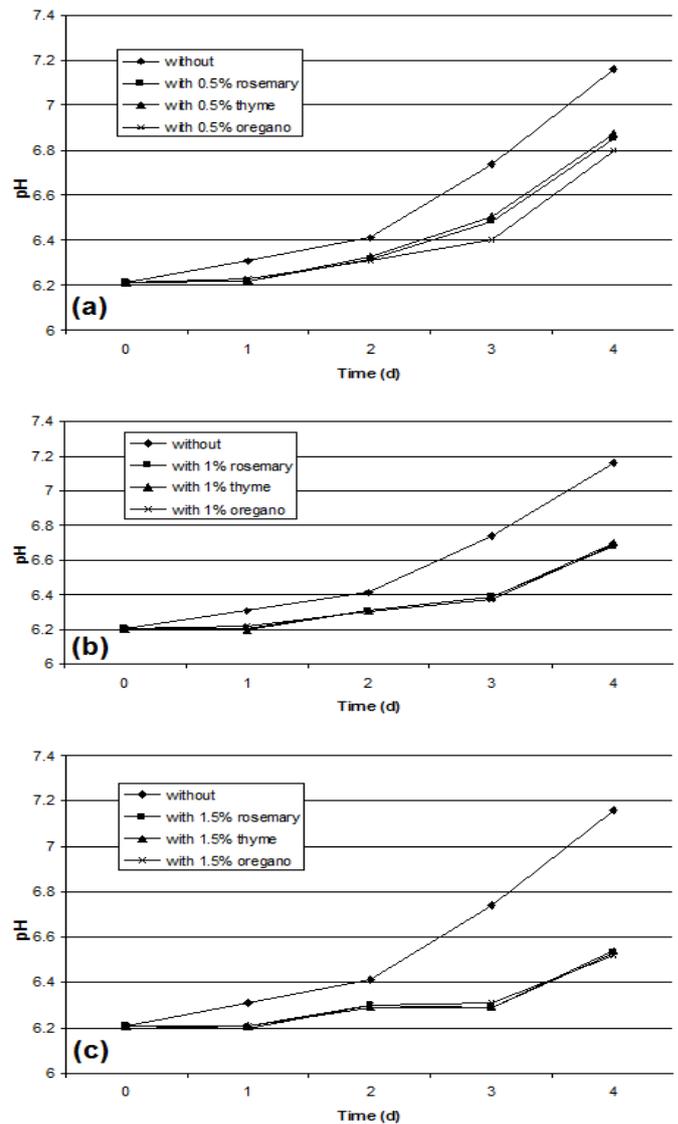


Figure 8 - Evolution of pH on days 0-4 depending on the type and amount of oil used (concentration of: (a) 0.5%, (b) 1% and (c) 1.5%)

CONCLUSION

In this paper was analysed the influence of three different oils rosemary, thyme and oregano on the physicochemical and microbiological properties of minced meat. The oils analysis were performed by applying DPPH method, also the microbiological activity was evaluated. From the analysis of experimental data and their statistical processing it can be concluded that the incorporation of essential oils in minced meat has differentiated effects depending on the parameter analysed. Sensory properties, with major impact on the purchasing decision, were maintained in the range of acceptability for the samples containing essential oils. Unlike these, the control sample at the end of the test, showed changes in odour and colour being unfit for consumption. Parameters such as humidity, total protein, total fat, are not affected by the type or content of essential oil. Indicators regarding the degree of meat degradation, easily hydrolysable nitrogen and pH showed increased values from day to day, but lower ones for the samples added with essential oils and much higher for the control sample. When increasing the concentration of essential oil added, the increase is balanced. No significant differences were found in the efficiency between the types of essential oil used.

It should be noted that for the previously named parameters, the rate of increase is not uniform throughout the testing period, an increasing in easily hydrolysable nitrogen accumulation and sample alkalinization after the third day were observed. In conclusion, the use of essential oils could improve both microbial stability and sensory quality. Thus, this study suggests the possibility of using thymus, oregano and rosemary oils as natural antioxidant in food industry, where they may be considered natural preservatives to replace synthetic preservatives of which consumers are increasingly distrustful. The pleasant taste, smell and low toxicity indicate their use as additives to prevent bacterial contamination.

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