J. Food and Dairy Sci., Mansoura Univ., Vol.5 (1): 15 - 31, 2014

ANTIOXIDANT ACTIVITY OF THYME AND SAGE AND THEIR ANTIMICROBIAL IMPACT AGAINST *Listeria monocytogenes* AND *Pseudomonas putida* IN BEEF BURGER

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ABSTRACT

The aim of this study was carried to evaluate the role of natural antioxidant and antimicrobial activity against *Listeria monocytogenes* and *Pseudomonas putida* from each thyme and sage powders as well as their ethanolic extracts in beef burger to help factories to extend the shelf-life of their products under storage at -18 ℃. Results revealed that by using 1.5 % of sage powder and 0.1% of ethanolic extract of thyme represented the optimum concentration for decreasing of *Listeria monocytogenes* count but the ethanolic extract of thyme at 0.1% gave the highest decreases in population of *Pseudomonas putida* in beef burger especially after 60 days of freezing storage condition. On the other hand, the results indicated that all treated samples with spices were recorded decreasing in thiobarbituric acid, peroxide and acid values compared with control sample. Moreover, moisture, protein and ash were decreased by increasing storage period. Meanwhile, only fat content was increased.

Keywords: Thyme - Sage - *Listeria monocytogenes* - *Pseudomonas putida* - Thiobarbituric acid - peroxide value – Acid value – beef burger.

INTRODUCTION

Raw meat can be easily contaminated by microorganisms and support the growth of pathogens leading to serious food-borne illness (Pokorny *et al*. 2001). The presence and growth of microorganisms in food may cause spoilage and result in reduction of food quality and reduce of quantity of food (Soliman and Badeaa, 2002). Meat and especially ground meat products are highly susceptible to both microbial growth and lipid oxidation because of their large surface to weight ratio, leading to rapid spoilage and development of rancid or warmed over flavor, respectively (Jay *et al*., 2005). Because there is an increasing consumer demand for minimally processed foods without chemical preservatives, the food industry is facing a constant challenge to develop alternative 'natural' methods to extend product shelf life and improve safety (Karabagias *et al*., 2011).

Food-borne illnesses associated with *Listeria monocytogenes* and *Pseudomonas* spp present a major public health concern throughout the world (Hall, 1997). The spoilage of refrigerated meat is caused in part by *Pseudomonas* species bacteria which are responsible for the off-odors, off-flavors, discoloration, gas production and slime production. *Listeria monocytogenes* has been shown to be responsible for outbreaks of food poisoning in France and in many countries (De Valk *et al*., 2000).

Microbial growth and lipid oxidation are primary factors of meat spoilage during refrigerated storage. To extend storage period, antimicrobial

and antioxidant additives especially of synthetic origin, are added in muscle foods. However, consumers and health authorities increasingly dictate that the use of chemical food additives should be phased out and, where possible, only natural products should be used. Many herbs, spices, and their extracts have been added in a variety of foods to improve their sensory characteristics and extend shelf-life (Bagamboula *et al*., 2003). Many natural plant extracts contain primarily phenolic compounds, which are potent antioxidants *(*Wong *et al., 1995).* Some phenolic compounds such as sage, rosemary, thyme, hops, coriander, tea, cloves and basil are known to possess antimicrobial effects against food-borne pathogens (*Elgayyar et al., 2001).* Herbs of the Lamiaceae family, mainly oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), and sage (*Salvia officinalis* L.), have been reported as having significant antioxidant capacity (Shan *et al*., 2005; Wojdylo *et al*., 2007). Nevas et al. (2004) reported that the essential oil of *Thymus vulgaris* contains various levels of thymol and / or carvacrol, phenolic derivatives with strong and wide- spectrum antimicrobial activity. Sage *(Salvia officinalis L)*, can be found worldwide and its leaves are commonly used as ingredient in food industry. Sage essential oil is applied in the treatment of a range of diseases and has been shown to possess different biological activities (Russo *et al.,* 2013*).*

Lipid oxidation, which occurs during the storage of raw materials, processing heat treatment and subsequent storage of final products, is one of the main causes of rancidity in food products (Tepe *et al., 2005)*. As well as , lipid oxidation and fatty acid composition, such as high proportion of highly unsaturated fatty acids, are also important factors influencing quality and acceptability of meat and meat products due to its more or less susceptibility to degrading process (Ahn *et al., 2007)*

The objective of the present study work was to evaluate the effect of dietary thyme and sage supplementation on growth of *Listeria monocytogenes* and *Pseudomonas putida* and the possibility of using as natural antioxidants to help beef burger industry to minimize lipid oxidation and extend the shelf –life of beef burger during freezing storage.

MATERIALS AND METHODS

Materials:

Spices : Thyme *(Thymus Vulgaris L.)* and Sage *(Salvia officinalis L.)* were obtained from SEKEM Company, Cairo Egypt.

Meat: Fresh meat (minced) used for beef burger was purchased from Metro Market, Giza.

Bacteria used: Two pathogenic bacteria strains i.e. *Listeria monocytogenes* and *Ps.putida* were collected from Dr. Abdel-Salam A.F. Regional Center for Food and Feed, ARC, Giza, Egypt, and Plant Pathology Dept., Faculty of Agriculture, Ain Shams Univ. respectively. *Listeria monocytogenes* and *Ps.putida* were maintained by transferring at regular intervals on tryptic soy agar (+0.6% yeast extract) and King's agar medium, respectively. Slants were kept at 4 $^{\circ}$C.

Methods:

Plants were first dried in an incubation oven at 40°C until a moisture content ~ 7-8% and milled in Moleneux, France, without sieving, and kept in glass brown till ethanol extraction.

Preparation of beef burger

Beef burger contained lean beef meat, cow fat, powdered rusk, dried onion, starch, salt and ice-water at a percentage of 78%, 11%, 4%, 0.4%, 0.9%, 1.25% and 4.45%, respectively, was prepared according to the method described by Khattab (1999). For preparing beef burger, meat and fat were twice minced and mixed well, with other ingredients. The natural additives (thyme and sage) were added individually to the prepared samples as follow:-

1-No addition (control)
2-Thyme powder at conc. 0.5% & 1.5%
3-Thyme ethanolic extract at conc. 0.05% & 0.1%
4-Sage powder at conc. 0.5% & 1.5%
5-Sage ethanolic extract at conc. 0.05% & 0.1%

Samples were packed in polyethylene bags and stored at -18°C for 60 days, and taken periodically each 30 days during storage to evaluate retardation of oxidative rancidity.

Ethanol extracts

Extracts were prepared by mixing spice preparation with sterile distilled water, ethanol in a 1:10 ratio followed by shaking in the dark for 24 h at room temperature using a universal shaker (Analytica Ltd, Ireland). The mixtures were vacuum filtered through a Whatman No. 4 filter paper, centrifuged at 3800g for 30 min, the supernatants were collected and filtered through 0.2 µm filters (Sarstedt, Germany). The filtrates were evaporated to dryness under vacuum at 70 °C for water extracts, and at 40 °C for ethanol extract, using a vacuum evaporator (Genvac Inc., NY, and USA). Concentrated extracts were subsequently stored at 4 °C until use. (Oliveira *et al*., 2010).

Determination of chemical constituents of ethanolic extracts of thyme and sage by using Gas chromatography

Gas chromatography – mass spectrometry (GC /MS) was used to separate and identify the components of thyme and sage ethanolic extracts. Analytical method described by Nath *et al*. (1996*).*

Antimicrobial activity of thyme and sage on the survival of inoculated *L. monocytogenes* and *Ps. putida* in beef burger:

a-Preparation of bacterial inoculum:-

L. monocytogenes and *Pseudomonas putida* were inoculated separately in 250 ml of a sterilized tryptic soy broth and nutrient both in 500 ml flask, respectively, and kept on shaking incubator for 2 days at 28 – 30 °C to achieve viable cell population 10^{11}cfu/ml for *L. moncytogenes* and 10^{9}cfu/ml for *Ps*. *putida*.

b- Preparation of inoculated samples:-

Beef burger sample (25g),which was treated by thyme and sage as mentioned before, was inoculated with the single strain of the pathogen by dipping in *L. monocytogen* (~10^{11} cfu/ml) or *Ps.putida* (~10^{9} cfu/ml) inoculum

for 5 min., then left for 10 min. under aseptic conditions at room temperature. Each sample was individually packed in sterile plastic bag before the samples were stored at -18°C. Cell count were converted to -10 logarithms of colony forming unit per gram (log cfu/g sample).

Measured parameters:

Chemical composition of beef burger

Fresh and stored beef burger were subjected to their chemical composition as follows: Moisture, protein, fat, ash and fiber of minced beef meat were determined according to the methods of A.O.A.C. (2005). While carbohydrates content were calculated from the following equation.

Carbohydrate = [100- (% moisture + % protein + % fat + % ash + % fibers)]

Chemical characteristic of lipid extracted from beef burger:

Determination of Thiobarbituric acid – reactive substances (TBARS)

Thiobarbituric acid (TBA) values was determined in beef burger samples at 0, 30, 60 days of storage at -18°C according to the described by Du and Ahn (2002) to evaluate efficiency of additives as natural antioxidants. The TBA values were calculated by multiplying the absorbance by the factor of 7.8 and the result was represented as mg of malonaldehyde per 1000g sample.

Determination of peroxide value (PV) and acid value (AV)

Peroxide value and acid value (AV) were determined according to method described by A.O.C.S. (1997).

Microbiological analysis

Populations of *L. monocytogenes* and *Ps. putida* in the samples were enumerated on tryptic soya agar (+0.6 % yeast extract) and King's broth media by plate count and most probable numbers (MPN) methods, respectively, after 0, 15, 30, 45, 60 days of inoculation.

RESULTS AND DISCUSSIONS

The data in Table (1) showed that 20 components were isolated from thyme and ethanolic extract, 9 components of them were identified. The identified compounds represented 97.41 % of the chemical components of thyme ethanolic extract and could be classified to three chemical categories. [monoterpene hydrocarbon (28.79%), monoterpene phenols (65.94%) and sesquiterpenes (2.70%)]. These data are in harmony with those reported by Vaz *et al.,* (2004*)* and Lee *et al.,* (2005).Also, 20 compounds were isolated from sage ethanolic extract. Only 12 compounds were identified from them. The identified compounds represented (94.27%) from the chemical components of sage ethanolic extract and were classified for 6 chemical groups, namely; monoterpene hydrocarbons (8.62%), oxygenated monoterpnes (51.72%), monoterpene ketones (25.25%), monoterpene esters (5.21%), monoterpene phenols (1.12%) and sesquiterpenes (2.35%). Amr and Dordevic (2000***)*** found that the essential oil from sage originating from Jordan contains α-pinene, camphene, limonene, 1, 8-cineol, α and β - thujone, camphor, lonalool, linalylacetate, bornyl acetate and humulene. In

the essential oil 29 components were detected, 28 of them were identified and a dominant share had α- thujone (29.9%) β-thujone (13.68%), camphor (15.74%) and 1, 8-cineole (12.31%). Moreover, *Martos et al. (2008)* found that GC-MS analysis of sage essential oils identified 37 constituents, representing 90% of the total oil. The main components were camphor (24.95%), 1, 8- cineole (24.75%) and camphene (7.63%).

Table (1): Chemical components of ethanolic extracts of thyme and sage (%)

Chemical compounds	Thyme	Sage
Monoterpene hydrocarbons:		
α-pinene	5.10	2.23
β-pinene	4.67	2.51
Camphene	ND	1.72
Myrcene	5.62	1.40
γ- Terpinene	6.63	0.76
Sabinene	1.42	ND
Limonene	3.37	ND
P-cymene	1.96	ND
Oxygenated monoterpenes :		
1,8 cineole	ND	43 52
Linalool	ND	ND
Bomeol	ND	5.20
Citronellol	ND	ND
α-terpinene	ND	ND
Monoterpene phenols:		
Thymol	65.94	ND
Estrogole	ND	1.12
Monoterpene esters:		
Bomylacetate	ND	5.21
Linaylacetate	ND	ND
Monoterpene ketones:		
Thujone	ND	16.31
Comphor	ND	8.94
Sesquiterpenes:		
β- Caryophyllene	2.70	2.35
Identified %	97.41	91.27
ND %	2.59	8.73

*** ND = Not Detected**

Effect of spices and their extracts on the chemical composition of beef burger during freezing storage:

Data in Table (2) shows that untreated beef burger samples resulted in moisture contents of 63.81%, protein 15.56%, total lipids 14.26% and ash 2.31% at zero time. These results were within Abd El Sattar (2001).

Addition of spices and their ethanolic extracts had a slight decrease effect on moisture contents of treated beef burger at zero time of storage compared with control sample. Moisture, content of the control sample decreased during storage to reach 62.35% after storage for 60 days at -18°C. Moreover, addition of thyme and sage powder at (0.5% and 1.5%) and their ethanolic extracts at (0.05% and 0.1%) decreased moisture content at zero time and

after storage compared with the control sample. On the other hand, the moisture content decreased during storage by adding the spices ethanolic extracts than spices powder. From the above data, it could be concluded that all samples had a decrease in moisture content during storage and this attribute to the evaporation of water and loss of drip during thawing of beef burger as well as the decrease due to the water holding capacity as reported by Abd El-Hamied *et al.,* (2009).

Protein content showed slightly decreases in the control sample than other all treated beef burger samples at zero time. The protein contents of the control sample were 15.56 % at zero time, which decreased during storage to reach 12.11% after storage for 60 days. Addition of thyme and sage extract reduce the degradation of protein contents during storage more than the addition of thyme and sage powder. This decrease in protein content of freezing beef burger samples might be attributed partially to the breakdown of protein by proteolysis enzymes, these results were in agreement with Ragheb (2005).

Table (2): Effect of different concentrations of spices powder and their ethanolic extracts on chemical composition of beef burger during freezing at -18°C for 60 days.

Addition %	Storage period (day)					
	0	30	60	0	30	60
	Moisture content (%)			Protein content (%)		
Control	63.81	62.83	62.35	15.56	14.28	12.11
Thyme powder 0.5%	63.71	61.85	61.51	15.62	14.34	14.21
1.5%	62.22	61.43	59.74	15.72	15.45	14.63
Thyme extract 0.05%	62.44	61.41	59.52	15.84	15.11	14.68
0.1%	62.12	61.31	59.37	15.83	15.46	14.82
Sage powder 0.5%	62.55	61.79	61.11	15.47	14.39	14.11
1.5%	62.31	61.31	61.01	15.62	15.01	14.34
Sage extract 0.05%	62.01	61.62	60.92	15.53	14.84	14.38
0.1%	61.91	61.36	60.82	15.75	14.93	14.43
	Fat content (%)			Ash content (%)		
Control	14.26	15.32	16.18	2.31	1.91	1.60
Thyme Powder 0.5%	13.23	14.72	15.61	1.88	1.60	1.52
1.5%	13.12	14.41	15.38	1.73	1.61	1.42
Thyme extract 0.05	13.69	14.51	15.66	1.98	1.67	1.43
0.1%	13.84	14.75	15.58	1.88	1.61	1.32
Sage Powder 0.5	14.01	14.94	15.78	2.12	1.86	1.58
1.5%	14.21	15.10	15.95	2.01	1.72	1.53
Sage extract 0.05	14.09	14.89	15.73	2.03	1.87	1.60
0.1%	14.13	14.92	15.84	2.01	1.73	1.56

Fat contents of the control sample were 14.26% at zero time which increased during storage to reach 16.18% after 60 days of storage. Although addition of thyme and sage powders and their extracts the fat contents increased during 60 days of storage, this increase in fat contents during freezing storage due to the decrease of the moisture content. Moreover, this increase in fat contents may be not true but it due to the degradation of some ingredients and produce new products soluble in solvent during solvent

extraction. These results agree with Abd-ELQader (2003); Hegazy (2004) and Ali (2008) who reported that the fat content increased during the freezing storage of meat products and there was a negative relationship between fat content and moisture content.

Ash content in the control sample was 2.31% which decreased gradually to reach 1.60% after storage. Addition of thyme and sage powders and their extracts led to decrease the ash content during storage (Table, 2).

It is worthy to mention that storage at -18°C for 60 days caused a decrease in moisture, protein and ash content while, fat content was increased. In general, it could be concluded that, adding of either spices powder or their extract were recorded more effect on the chemical composition of treated beef burger samples than control beef burger during freezing at -18°C for 60 days.

Effect of spices and their extracts on Thiobarbituric acid, peroxide and acid values of beef burger during freezing

Thiobarbituric acid (TBA):

Thiobarbituric acid (TBA) test is used as an index for measuring oxidative rancidity which takes place in minced meat. The TBA test is a sensitive test for the decomposition products of highly unsaturated fatty acids which do not appear in peroxide value determination, as reported by Mohamed (2005).

Data presented in Table (3) showed that, during freezing storage, the TBA value of the control sample showed continuous progressive increases to reach the highest value (0.71 mg malonaldehyde /kg) at the end of freezing storage periods. Although the other burger treatments showed increment in TBA values during freezing storage, these increments were largely lower than that of control sample with a difference in some cases. In regard to these results, burger samples formulated with spices powder at level 1.5% had the lowest TBA values at the end of freezing storage, while those samples formulated with spices powder at level 0.5% had higher TBA values. On the other hand, addition of thyme and sage extract were more effective to decrease the TBA value at zero time compared with the control sample and spices powder.

Generally, during storage the TBA values of all samples increased with some differences as storage periods increased. These results were in line with Darwish *et al.* (2012). It could be concluded that addition of spices powder at level 1.5% caused a decrease in TBA. values in fresh and freezing beef burger compared with beef burger formulated with the same spices powder at level 0.5% and control. Also, it could be noticed that thyme showed the highest antioxidant effect for reduction TBA. values in samples, the effectiveness of decrement followed the sequence: Thyme extract > thyme powder > sage extract > sage powder. Similar results were obtained by Zheng and Wang (2001). On the other hand, the control sample was acceptable for human consumption after 60 days of storage as their TBA value reached (0.71 mg malona ldehyde/ kg sample). The E.O.S.Q.C (2005) rejected freezing chicken sausage samples which had more than 0.9 mg malonaldehyde/kg sample).

Table (3): Effect of different concentrations of powder spices and their ethanolic extracts on Thiobarbituric acid of beef burger during freezing at -18°C for 60 days.

Addition %		Storage period (days)		
		0	30	60
		Thiobarbituric acid (TBA) (mg malonaldehyde /kg)		
Control		0.47	0.59	0.71
Thyme powder	0.5%	0.35	0.38	0.43
	1.5%	0.27	0.32	0.39
Thyme extract	0.05%	0.30	0.32	0.36
	0.1%	0.25	0.27	0.31
Sage powder	0.5%	0.36	0.40	0.46
	1.5%	0.33	0.36	0.41
Sage extract	0.05	0.33	0.35	0.39
	0.1%	0.30	0.32	0.36

Peroxide value (PV):

The peroxide values are used as an index of the degree of oxidative rancidity of lipids. According to Liberman and Petrovski *(1972).*They reported that fresh edible animal fats which might be stored for long periods, show peroxide values of 2.34 m equiv./kg fat or less. In comparison with vegetable oils, this value was lower than the maximum permissible amount given for sun flower seed oil (not more than 10m.equiv/kg fat).

The data in Table (4) cleared that peroxide value of control sample was 4.11, which increased during storage to reach 5.14 after storage. Addition of thyme and sage powders decreased the (PV) at zero time compared with the control sample. During freezing storage the beef burger samples formulated with powder at level 1.5% had lower PV values than those samples formulated with powder at level 0.5%. Moreover the thyme powder showed more effect than sage powder at the same percentage, while the addition of thyme and sage extracts were the most effective in reducing the (PV) during storage compared with the control sample and spices powder samples.

Table (4): Effect of different concentrations of spices powder, their ethanolic extract on peroxide values of beef burger during freezing at -18°C for 60 days.

Addition %		Storage period (days)		
		0	30	60
		Peroxide value (PV) (m equiv./kg)		
Control		4.11	4.89	5.14
Thyme powder	0.5%	3.16	3.58	3.94
	1.5%	3.09	3.20	3.50
Thyme extract	0.05%	2.92	3.97	4.42
	0.1%	2.57	2.73	2.91
Sage powder	0.5%	3.27	4.01	4.50
	1.5%	3.16	3.61	3.97
Sage extract	0.05%	3.10	3.28	3.67
	0.1%	2.76	2.79	2.96

The ethanolic extract was more effective to reduce the peroxide value more than the spices powder and control sample. Moreover, this reduces increased by increasing the concentration of species powders and their extract. These results were in agreement with Dapkevicius *et al*. (1997) and Abd El-Sattar (2001).

Acid value (AV):

The test for acid value uses the principles of acid –base chemistry to determine the acidity of tested samples. Acid value of the control sample was 2.38 at zero time which increased to 3.21 after storage (Table, 5). Adding of both thyme and sage spices decreased the (AV) at zero time compared with the control sample. The lower (AV) was obtained at 1.5% in thyme powder than sage powder which were 1.59 and 1.62 respectively. These values increased during storage to reach 2.50 and 2.65 for the same above mentioned treatments after 60 day of storage. Meanwhile addition of thyme and sage extracts were more effective to reduce the acid value than spices powder during storage compared to values of control treatments. The lower (AV) was obtained at 0.1% in both spices extract, which were 1.69 and 1.75 at zero time. These values increased gradually during storage to reach 2.13 and 2.17 for the same spices extract respectively after storage period.

Table (5): Effect of different concentrations of spices powder and their ethanolic extract on acid value of beef burger during freezing at -18°C for 60 days.

Addition %		Storage period (days)		
		0	30	60
		Acid value (mg KOH / gm)		
Control		2.38	2.92	3.21
Thyme powder	0.5%	1.87	2.31	2.78
	1.5%	1.59	2.26	2.50
Thyme extract	0.05%	1.78	2.14	2.40
	0.1%	1.69	1.98	2.13
Sage powder	0.5%	1.91	2.39	2.81
	1.5%	1.62	2.31	2.65
Sage extract	0.05%	1.82	2.18	2.46
	0.1%	1.75	2.00	2.17

From the results in Table (5) it could be concluded that two spices extract were more effective to reduce the acid value more than spices powder samples. These results were in agreement with Khattab (1999).

In general, it could be concluded that spices extract were higher antioxidant effect than spices powder samples and control sample. Thyme extract was higher antioxidant than sage extract. Moreover, their antioxidant activity increased by increasing their concentration.

Microbiological determination in treated beef burger:

Many naturally occurring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Bagamboula, *et al*., 2003). More particularly, essential oils and their components are known to be active against a wide variety of microorganisms, including Gram-negative bacteria (Helander *et al.,* 1998). *Pseudomonas putida* and *L. monocytogenes*, the most important spoilage bacteria in meat, were used to evaluate the antibacterial activity of thyme and sage.

1- **Population of *L. monocytogenes***

The results recorded in Fig. (1) showed that thyme powder at concentrate 1.5 % decreased *L. monocytogenes* counts from 11.77 to 5.30 and 4.60 log cfu g^{-1} after 45 and 60 days of storage at -18 °C respectively, while thyme extraction at concentrate 0.1% decreased *L. monocytogenes* counts from 11.77 to 4.95 and 4.30 log cfu g^{-1} at the same conditions. Sage powder at concentrate 1.5 % induced pointed decrease of *L. monocytogenes* from 11.77 to 3.30 log cfu g^{-1} after 60 days of storage at -18 °C. Also sage extraction at concentrate 0.1 % paid to decreasing of *L. monocytogenes* counts from 11.77to 4.95 and 4.60 log cfu g^{-1} after 45 and 60 days respectively of storage at -18 °C. The concentration 1.5 % of sage powder represented the optimum concentration for decreasing *L. monocytogenes* counts in beef burger. On the other hand L*. monocytogenes* counts were recorded to be 8.69 and 7.60 log cfu g^{-1} after 45 and 60 days respectively at -18 °C without any treatment. The same observation regarding the effect of freezing on *L. monocytogenes* was reported by (Rodgers ***et al*** 2004**)**. Also addition of sage showed a higher inhibitory effect against psychrophilic count in minced meat for 100 days at -18 °C **(**Ab del- Hamied *et al*., 2009**).** Plant extracts and essential oils constitute a natural source of antimicrobial mixtures or pure compounds for centuries **(**Oke *et al*., 2009**).** Thymol is active against *L. monocytogenes*, *Staph. aureus* and *E. coli* **(**Chiasson *et al*., 2004**).** The antimicrobial activity of essential oils is benefited by a decrease in storage temperature (Burt 2004**).** The inhibitory effect on the growth of Staph*. aureus* recorded by thyme which is a dominant component of *Zataria multiflora* oil **(**Choobkar *et al*., 2010**)**. Treatment of minced beef meat with *Zataria multiflora* essential oil at 0.6% essential showed stronger inhibitory activity against *Listeria monocytogenes* (Solomakos *et al*., 2007). Differt plant essential oils against *L. monocytogenes* in hotdogs (rigid media), was reduced the bacterial population but the antimicrobial action was highly dependent on the fat content of hotdogs (Singh *et al*., 2003). This effect may be due to the deleterious effect of thyme and sage spices on the cytoplasmic membrane structure and function.

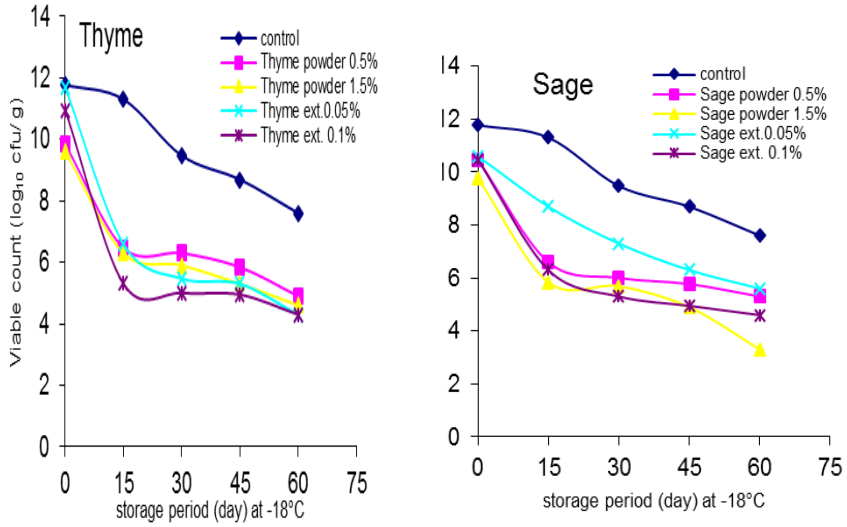

Fig. (1): Survivor curves of *Listeria monocytogenes* in beef burger containing different treatments of thyme or sage during freezing storage.

2-Population of *Pseudomonas putida*

The log cfu g^{-1} of *Pseudomonas putida* in inoculated beef burger treated with thyme or sage as a powder form (at 0.5% and 1.5% conc.) or alcoholic extract(at 0.05% and 0.1% conc.) during storage time at -18°C are show in Fig.(2).The obtained results showed that the bacterial load of *Pseudomonas putida* in samples treated with thyme and sage had significant difference compared with control samples(untreated with thyme or sage) .As general trend ,the gradual decrease in the microbial load (log cfu g^{-1} value) of treated samples(contain thyme or sage) was encountered with storage time and showed the lowest log cfu g^{-1} after 60 days of storage under freezing condition. In the treated samples with thyme powder, the count decreased from initial 8.8 to 3.2 log cfu g^{-1} at 0.5%. whereas the samples contained 1.5% thyme powder gave 2.9 log cfu g^{-1} ,by the end of 60 days storage , under freezing condition .However , the corresponding values of its alcoholic extract are 2.0 & 1.2 0 log cfu g^{-1} at 0.05% and 0.1% conc., respectively, under the same condition. The alcoholic extract of thyme exhibited the highest inhibitory effect on the *Pseudomonas putida* count at 0.1% under freezing. The similar trend was observed with sage treatments, the samples treated with sage gave the lowest values of log cfu g^{-1} with alcoholic extract treatments followed by its treatments by powder form.

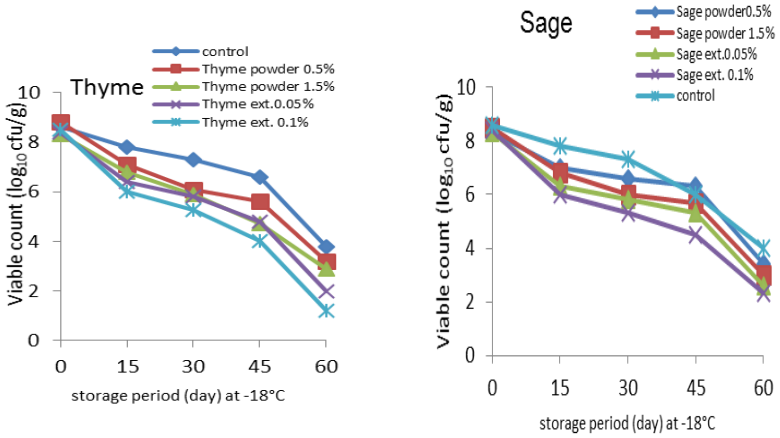

Fig.(2):Survivor curves of *Pseudomonas putida* in beef burger containing different treatments of thyme or sage during freezing storage

The corresponding figures of viable count of *Pseudomonas putida* are 2.3 and 2.6 log cfu g^{-1} with alcoholic extract at 0.1% and 0.05% and were 3.0 and 3.4 log cfu g^{-1} in presence of sage powder at 1.5% and 0.5%, respectively, after 60 days of storage under freezing condition. The similar results are obtained by Abd El-Hamied *et al*., (2009) who found that adding the natural extracts of rosemary, sage to minced meat as natural antimicrobial led to retard the microbial growth during frozen and refrigerated storage. On comparison of both treatments by medicinal plants (thyme and sage), the bacteriostatic effectives found to be more significant in the presence of thyme than sage. Oussalah *et al*., (2006) showed that the oils containing mainly components with phenolic structures, such as carvacrol, thymol and eugenol were highly active against *P. putida*. Powder of *Thymus ciliatus* has shown a strong antibacterial effect; this activity could be due to that the total powder contains all active components which may act synergistically against bacteria. It has been reported that carvacol and thymol which are the main compounds of *Thymus* have antibacterial properties (Ettayebi, 2000). Amarti *et al*. (2009) found that the yield of essential oils in *Thymus ciliatus* was 1.2%, and thymol represents 44.2% of this oil. Dorman and Deans (2000) demonstrated that thymol have a wide array of antibacterial activity against 25 tested bacterial strains. Another study realized by the World Health Organization (1999) showed that this compound (thymol) has an important antifungal and antibacterial activity against many microbial strains. Lambert *et al.* (2001) explained this phenomena by the fact that thymol binds to the membrane protein and increases the permeability of bacterial cell membrane. Helander *et al.* (1998) attributed the thymol antimicrobial action to its phenolic character, which can cause membrane-disturbing activities. Other studies suggested that this volatile compound was responsible for the inactivation of an enzyme implicated in syntheses of structural constituents (Trombetta, 2005).

Conclusion

The results of this study support many recommendations for using natural spices in preserving meat. The present study reveals potential application of thyme and sage as a good natural source of both anti-oxidants that seemed to be effective in minimizing the lipid oxidation in freezing beef burger and an efficient means for inhibiting the growth of *L. monocytogenes* and *Pseudomonas putida* .

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النشاط المضاد للأكسدة الزعتر و المريمية و تأثيرهما المضاد لبكتريا الليستريا مونوسيتوجينس و السيدوموناس بيوتيدا فى البيف برجر

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*** المركز الاقليمى للأغذية و الأعلاف – مركز البحوث الزراعية – الجيزة – مصر**

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الهدف الأساسى فى هذه الدراسة هو تقييم دور مضادات الأكسدة الطبيعية و النشاط المضاد لبكتريا الليستريا مونوسيتوجينس و السيدوموناس بيوتيدا بواسطة مسحوق الزعتر و المريمية بالإضافة إلى المستخلص الإيثانولى لهما فى البيف برجر و ذلك لمساعدة المصانع لإطالة مدة حفظ منتجاتها تحت ظروف التخزين على ⁻ ١٨ م°. كشفت النتائج إلى أن إستخدام مسحوق المريمية عند تركيز ١.٥ % و المستخلص الإيثانولى للزعتر عند تركيز ١,. % يمثل التركيز الأمثل لانخفاض العد الميكروبى للمونوسيتوجينس ، و لكن المستخلص الإيثانولى للزعتر عند تركيز ١,. % أعطى أعلى انخفاض فى أعداد بكتريا السيدومونس بيوتيدا فى البيف برجر و خاصة بعد ٦٠ يوم من ظروف التخزين . من ناحية آخرى ، أشارت النتائج أن كل العينات المعاملة بالتوابل سجلت انخفاض فى حمض الثيوباربيتيوريك و قيم البيروكسيد و الحموضة مقارنة بالعينة الكونترول . علاوة على ذلك فقد أنخفضت قيم الرطوبة ، البروتين و كذلك الرماد و ذلك بزيادة فترة التخزين و فى الوقت نفسه ازداد محتوى الدهون فقط .

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