IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 6 Ver. II (Jun. 2016), PP 63-68
www.iosrjournals.org

Preservative Effects of *Thymus Citriodorus* and *Rosmarinus Officinalis* Volatile Oils on Prolonging Shelf Life of Raw Chicken

Dara M. Jamil[1]
Faculty of Agricultural Sciences-Sulaimani University, Bakrajo Street, Sulaimaniyah - Iraq

Abstract: *The volatile oils of Rosemarinus Officinalis (rosemary) and Thymus citriodorus (Lemon thyme) at different concentration (0.1, 0.25, and 0.5) % were used to examine their preservation impacts on raw chicken.). Chicken pieces were immersed in ethanolic solutions (10 %) of the volatile oils of rosemary and lemon thyme for 30 minutes stored in (30^{o}C) and in a fridge (4^{o}C.After the first day, control samples were found completely putrefied, whereas samples treated with VOs (0.25 and 0.5 %) were found with acceptable organoleptic attributes and significant microbial prohibition for five days at (30^{o}C) and for 10 days in the fridge (4^{o}C). It can be concluded that beside their desirable flavours, the herbal VOs used can be considered as a natural preservative to prolong shelf life of raw poultry and this could have an economical potential on raw chickens at industrial level.*

Keywords: *Food preservation, raw chicken, Rosmarinus Officinalis, Thymus citriodorus, Volatile oil*

I. "Introduction"

Raw meats (cattle, poultry and sea foods) are known as perishable foods because they are ideal mediums for microbial growth, so they get spoiled and/or poisoned in storage unless they are freshly consumed or preserved, thus a number of preservation techniques, including freezing, heat treatment, salting, acidification, and drying have been used in the food industry [1] and [2].

Meat spoilage is the deterioration of texture and change in flavor and color. The use of natural antibacterial extracts of herbs and spices, essential oils, organic acids, salts, and bacteriocins is reported in the literature to improve the shelf life of meat [3] and [4]. Since plant essential oils are Generally Recognized as Safe (GRAS) [5], a wide interest in food industry as well as consumers have shown to replace the synthetic preservatives for their potential as decontaminating agents.

Rosemary (*Rosmarinus officinalis L.*) essential oil is widely used for preservation by the food industry because the oil has shown to inhibit the growth of various foodborne pathogens in vitro [6] and [7]. Further, the impacts of rosemary essential oil as a natural antioxidant that prevented color deterioration and/or lipid oxidation have been reported by many researchers [8] and Yu [9], however, its effectiveness when applied to food has not been extensively investigated.

Chemical composition of essential oils of rosemary after hydro distillation have been characterised using GC/MS. The main terpens and terpenoids found were α-pinene (43.0 – 46.1%), 1, 8-cineole (11.1%), camphene (8.6 – 9.6 %), camphor (2.4 – 5.3 %), broneol (3.4%), bornyl acetates (2.8%), sabinene (4.6%), myrcene (3.9%), verbenol (2.3%) and linalool (2.1%) [10]. The main compounds responsible for the antimicrobial activity are α-pinene, bornyl acetate, camphor and 1,8-cineole [11] and[12], whereas carnosic acid and carnosol has been related to the antioxidant activity of rosemary extracts [13].

Another herb which is less investigated in food preservation is lemon thyme (*Thymus citriodorus*) with a desirable lemon scented mainly used in some culinary foods. Though the applications and researches on this herb is electronically limited, some researchers however has tested the herbal antimicrobial properties. For instance, Gianni et al. tested eleven essential oils from herbs and spices for their antimicrobial properties on five food spoilage yeasts, *Thymus citriodorus* and *Cymbopogon citratus* (Poaceae) were found as the most effective against the tested strains [14].Importantly, a report was published on the chemical composition of essential oil of *Thymus citriodorus* and its toxic effect on liver cancer cells, showing the essential oil have strong toxic effects on the cancer cells [15] and [16].

The hydro-distilled essential oil of lemon thyme (*Thymus citriodorus* L.) cultivated in Iran was analyzed for its main constituents including geraniol (54.4%), geranial (13.9%), neral (10.1%), nerol (5.2%), 3-octanone (3.3%) and borneol (3.2%) [17]. Earlier the essential oil of *Thymus* x *citriodorus* was analysed and Geraniol was found to be the main compound (> 60%), among other compounds detected were Geranyl acetate (1.0%), geranyl butyrate (0.8%), nerol (2.8%), and citronellol (0.3%). Further the lemon-scented compounds geranial and neral amounted to 8.2% and 5.5% respectively. [18].

Consumer awareness and concerns regarding the adverse impact of synthetic chemical additives, have been the driving forces for preserving food with natural preservatives. The aim of this study is to evaluate the preservative impact of *Rosmarinus Officinalis* and *Thymus citriodorus* volatile oils as natural preservatives in

delaying the spoilage of raw chicken. It is worth to mention that this is for the first time to investigate the antimicrobial activities of lemon thyme and probably rosemary too on raw poultry in Kurdistan and Iraq.

II. "Materials and Methods"

2.1. Materials

The aerial parts of rosemary and lemon thyme were collected from the author's home garden from which specimens were given to the faculty of agricultural sciences, fresh whole chickens purchased from local grocery shop that were cut in to pieces and divided into seven equal portions (wings, breasts and legs i.e. thighs and drumsticks). Pure cultures of *Escherichia coli* and *Pseudomonas aeruginosa* obtained from microbiology's laboratory of the food sciences department.

2.2. The Methods

Following shade drying the herbal leaves of rosemary and lemon thyme, the volatile oils were extracted by hydro distillation using Clevenger in several successive times [19]. The volatile oil (VO) contents were measured in the graduated trap followed by transferring it into a vial using a pasture pipette and stored in the freezer until used.

2.2.1. Preparation of Rosemary and lemon thyme VO solution

Prior selecting the concentrations used, the minimum inhibitory concentration (MIC) of the VOs against total counts of *E.coli* and *P.aeruginosa*growth on agar plates was determined, upon which three concentrations (0.1 %, 0.25 % and 0.5 %) of Rosemary and Lemon thyme VOs in ethanolic solutions (10 %) were prepared in three different sterile containers. Volumes of 300µL, 750µL and 1.5mL of the VOs were precisely measured and added to 300 mL ethanolic solution in the containers using cylinder measurements, followed by a vigorous mixing.

2.2.2. Measurement of initial microbial load on raw chicken

The seven portions of raw chicken were equally divided on 3 concentrations of each herbal VO at two different temperatures (4^oC and 30^oC) and one group for control. Prior cutting into pieces, the poultry (three 25 cm^2 areas of chicken carcass) were swabbed with sterile cotton tipped swabs at all sides followed by immersing the swab in 10 mL sterile normal saline to release the bacteria. The swab rinsed solution is then diluted appropriately and inoculated on nutrient agar plates using Miles and Misra technique [20]. The duplicate plates were incubated at 35^oC for 48 hr. This total microbial count was used as an initial biota (Control 1) for both treatments of the raw chicken stored at 4^oC and 30^oC.

2.2.3. Measurements of antimicrobial activities of the VOs

The chicken portions (wing, legs and breast) were separately immersed in the VO solutions (0.1, 0.25, 0.5 %) in two treatments and left for 30 minutes. The same procedure was applied to the chicken pieces in ethanolic solution (10 %) without VOs (Control 2). The samples and control were aseptically drained and separately placed on aluminum foil sheets that were carpeted on trays. The samples were carefully covered with extra aluminum sheets and clearly labelled to each herbal concentration and were kept at two different temperatures (an incubator at 30^oC and a fridge at 4^oC). The time taken from raw chicken swabbing to the first incubation was estimated by three hours. Then, samples were tested and swabbed in hygienic manner on day interval basis (1, 3, 5, 7 and 10) for microbial growth and sensory aspects.

2.2.4. Sensory analysis

Raw chicken samples treated with both herbal VO concentrations and controls were analysed for sensory evaluation including appearance (visual colour and texture) and odour on daily basis.

2.2.5. Statistical analysis

The data were statistically analysed according to the method of analysis of variance as a general test. A factorial experiments with three replicates were used by XLSAT program ver. 7.5.2 and conducted using Complete Randomised Design (CRD). All possible comparisons were carried out by using Least Significant Difference (LSD) at the significant level of 0.05, after they showed their significant differences in the general test.

III. "Results and discussion"

Following several successive hydro distillations of the shade dried herbs of *Rosmarinus Officinalis* and *Thymus Citriodorus*, the average volatile oil contents were 3.5 % and 2.5 %, and the average moisture contents were 63.0 % and 55.0 % respectively.

The VOs of the herbs were used at different concentrations to measure their antimicrobial activities on the raw chicken kept in a fridge (4^oC) and in an oven (30^oC). The results showed all the VOs were significantly inhibited microbial growth on the raw poultry against control in a dose dependent manner (Table 1 and Table 2.).

Most of the microorganisms of chickens are on the surface, so the surface counts/cm^2 are generally more valid than counts on surface and deep tissues [21]. The mean of initial total surface count of whole chicken was (1.3. x 10^8 cfu/cm^3). This microbial load found to be too high, probably because of the previous unhygenic handling during slaughter, transport and storage conditions of the poultry that were purchased from the mini market shop, rather from a direct slaughter house. Factors that support growth and survival of common spoilage and pathogenic microorganisms in packaged food were attributed to intrinsic factors (pH and presence of oxygen) and extrinsic factors associated with storage (temperature, time and relative humidity) [22], [23] and Rydlo [24].

Three doses (0.1, 0.25, 0.5 %) were selected in this work after an earlier pretest for MIC (0.05 %) determination of the herbal voltile oils against *E. coli* and *P. aeruginosa*. Obviousely, the VOs work differently in food systems comparing to laboratorial agar plates due to the presence of other compositional compounds in the food including fat and proteins. This is in line with some researchers report of the limitation of essential oils application in foods due to their interaction with other food ingredients and the strong flavor they impart. In this work, the preservative effects of the VO concentrations was evaluated alone, without combination with other preservation strategies, such as modified-atmosphere packaging (MAP) [25].

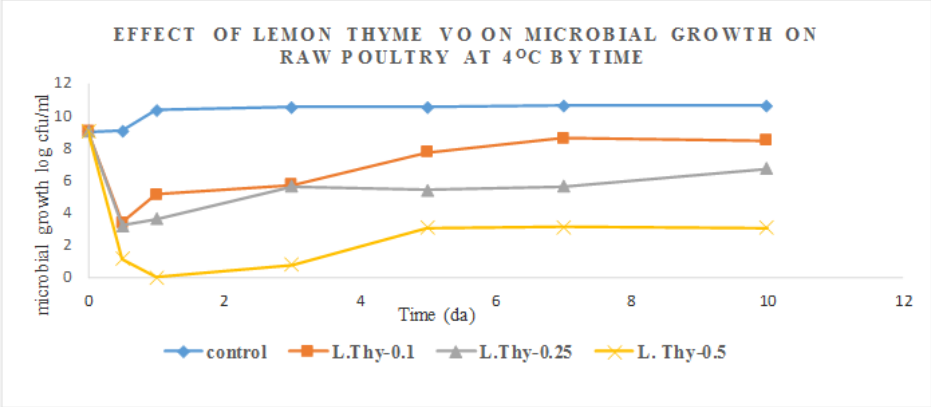
The results showed a steady preventitive trend of microbial growth by the VOs (0.25 and 0.5 %) and this can be noticed throughout the ten days of storage of the raw poultry in the fridge ($4\ ^oC$) (Table 1).The reason for that is probably due that the voatile compounds have retained on the poultry surface in such a temperature and weren't volatelise quickly, further, such a cooling temperature didn't support much all kinds of microbial multiplication too. However, the mean of the microbial load (1.1 x 10^8 cfu/cm^3) of (2^{nd} Control) was too close to the microbial load (1.3 x 10^8 cfu/cm^3) in (1^{st} Control), indicating ethanolic solution (10 %) didn't show signigficant reduction in microbial growth, probably due to ethanol's low concentration. Therefore the total count (1.1. x 10^8 cfu/cm^3) of ethanolic solution was used as a control throughout the work, Further, the efficacy of herbal VOs (0.1 %) on microbial log reduction didn't show as effective as (0.25 and 0.5 %) particularly after 3 days of incubation, therefore the VO concentration of 0.1 % was not considered as an effective dose at least for this work on raw poultry (Table 1. And Table 2.).

Table. 1. The effects of Rosemary and Lemon thyme VOs (0.1, 0.25, 0.5 %) on microbial grwoth on raw chicken at 4^oC by time (days) against control (10 % ethanolic solution). Values are averages of six replicates (Miles and Misra).

Time	Microbial growth (cfu /cm^2) on meat treated with rosemary and lemon thyme VOs						
	Rosemary				Lemon thyme		
(day)	Control	0.1	0.25	0.5	0.1	0.25	0.5
After 3 hr	1.1 x 10^8	$3.3x10^7$	2.0 x 10^2	TFTC*	$2.3x10^6$	$1.6x10^2$	TFTC*
1		$1.1x10^7$	1.3 x 10	0 cfu.	$1.4x10^6$	$4.1x10^2$	0 cfu.
3		$3.3x10^6$	3.3 x 10^3	0.3 x 10^1	$5.1x10^6$	$4.3x10^4$	$0.6x10^1$
5		$6.2x10^7$	2.1 x 10^4	0.1 x 10^2	$5.5x10^7$	$2.5x10^4$	$1.2x10^3$
7		$4.3x10^8$	2.2 x 10^4	0.2 x 10^2	$4.3x10^7$	$4.5x10^4$	$1.3x10^3$
10		$4.2x10^8$	4.4 x 10^6	6.2 x 10^2	$3.1x10^8$	$5.2x10^5$	$1.1x10^3$
LSD (P<0.05)			$1.46x10^{-6}$	(± SD)			

* Too few to count.

All the raw chicken pieces treated with VOs were found significantly different ($p<0.05$) between all the VO doses against the control. Although the dose of 0.25 % of both herbal VOs showed significant ($p < 0.05$) inhibition of microbial growth, the higher dose (0.5 %) has reduced microbial growth by four log. at 30 ^oC for 3 days (fig. 3 and fig. 4) and five log in refrigration for 10 days (fig. 1 and Fig. 2). Further, the sensory quality



Fig, 1. The effect of *Thymus citriodorus*VO on preserving raw poultry in a frdige by time. parameters (colour, flavour and appearance) were also acceptable.

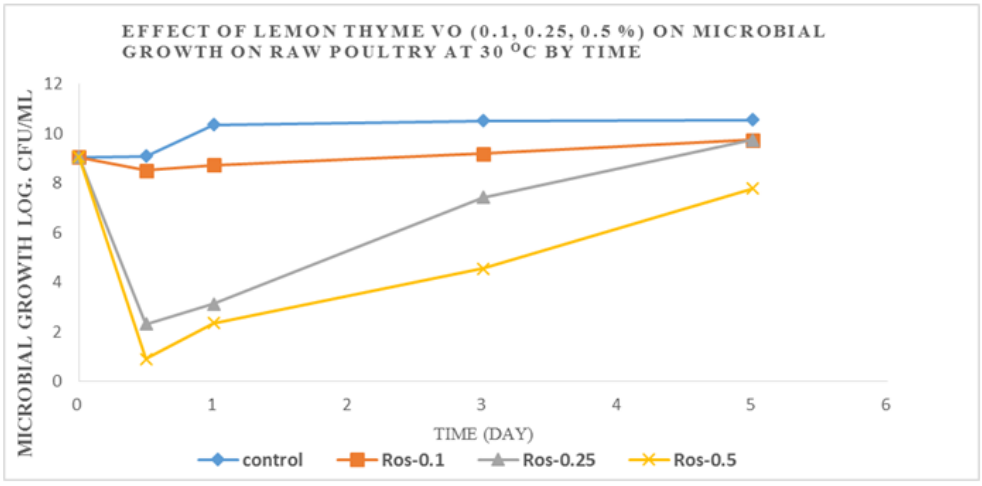


Fig. 2. The effect of *Rosemarinus officinalis* VO on preserving raw poultry in a fridge by time.

It was also observed that the rosemaary VO has shown stronger antimicrobial activity comparing to lemon thyme VO at concentrations (0.25 and 0.5 %) for ten days in the fridge except for rosmary (0.25 %) at 10^{th} day, probably due the anti microbial as well as antioxidant activities of rosemary VO's components that was well documented by many researchers [8] and [9].

However, when the same herbal VOs were applied to the raw poultry at 30 ^{o}C, expectably the extending shelf life of the poultry were reduced to a maximum of 5 days, as the VOs (0.5 %) reduced the microbial growth by almost only 2 log (Table 2) comparing to the control. The raw chicken pieces used as a control (dipped in 10 % ethanolic solution) was putrified after 24 hours to an extend that organoleptically was not reasonable for consumption, so was safely discarded.

However, the VOs (0.25 and 0.5 %) have reduced the bacterial load by almost 6 log. after one day and by 4 Log. after three days, while the poultry were found in a satisfactory organoleptic conditions.

Table. 2. The effect of Rosemary and Lemon thyme VOs (0.1, 0.25, 0.5 %) on microbial grwoth on raw poultry at 30^{o}C by time (days) against control (10 % ethanolic solution).

	Microbial growth (cfu/mL) on poultry treated with rosemary and lemon thyme VOs						
Time	Rosemary				Lemon thyme		
(Day)	Control	0.1	0.25	0.5	0.1	0.25	0.5
After 3 hr	1.1×10^{8}	3.3×10^{7}	2.0×10^{2}	TFTC	2.3×10^{6}	1.6×10^{2}	TFTC
1		5.3×10^{7}	1.3×10^{3}	2.2×10^{2}	2.610^{6}	4.4×10^{2}	1.5×10^{2}
3		1.5×10^{8}	2.6×10^{6}	3.5×10^{4}	4.5×10^{7}	7.5×10^{4}	3.6×10^{3}
5		5.5×10^{8}	5.5×10^{8}	$6.0\ 10^{6}$	2.5×10^{8}	5.5×10^{6}	6.4×10^{6}
LSD (P<0.05)		9.73×10^{-7} (±SD)					

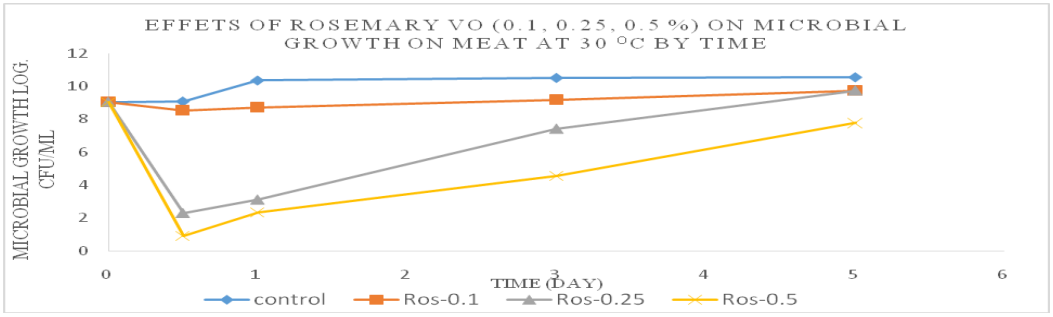


Fig.3. The effect of *Rosmarinus officinalis*VO on preserving raw poultry at 30 °C by time.

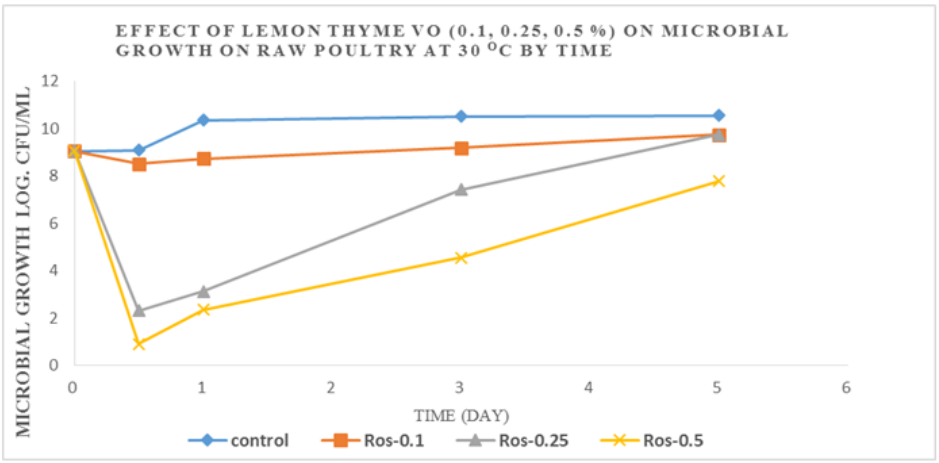


Fig. 4. The effect of *Thymus citriodorus* VO on preserving raw poultry at 30 °C by time.

The antimicrobial and antioxidant activities of Rosemary has been well established by many researchers **[11]**, [12] and [13], due to its terpene(oid)s main compounds including 1,8-cineole, α-pinene, bornyl acetate, camphor, carnosic acid and carnosol. The VOs activities were ascribed to the presence of hydrophilic functional groups, such as hydroxyl groups of phenolic components and/or lipophilicity of some essential oil components [26]. However, it was also reported that the VOs are more inhibitory against Gram-positive than Gram-negative bacteria [27] and Marino [28].

The results also showed the action of VOs (0.25 and 0.5 %) on microbial growth at 30°C is significant ($p < 0.05$) after 24 hours as well as after 3 days. However, the extended shelf life of the raw poultry at 30 °C found to be shortened comparing to the same in the fridge and this can mainly be attributed to the factor of temperature, as the VO aroma compounds volatalise easily in the temperature (30 °C) comparing to the fridge (4 °C), consequently the VOs contributioin to inhibit microbial growth not only was ineffective but the regrowth was noticed after 3 days of storage. Further, the temperature (30 °C) falls in the danger zone that highly support the microbial growth particularly E. coli and other mesophyllic microorganisms.

A researcher has reported that geraniol (the main constituent of lemon thyme) was shown a large bactericidal activity and greater than the cinnamic acid for Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Salmonella enterica [29].

In another research studying the antimicrobial activities of thymus pannonicus (containing geranial 41.4%, and neral 29.6 %) on a range of pathogenic bacteria and moulds. They found the essential oil of T. pannonicus was shown remarkable antimicrobial activity against several strains of medicinally important pathogens. The researchers attributed the results to lemon-scented citral which is a mixture of geranial and neral [30], both compounds interestingly are the main constituents of lemon thyme volatile oil.

In conclusion, both herbal VOs can be used as natural preservatives to prolong shelf life of raw poultry kept in fridge (4°C) for at least a week at concentrations (0.25 %) and at most for 10 days at (0.5 %), also the herbal VOs can preserve raw poultry in an ambient temperature (30 °C) for at least one day at concentration (0.25 %) and for 3 days at (0.5 %). These findings could also serve in preserving raw meats in the regions that lack of constant electrical power supply, particularly those inhibit in tough mountainous districts. Further, these herbs among several others grow abundantly in wild and have been domesticated in Kurdistan, so can be employed industrially, where beside their desirable flavors and beneficial health impacts, they could save a great loss of our national economy.

Further works can be carried out for characterization of the indigenous herbs and their application on poultry, meat and fish with extending dipping time, using different concentrations at different ambient temperatures.

"References"

[1] P. M. Davidson and M. T. Taylor, Chemical preservatives and natural antimicrobial compounds, in Food Microbiology, *American Society for Microbiology Press*, 2007, 713–734.

[2] J. Farkas, Physical methods of food preservation, in Food Microbiology, *American Society for Microbiology Press*, 2007, 685–705.

[3] M. B. Jamilah, K. A. Abbas, R. A. Rahman, A review on some organic acids additives as shelf life extenders of fresh beef cuts, *Am. J. Agric. Biol. Sci.,3 (3),* 2008, 566–574.

[4] M. Jałosńska, J. and Wilczak, Influence of plant extracts on the microbiological shelf life of meat products, *Pol. J. Food Nutr. Sci. 59(4),* 2009, 303–308.

[5] US – FDA, *CFR- Code of federal Regulations, 21 (3),* (2015).

[6] K. A. Hammer, Carson C.F. and T.V. Riley, Antimicrobial activity of essential oils and other plant extracts, *J ApplMicrobiol, 86(6),* 1999, 985–990.

[7] A. Smith-Palmer, J. Stewart and L. Fyfe, Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens, *Lett Food Microbiol.,26(2),* 1998, 118–122.

[8] A.I. Hussain, F. Anwa, S.A.S.Chatha, Rosmarinus officinalis essential oil: antiproliferative, antioxidant and antibacterial activities, *Braz J Microbiol, 41(4),* 2010, 1070–1078.

[9] L. Yu, L. Scanlin and J. Wilson, Rosemary extracts as Inhibitors of lipid oxidation and color change in cooked turkey products during refrigerated storage, *J Food Sci. 67(2),* 2002, 582–585.

[10] R. Jamshidi, Z. Afzali and D. Afzali, Chemical Composition of Hydrodistillation "Essential Oil of Rosemary in Different Origins in Iran and Comparison with Other Countries, *American-Eurasian J. Agric. & Environ. Sci., 5 (1),* 2009, 78-81.

[11] D. J. Daferera, B. N. Ziogas, AND M. G. Polissiou, GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicilliumdigitatum, *Journal of Agricultural and Food Chemistry, 48(6),* 2000, 2576-2581.

[12] G. Pintore, Chemical composition and antimicrobial activity of Rosmarinus officinalis L. oils from Sardinia and Corsica, *Flavour and Fragrance Journal, Sassari/Ajaccio, 17(1),* 2002, 15-19.

[13] E. N. Frankel, Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion, *Journal of Agricultural and Food Chemistry, 44(1),* 1996, 131-135.

[14] S. Gianni, S. Maietti, M. Muzzoli , M. Scaglianti, S. Manfredini, M. Radice and R. Bruni, Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods, *Food Chemistry, 91 (4),* 2005, 621–632

[15] S. Wu, FX. Wei, HZ. Li, XG. Liu, JH. Zhang, JX. Liu , Chemical composition of essential oil from Thymus citriodorus and its toxic effect on liver cancer cells, *Zhong Yao Cai. 36 (5),* 2013,756-9.

[16] O. Yesil-Celiktas, C. Sevimli , E. Bedir , F. Vardar-Sukan, Inhibitory effects of rosemary extracts, carnosic acid and rosmarinic acid on the growth of various human cancer cell lines, *Plant Foods Hum Nutr., 65(2),* 2010, 158-63.

[17] R. Omidbaigi, F. Sefidkon, and M. Hejazi, Essential oil composition of Thymus* citriodorus L. cultivated in Iran, *Flavour and Fragrance Journal. 20 (2), 2005,* 237–238.

[18] B. E. Stahl and J. Holthuijzen, Essential oil and glycosidically bound volatiles of lemon scented thyme, Thymus × citriodorus (Pers.) Schreb, *Flavour and Fragrance Journal, 10 (3),* 1995, 225–229.

[19] ASTA. "*Official analytical methods of the American Spice Trade Association*". ASTA, Englewood Cliffs, pp. 8-11, (1968).

[20] A. A. Miles, and S. S. Misra, *Journal of Hygiene. 38 (6),* 1938, 732 - 49.

[21] K. N. May, Bacterial contamination during cutting and packaging chicken in processing plants and retail stores, *Food Technol,16,* 1962, 89-91.

[22] T. K. Singh, M. A. Drake and K. R. Cadwallader, Flavour of Cheddar cheese: a chemical and sensory perspective, *Comp. Rev. Food Sci. Food Saf. 2,* 2003, 139–162.

[23] M. A. Lopez, A. S. Maris and E. Palou, Aspergillus flavus growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds, *Int. J. Food Microbiol, 99,* 2005, 119 -128.

[24] T. Rydlo, J. Miltz and A. Mor. Eukaryotic antimicrobial peptides: promises and premises in food safety, *J. Food Sci. 71 (9),* 2006, 125–135.

[25] M. Mastromatteo, A. Conte and MA. Del Nobile, Combined use of modified atmosphere packaging and natural compounds for food preservation, *Food Eng Rev. 2(1),* 2010, 28–38.

[26] H. J. D. Dorman and S. G. Deans, Antimicrobial agents from plants: antibacterial activity of plant volatile oils, *J. Appl. Microbiol.* 88, 2000, 308–316.

[27] T. Mangena, and N. Y. O. Muyima, Comparative evaluation of the antimicrobial activities of essential oils of Artemisia afra, Pteroniaincana and Rosemarinus officinalis on selected bacteria and yeast strains. *Lett. Appl. Microbiol. 28(4*), 1999, 291–296.

[28] M. Marino, C. Bersani, G and Comi, Impedance measurement to study antimicrobial activity of essential oils from Lamiaceae and Compositae, *Int. J. Food Microbiol. 67,* 2001, 187–195.

[29] M. Zanetti, Z.R. Ternus, DM. Dalcanton, D. de Oliveira, Microbiological Characterization of Pure Geraniol and Comparison with Bactericidal Activity of the Cinnamic Acid in Gram-Positive and Gram-Negative Bacteria, *J Microb BiochemTechnol. 7*, 2015, 186-193.

[30] Z. Maksimovic, M. Milenkovic, D. Vuicevic and M. Ristic, Chemical composition and antimicrobial activity of Thymus pannonicus All, *Lamiacea essential oil. Open life science, 3(2),* 2008, 149-154.