JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2013. Vol. **7**(1), p. 221-228

Effect of *Origanum heracleoticum* L. Essential Oil on Marinated Chicken Meat Shelf-life

Ivana Cabarkapa[1]*, Marija Skrinjar[2], Nevena Blagojev[2], Jasmina Gubic[1], Dragana Plavsic[1], Bojana Kokic[1] and Tanja Radusin[1]

[1]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.
[2]Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.

(Received: 13 August 2012; accepted: 22 September 2012)

Poultry meat is a food which has been accepted worldwide throughout the ages, mostly due to its relatively low cost of production, low fat content and the high nutritional value. The consumption of poultry products is increasing every year and consumers want a safe product. At present, meat industry uses chemical additives to prevent growth of food-borne pathogens and extend the shelf life of refrigerated storage. Since concern over the safety of chemical additives has arisen in recent years, consumers increasingly demand use of natural products as alternative preservatives in foods. Among several essential oils that may be useful as antimicrobial agents, oregano oil (*Origanum heracleoticum* L.) may have the greatest potential for use in industrial applications. In this context, the aim of the present work is to evaluate the effect of addition of oregano essential oil in marinade in three concentrations (1.25%, 0.625% and 0.156%) on the shelf - life of marinated chicken fillet and their sensory properties.

Key words: Essential oil, *Origanum heracleoticum* L., Marinated meat, Shelf-life.

Poultry meat is a food which has been accepted worldwide throughout the ages, mostly due to its relatively low cost of production, low fat content and the high nutritional value[1]. The consumption of poultry products is increasing every year and consumers want a safe product. Fresh meat and meat products are very susceptible to microbial contamination, which leads to quality loss and public health hazards. The main problems that poultry producers need to solve are: reduce spoilage, avoid food borne diseases and preserve or develop an attractive flavor, taste and appearance of the meat. Refrigeration storage is usually the most common preservative method of fresh meat and meat products[2]. As in many refrigerated foods, microbial growth is generally responsible for the spoilage in meats and meat products together with biochemical and enzymatic deteriorations[3]. At present, meat industry uses chemical additives to prevent growth of food-borne pathogens and extend the shelf life of refrigerated food. Since concern over the safety of chemical additives has arisen in recent years, consumers increasingly demand use of natural products as alternative preservatives in foods. Particular interest has been focused on the potential application of plant essential oils as safer additives for meat[4]. The modern trends in nutrition suggest the limitation of synthetic food additives or the substitution with natural ones. Aromatic herbs are rich source of antimicrobial compounds, among which genus *Origanum* occupies special position.

Chemical analysis of the oregano essential oil (EO) revealed the presence of several

* To whom all correspondence should be addressed.
Tel.: +381 21 485 3822, Fax: +381 21 450 725
E-mail: ivana.cabarkapa@fins.uns.ac.rs

ingredients, most of which have important antioxidant, antibacterial and antifungal properties[5,6,7]. This is basically due to major components being carvacrol and thymol, which act as preservatives. Carvacrol and thymol, two main phenolic compounds that constitute about 78–85% of oregano oil, are principally responsible for the antimicrobial activity of the oil[8].These phenolic compounds making reasonable that their mechanism of action is the disturbance of the proton motive force (PMF), electron flow, active transport and coagulation of cell contents. Instead, enzymes such as ATP_{ases} are known to be located in the cytoplasmic membrane and be bordered by lipid molecules. In addition, other minor constituents such as monoterpene hydrocarbons γ-terpinene and p-cymene also contribute to antimicrobial activity of the oil[9]. Carvacrol and thymol do not exhibit adverse effects on human health, and are proven not to cause either significant or marginal toxic effects at cellular level. Also, the concentrations at which they exhibit antimicrobial activities are not at possible genotoxic level[10]. Several investigations have confirmed the antimicrobial action of essential oils against foodborne pathogens and spoilage bacteria in synthetic media, food systems and real foods[4,11]. However, higher concentrations of essential oils are needed to achieve the same effect in foods as in synthetic media. These higher concentrations needed to inhibit spoilage and pathogen bacteria in food matrixes could often exceed the flavor threshold acceptable to consumers. Considering the above, the aim of the present study was to investigate an effect of addition of oregano essential oil in marinade in three concentrations (1.25%, 0.625% and 0.156%) on the shelf - life of marinated chicken fillet and their sensory properties.

MATERIALS AND METHODS

Plant Material

Aerial parts of *O. heracleoticum* L. (*O. vulgare* L. ssp. *hirtum*) were collected during blooming stage (August 2009) from the locality Kamendol near Smederevo, Serbia. The plant material was dried under laboratory conditions (20-25ºC). Institute of Medicinal Plant Research Dr. Josif Pancic identified the plants and voucher specimens were stored in the herbarium of the Institute of Medicinal Plant Research Dr. Josif Panèiæ.

Isolation of the essential oil

The essential oil was isolated from dried plant material by hydro-distillation according to the standard procedure reported in the Sixth European Pharmacopeia[12]. Distillation was performed using Clevenger type apparatus, for 2.5 hours. The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4ºC.

Marinated meat preparation

Marinades were prepared of fresh oregano oil distillate diluted with sunflower oil so that the final concentration of the oregano essential oil in marinade was 1.25%, 0.625%, 0.156%. On each 15 ml of marinade, 5 g of spice mixture (with vegetables) were added. Fresh chicken filets, average mass 150 g, were packed in polystyrene containers, dimensions 100 x 150mm, poured with 15 ml of marinade and covered with PVC foil. For each concentration, 21 samples were prepared, as well as 21 control samples, containing all components except oregano oil. All samples were stored at 4°C. Sampling was carried out every 24h during 7 days.

Microbiological analyses

The total viable counts (TVC) were monitored during 7 days of storage, using horizontal method for the enumeration of microorganisms – colony count technique at 30°C[13]. Twenty-five grams of each sample were measured and aseptically transferred into stomacher bags containing 225 ml peptone salt dilution fluid, and then homogenized using stomacher homogenizer (Easy MIX) during 60 s. Serial decimal dilutions (10^{-1} to 10^{-4}) were than prepared for each sample. Sterile Petri dishes were inoculated with dilutions, and poured with 15 ml of Plate Count Agar. Experiment was done in triplicates, while microbiological analyses of each sample were carried out in duplicates. Obtained data were expressed as decade logarithm of colony forming units (log cfu/g). Data were subjected to the analyses of variance (ANOVA) with 95% confidence interval ($p \leq 0.05$), and mean comparisons were carried out by Duncan's multiple range test. Analyses were performed using Statistics package StatSoft, Inc.[14].

Sample preparation for sensorial analyses

Marinated chicken filets prepared using the same concentrations of oregano oil in marinade as for the microbiological analyses (1.25%, 0.625%

and 0.156%), as well as control samples, were submitted for sensorial analyses. Square pieces (2 x 2cm) of each sample were cut off. Samples were then heat treated by dry baking (in oven, at 175°C, on thin layer of grease, until the temperature in the middle of the meat reaches 72°C). Samples were prepared and served to panelists at unified temperature. Each sample was coded by random three digit number, and they were presented to the panelists also randomly. Within one session, one sample of each tasted concentration and one control sample were given to the panelists.

Table 1. Sensory quality evaluation of heat treated meat

Point	Odour	Taste	Softness	Juiciness	Overall acceptability
1	Extremely bad	Extremely bad	Tough	Extremely bad (very dry)	Enough
2	Bad	Bad	Moderately tough	Bad (dry)	Neither good or bad
3	Neither good or bad	Neither good or bad	Moderately tender	Moderately juicy	Good
4	Good	Good	Tender	Juicy	Very good
5	Very good	Very good	Very tender	Very juicy	Very good

Sensorial analyses

Sensorial analyses of marinated chicken filets were carried out according to general sensory analysis guidance, given in ISO 6658/2002[15]. In order to evaluate the influence of different concentrations of oregano oil in marinade on the sensory characteristics, samples were assessed by a trained panel of 8 members. Sensory quality evaluation was performed using analytical descriptive scoring system, with a scale of 0-5 points. Every point is defined descriptively (Table 1). Sensorial characteristics that were evaluated are: odour, taste, softness, juiciness and overall acceptability.

Test has been formed on the basis of sensory profile, i.e. descriptions of sensorial characteristics of samples, in order of their perception, assigning the proper value for each characteristic. The procedure was repeated for each concentration and control sample on the 1st, 3rd, 5th and 7th day of storage. Using the average values obtained by 8 panelists, sensory profile of each tested sample has been formed.

RESULTS AND DISCUSSION

From the collected plant material of *O. heracleoticum* L. total of 2.05% (v/w) of essential oil has been isolated by the process of hydro-distillation. In our previous investigation twenty six components (92.86 %) were identified as constituents of this essential oil by GC/MS analysis[7]. The major components were carvacrol (69.00%), p-cymene (10.50%), thymol (7.94%) and γ-terpinene (2.86%). Except β–caryophyllene (1.53%) and β–bisabolene (1.01%) the amount of all remaining oil components was less than 1%.

Microbiological changes of marinated chicken filets, during the 7 days of storage at 4°C, have been shown in the Table 2. Initial number of total viable counts (TVC) (day 0) was 4.37 log cfu/g, which indicated that chicken filets used for analyses were of acceptable quality. In all tested samples containing different concentrations of oregano oil, TVC value after the 1st day of storage was reduced. TVC reduction was directly related to the oregano oil concentration in marinade. The highest TVC reduction was achieved in samples containing 1.25% of oregano oil in marinade, after the 1st day of storage, and it was 1.37 log cfu/g. After the 1st day of storage, reduction of TVC value in samples with 0.625% and 0.156% oregano oil in marinade was 0.18 log cfu/g and 1.06 log cfu/g, respectively, comparing to the control sample (Table 2).

Obtained results are in accordance with results given by Chouilara *et al.* (2007)[1], who reported that TVC value in chicken meat containing 1% of oregano oil in marinade after the 2nd day of storage was reduced for 2.28 log cfu/g. Results are

also in accordance with investigations of Tsigarida *et al*. (2000)[16], who achieved the TVC value reduction of 2-3 log cfu/g, in beef filets containing 0.8% essential oil of oregano. Reduction of initial micropopulation of 1 log cfu/g in meat was achieved in research of Scandamis and Nychas, (2001)[17], by treating the minced beef meat with 1% essential oil of oregano.

Table 2. Effects of three tested concentrations of oregano oil in marinade on TVC in marinated chicken filets

Storage duration (day)	Control	1.25%	0.625%	0.156%
0	4.37 ± 0.04^{h}	4.37 ± 0.04^{h}	4.37 ± 0.04^{h}	4.37 ± 0.04^{h}
1	4.55 ± 0.06^{ij}	3.0 ± 0.2^{a}	3.31 ± 0.19^{b}	4.19 ± 0.22^{g}
2	4.65 ± 0.03^{j}	3.52 ± 0.18^{c}	3.60 ± 0.24^{cd}	4.28 ± 0.10^{gh}
3	4.82 ± 0.08^{k}	3.73 ± 0.16^{dc}	3.97 ± 0.18^{f}	4.43 ± 0.04^{hi}
4	4.97 ± 0.08^{l}	3.80 ± 0.11^{e}	4.18 ± 0.13^{g}	4.79 ± 0.07^{k}
5	5.12 ± 0.03^{m}	4.38 ± 0.12^{h}	4.60 ± 0.13^{j}	4.93 ± 0.08^{kl}
6	5.94 ± 0.04^{op}	4.55 ± 0.14^{ij}	4.80 ± 0.06^{k}	5.28 ± 0.15^{n}
7	6.02 ± 0.07^{p}	4.89 ± 0.09^{kl}	5.28 ± 0.08^{n}	5.85 ± 0.19^{op}
8	NT	5.21±0.10	5.59±0.03	NT

The values are represented as mean (log cfu/g) ± *SD, n=3*
NT-not tested
Mean values marked with different letters are significantly different (P<0.05)

As it can be seen in Table 2, TVC in control sample after the 1st day of storage was 4.55 log cfu/g, while that value was achieved in samples with 1.25% of oregano oil in marinade after the 6th day of storage. In samples with lower concentrations of oregano oil in marinade, 0.156% and 0.625%, this value was achieved between 3rd and 4th, and between 4th and 5th day, respectively. After the second day of storage, in all tested samples slight increasing of TVC was noticed. In control samples after the second day of storage TVC was 4.65 log cfu/g, while in samples containing 1.25% and 0.625% of oregano oil in marinade this number was registered between 6th and 7th day and on 5th day, respectively. After the 3rd day of storage, TVC in control samples reached the value of 4.82 log cfu/g. This value was achieved in samples containing 0.156%, 0.625% and 1.25% oregano oil in marinade on 4th, 6th and 7th day of storage, respectively. According to recommendations of Health Protection Agency (HPA), microbiological limit for total bacterial count in commodities intended for heat treatment before consumption has been set to 5 log cfu/g [18]. In this experiment, the limit was reached between 4th and 5th day of storage in control samples only. Considering the fact that in samples containing 1.25% of oregano oil in marinade, microbiological limit have not been achieved even on the 7th day of storage, the TVC was tested in the samples with 1.25% and 0.625% of oregano oil in marinade on the 8th day as well. In samples with 0.156%, 0.625% and 1.25% oregano oil in marinade, microbiological limit was reached on 6th, 7th and 8th day of storage, respectively. TVC in control samples after the 5th day of storage was 5.12 log cfu/g. Microbiological limit of 5 log cfu/g in samples treated with oregano oil marinade was achieved on 8th day of storage in samples containing 1.25% of oregano oil in marinade, on 7th day in samples with 0.625% oil, and on 6th day in samples with the lowest concentration of oregano oil in marinade (0.156%). Chouliara *et al*. (2007)[1] investigated the effect of oregano oil (0.1% and 1% w/w) on poultry meat shelf life. In their research, microbiological limit was set on 7 log cfu/g, and it was reached on 6th day in samples containing 0.1% of oregano oil, and on 25th day in samples containing 1% of oregano oil. In this research the number of 5 log cfu/g was exceeded on 3rd day in control samples and in samples with 0.1% of oregano oil, and on 15th day in samples with 1% of oregano oil. Obtained results showed that oregano oil extended lag phase of bacterial growth, which has been already confirmed in some other studies[1, 4, 19]. Therefore, it could be observed that shelf life of

marinated poultry products can be extended for two and three days using marinade with 0.625% and 1.25% of oregano oil, respectively. According to Burt (2004)[9], bacterial reduction of 1.5 log cfu/g comparing to the control, has been considered as slight. Reduction of antimicrobial properties of oregano oil in this study could arise from mixing oregano oil with sunflower oil, because dilution of essential oils in lipid phase attenuates their effects. Besides, in some other investigations where higher bacterial reduction was observed, essential oils were directly applied on meat, which resulted in higher bacterial reduction, but sensorial properties of that meat were poor. Investigations concerning antimicrobial properties of essential oils in food models revealed that significantly higher concentrations of oils are needed to obtain the same effect as in nutritive media[20], which was also confirmed in this study. This could be explained by high diversity of food components (lipids, proteins, water, antioxidants, preservatives, salt and other additives), unlike laboratory nutritive media[21].

Sensorial analyses of heat treated marinated chicken filets was done after 1st, 3rd, 5th and 7th day of storage. Results are showed on Figs. 1-5.

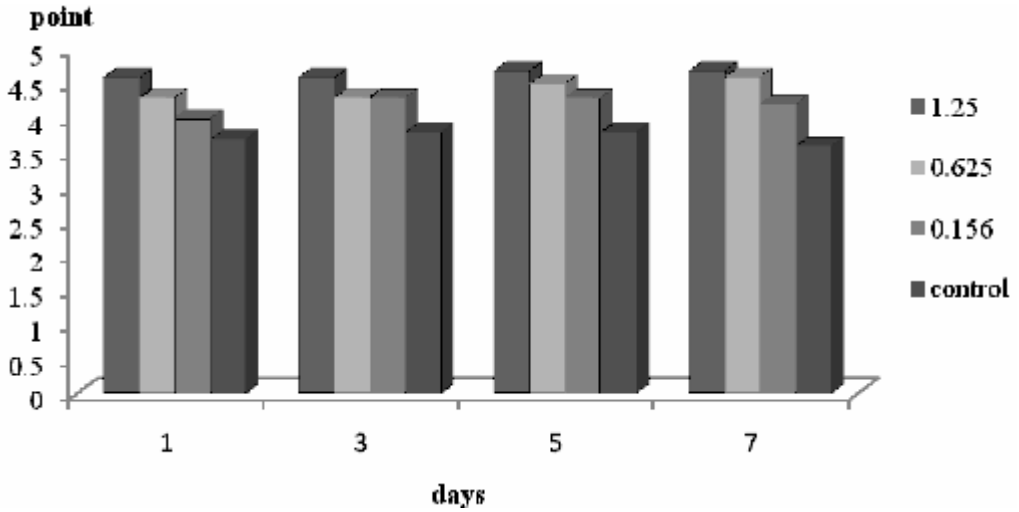

Fig. 1. Sensory evaluation of odour heat treated marinated chicken filets

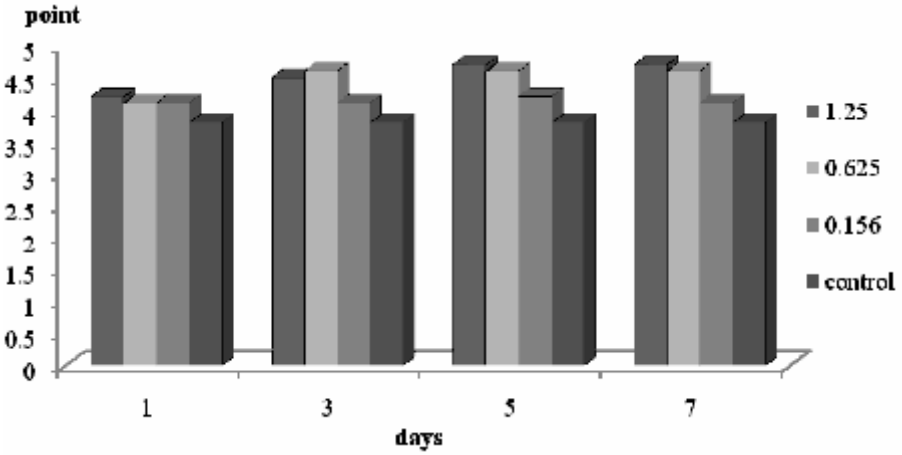

Fig. 2. Sensory evaluation of taste heat treated marinated chicken filets

Results of sensorial analyses of chicken filets marinated with oregano oil marinade showed that addition of marinade has positive effects on tested sensorial properties odour and taste, comparing to control samples, during the 7-day period of storage. Obtained results are in accordance with results given by Skandamis and Nychas (2001)[17], who reported that addition of 1% essential oil had positive effects on odour and colour of beef minced meat, during the storage at 5ºC. Positive effect of essential oregano oil addition on meat sensorial properties has been also reported by some other researches. Govaris *et al*. (2010)[4] treated minced lamb meat with oregano oil in concentrations of 0.6% and 0.9% and also pointed out positive effect of essential oil on sensorial properties of meat. Within this research, samples treated with 0.9% oregano essential oil showed better odour properties during storage comparing with control sample, as well as better taste and overall acceptability. Authors of this study also reported that samples which obtained the highest values for sensorial properties odour, taste and overall acceptability were treated with 0.6% of oregano oil[4].

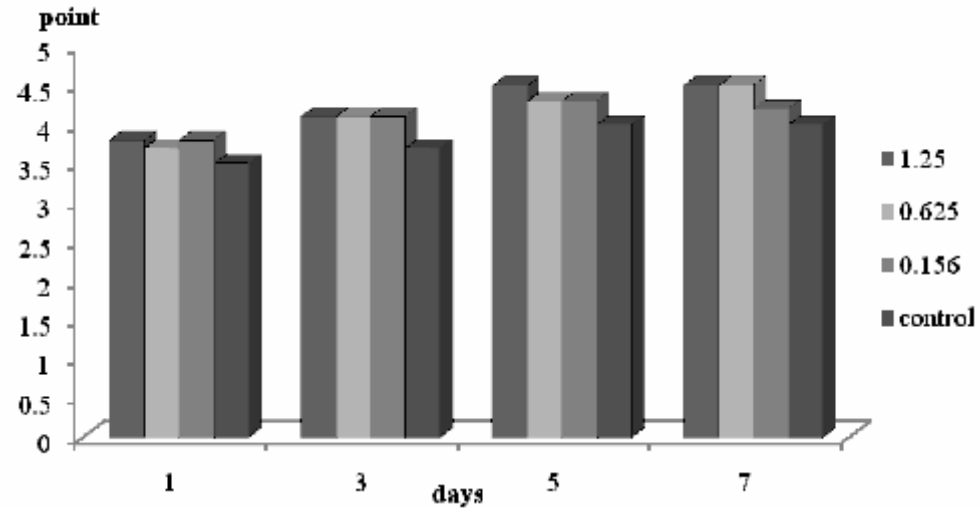

Fig. 3. Sensory evaluation of softness heat treated marinated chicken filets

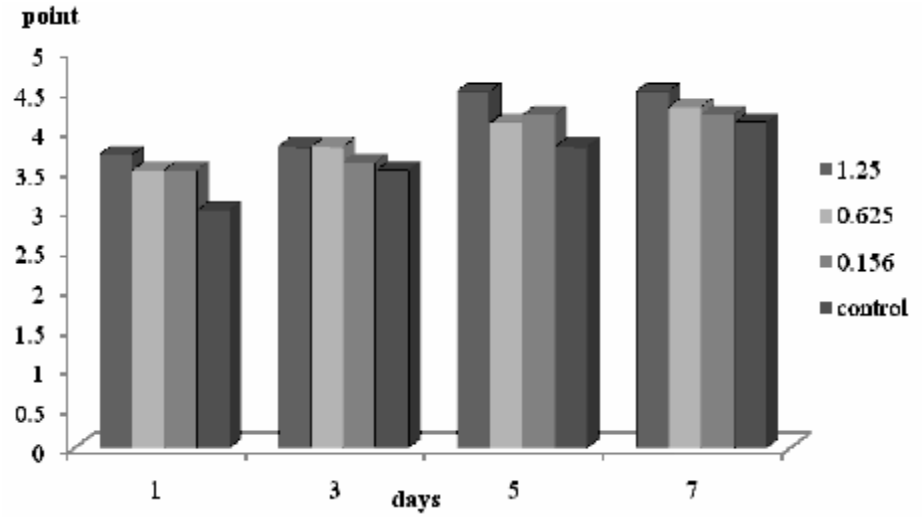

Fig. 4. Sensory evaluation of juiciness heat treated marinated chicken filets

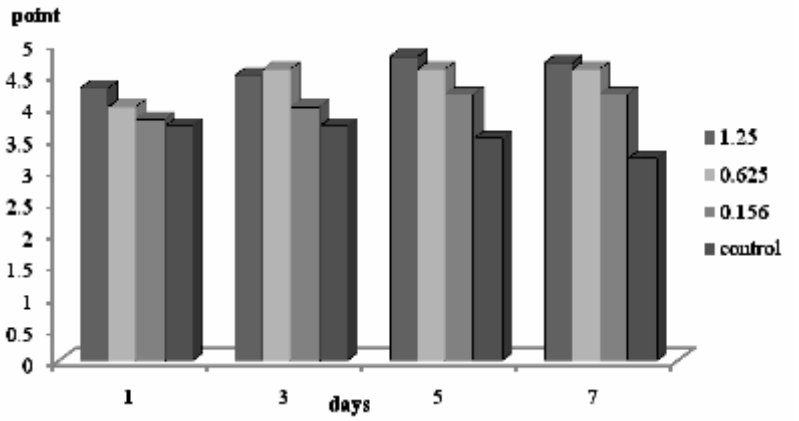

Fig. 5. Sensory evaluation of overal acceptability heat treated marinated chicken filets

On the contrary, Chouliara *et al*. (2007)[1] investigated the effect of oregano oil in concentrations 0.1% and 1% w/w on sensorial properties (odour and taste) of poultry, and they eliminated all samples with 1% w/w of oregano oil from further sensorial analyses because of too strong odour. Control samples and samples with addition of 0.1% w/w of oregano oil were valuated as unacceptable, with 3.5 grades within this study, after 6th and 9th day, respectively, regarding both tested properties.

Results of sensorial analyses of heat treated marinated chicken filets showed that addition of marinade containing 1.25% and 0.625% of oregano oil in final concentrations had positive effects on sensorial properties odour and taste, comparing with control samples, during the period of storage. Addition of oregano oil also affected the texture (softness and juiciness) of heat treated marinated chicken filets, and had positive effects comparing with control samples.

Addition of oregano essential oil in the marinade had significant effect on sensorial properties (odour and taste) and texture (tenderness and juiciness) of heat-treated chicken fillet marinated in comparison with control samples during storage.

CONCLUSION

Results obtained during this study indicated the possibility to extend shelf-life of marinated meat for two days and three days by adding the marinade with the addition of oregano essential oil in a final concentration of 0.625% and 1.25%, respectively. Thus, the addition of oregano essential oil in marinade appears to be a promising method for enhancing the shelf-life of the meat products. Application of essential oil in food could provide a more natural and attractive alternative to industry, meaning an additional barrier to inhibit the growth and survival of microorganism in foods. Chemical preservatives can be replaced with essential oils; this provides the opportunity for 'Green labelling' to which consumers are attracted by their 'natural image'. This is highly relevant because food quality and safety are of prime importance in the current world environment.

ACKNOWLEDGMENTS

These results are part of a research on the Project III 046009, financed by the Ministry of Education and Science, Republic of Serbia.

REFERENCES

1. Chouliara, E., Karatapanis, A., Savvaidis, I.N., Kontominas M.G. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 °C. *Food Microbiol.,* 2007; **24**: 607-617.
2. Solomakos, N., Govaris, A., Koidis, P., Botsoglou, N. The antimicrobial effect of thyme essential oil, nisin, and their combination against *Listeria monocytogenes* in minced meat. *Food*

Microbiol., 2008; **25**: 120-127.
3. Zehra, K.E, Gökce, P.Y, Betûl, K.C., Kezban C. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. *Meat Science,* 2010*;* **86**: 283-288.
4. Govaris, A., Solomakos, N., Pexara, A., Chatzopoulou, P.S. The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella Enteritidis* in minced sheep meat during refrigerated storage. *Int. J. Food Microbiol.,* 2010; **137**: 175-180.
5. Tsimogiannis, D., Stavrakaki, M., Oreopoulou, V. Isolation and characterization of antioxidant components from oregano (*Origanum heracleoticum* L.). *Int. J. Food Sci. Tech.,* 2006; **41**: 39-48.
6. Dzamic, A., Sokovic, M., Ristic, M.S., Grujic-Jovanovic, S., Vukojevic, J., Marin, P.D. Chemical composition and antifungal activity of *Origanum heracleuticum* essential oil. *Chem. Nat. Comp.,* 2008; **44**(5): 659-660.
7. Cabarkapa, I., Skrinjar, M., Blagojev, N., Milovanovic, I. Effect of *Origanum heracleoticum* L. essential oil on food-borne *Penicillium aurantiogriseum* and *Penicillium chrysogenum* isolates. *Proc. Nat. Sci.,* 2011; **120**: 83-93.
8. Kokkini, S., Karousou, R., Dardioti, A., Krigas, N., Lanaras, T. Autumn essential oils of Greek oregano. *Phytochemistry.,* 1996*;* **44***:* 883–886.
9. Burt S. Essential oils: their antibacterial properties and potential applications in food – a review. *Int. J. Food. Microbio.,* 2004; 94: 223-253.
10. Stammati, A., Bonsi, P., Zucco, F., Moezelaar, R., Alakomi, H.L., Von Wright, A. Toxicity of selected plant volatiles in microbial and mammalian short-term assays. *Food Chem. Toxicol.,* 1999; **37**: 813–823.
11. Tassou, C., Drosinos, E.H., Nychas, G.J.E. Inhibition of resident microbial flora and pathogen inocula on cold fresh fish fillets in olive oil, oregano, and lemon juice under modified atmosphere or air. *J. Food Protect*., 1996; **59**(1): 31–34.
12. European pharmacopoeia (ed): 6th, Strasbourg, Council of Europe, 2008.
13. ISO 4833. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count technique at 30°C. 2003; ISO, Geneva.
14. StatSoft, Inc. (2010). STATISTICA (data analysis software system), version 9.1. www.statsoft.com
15. SRPS ISO 6658. Sensory Analysis, Methodology, General Instructions, 2002
16. Tsigarida, E., Skandamis, P., Nychas, G.J.E. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 ÚC. *J. Appl. Microbiol*., 2000; **89**: 901–909.
17. Skandamis, P.N., Nychas, G.J.E. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. *J. Appl. Microbiol.,* 2001; **91**: 1011–1022.
18. HPA draft (2009). Guidelines Microbiological Safety of Ready to Eat foods, Consultation from 01/12/08 to 20/02/09.
19. Gutierrez, J., Bary-Ryan, C., Bourke, P. Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. *Food Microbiol*., 2009; **26**: 142-150.
20. Smid, E.J., Gorris, L.G.M.: Natural antimicrobials for food preservation. In: *Handbook of Food Preservation* (Rahman MS, ed). New York: Marcel Dekker, 1999; pp 285–308.
21. Gill, A.O., Delaquis, P., Russo, P., Holley, R.A. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *Int. J. Food Microbiol.,* 2002;**73**: 83-92.