Impact of some natural preservatives on Bacterial Profile of Minced Meat in Egypt

Fahim A. Shaltout[a], Amani. M. Salem[a], Dalia F. Khater[b] and Radwa. A Lela[b]

[a] Department of Food Control, Faculty of Veterinary Medicine, Benha University, Egypt. [b] Department of Food Hygiene., Animal Health Research Institute., Tanta branch.

ABSTRACT

In this study, the antioxidant and antibacterial effect of Marjoram and Cumin oils (0.2%&0.3%Vol/W) of each and Mixture from both oils (0.2% Vol/W) were investigated in refrigerated fresh minced meat. Both oils used had considerable effectiveness in decreasing Aerobic Plate Count (APC), Coliform Count and Staphylococci Count as well as chemical indices as pH, Total Volatile Nitrogen (TVN) and Thio Barbituric Acid (TBA) values. Meanwhile, these values decrease as the concentration of the oil increases with highly significant differences (P<0.05) between these different oils. Marjoram oil (0.3% Vol/W) gives the best effectiveness with a significant advantage in extend shelf life of refrigerated minced meat to 7 days compared to control group 4 days. Consequently, essential oils can play an important role as antimicrobial agent in refrigerated minced meat and potentially it might be used as a natural preservative ingredient for longer periods without the need to use hazardous preservatives in food industry, but Marjoram oil is the best one.

KEY WORDS: Marjoram, Cumin, Staphylococci, Minced meat

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1. INTRODUCTION

Meat and its products have experienced increasing popularity and become widely spread all over the world. The appearance of food is one of the major determinants of its appeal to consumers and consequently, sales of the product. However, during storage, quality attributes of the products deteriorate due to lipid oxidation and bacterial growth which are the main factors that determine food quality loss and shelf life reduction. Lipid oxidation leads to the degradation of lipids and proteins which, in turn, contribute to the reduction in nutritional quality as well as deterioration in flavor, color and texture of displayed meat products (Aguirrezábal *et al.,* 2000), while bacterial contamination can precipitate major public health hazard and economic loss in terms of food poisoning and meat spoilage (Fernández – López *et al.,* 2005). Consumers need to feel reassured that the foods they consume are safe; there is therefore increasing pressure on food manufacturers and authorities to eliminate harmful chemical preservatives from food preparations, as well as to prioritize research activity that may generate alternative − more effective, non-toxic, natural or synthetic − preservatives. (Nychas et al.,2003). Essential oils are regarded as natural alternatives of chemical preservatives and their use in food meets the demands of consumers for mildly processed or natural products, since in modern food industries, mild processes are applied in order to obtain safe products which have a natural or "green" image (Burt-sara, 2004).

Among several essential oils that may be useful as antimicrobial agents, Marjoram (*Origanum majorana L.)* essential oil belonging to the family Lamiaceae (Mohamed and Mansour,2012). It is characterized by a wide range of volatile secondary metabolites. It is commercially used as a spice. It is traditionally used to treat asthma, indigestion, headache, rheumatism, dizziness, gastrointestinal disorder and migraine (Abdel-Massih and Abraham, 2014). The major compound of Marjoram essential oil was terpinen-4-ol, which is believed to work by inhibiting oxidative respiration, inducing membrane deformation (dilatation) with consequent changes in membrane permeability of bacteria cells (Cox et al., 2000). Cumin *(Cuminum cyminum L.)* is aromatic plants included in the Apiaceae family and is used to flavor foods, added to fragrances, and for medical preparations. In addition, *C. cyminum* is used as antispasmodic, carminative, and appetite stimulant

agents (Morton,1976). Cumin essential Oil has antimicrobial activity as contain a high level of Cumin-aldehyde and to other minority compounds that may contribute to the antimicrobial activity, such as β-pinene, limonene and α-pinene (Iacobellis et al., 2005). The objective of the present study was to investigate the antioxidant as well as the antimicrobial effectiveness of two essential oils (Marjoram, Cumin& Mixture of both oils) at various concentrations on the quality of fresh minced beef during refrigerated storage (4ºC).

2. MATERIAL AND METHODS

A total amount of 6 kg of raw fresh minced meat was purchased from a butcher shop from Tanta city, taken and transferred directly to the laboratory under complete aseptic conditions without undue delay. The minced meat sample was divided to 6 equal groups (1 kg for each)1st groups as control (no treatment), 2nd &3rd groups were treated with Marjoram oil (0.2%-0.3% Vol/W), respectively.4th &5th groups were treated with Cumin oil (0.2%-0.3% Vol/W), respectively, while 6th group treated with Mixture of both oils (0.2%Vol/W), both oils (Marjoram-cumin) provided by "Phar-trade Company, Obour City, Cairo, Egypt " by hydro-distillation method. Tween 80 added to the oils before applying in minced meat groups as diluent and therefore, easily distribution and dissolving (Cosentino et al., 1999). The control as well as the treated groups were packed in a separate sterile polyethylene bags and stored in domestic refrigerator at nearly 4±1˚c. each sample was analysed at zero, 2nd, 4th, 5th, 6th, 7th and 8th days during storage. This work was conducted in triplicate. as follows:

2.1. Sensory examination:

The colour, odor and overall acceptability were determined for each sample of minced meat and were tested according *to* (Hemin,2013) at each inspection day.

2.2. Chemical examination includes:

- PH values were carried out according to (ISO,1974).
- TVN was done according to (FAO ,1980).
- TBA was carried out according to (Vyncke, 1970).

2.3. Bacteriological examination:

- Determination of APC according to (APHA, 1992).
- Determination of Coliform Count according to (APHA, 1992).
- Determination of Staphylococci Count according to (ICMSF, 1996)

2.4. Statistical Analysis:

ANOVA was carried out on data of the sensory, chemical and microbiological evaluations. Data are expressed as mean + SE (Gomez and Gomez, 1984).

3. RESULTS

The current results showed the effects of various concentrations of essential oils on acceptability of minced meat during storage at 4±1˚C.Marjoram oil (0.3% Vol/W) showed overall acceptability extended to 7th day of cold storage. While, lower concentration (0.2% Vol/W) showed acceptability till 6th day. In comparison Cumin oil (0.2% and 0.3%) and Mixture of both oils (0.2% Vol/W) showed overall acceptability till 4th, 5th and 5th days, respectively. In contrast, the control groups showed signs of unacceptability at 4th days (table1). A significant (P<0.05) increase in pH mean values in different treatments during storage by different rates. The highest ones were found in the untreated (control) samples, While, the treated samples with Marjoram oil (0.3% Vol/W), showed the highest significant effect on lowering PH values than those of other samples, followed by samples treated with Marjoram oil (0.2% Vol/W), Mixture of both oils (0.2% Vol/W), Cumin oil (0.3% Vol/W) and Cumin oil (0.2% Vol/W), respectively (table 2). Also, the evaluation of TBA mean values of untreated and treated samples during storage at 4ºC were shown in Table (3). The highest incremental rate was recorded in the untreated (control) sample which reached to 0.50±0.02 mg Melanoaldehyde /Kg in 2nd day of storage compared to zero day was 0.03±0.01 mg Melanoaldehyde /Kg, while the lowest incremental rate was recorded in samples treated with Marjoram oil (0.3% Vol/W) reached to 0.58±0.05 mg Melanoaldehyde /Kg at the end of 7th day of storage followed by samples treated with Marjoram oil (0.2% Vol/W), Mixture of both oils (0.2% Vol/W), Cumin oil (0.3% Vol/W) and Cumin (0.2% Vol/W), respectively.

The mean values of TVN are summarized in table (4), where the initial value for control group was 6.43±0.08 mg /100g that indicate good quality meat. Moreover, as the period of cold storage increased the TVN of all groups progressively increased with different rates depending on the nature of treatments. However, control group showed the highest incremental rate compared to other treated groups which had TVN value reached

to 19.6±0.86 mg /100g at end of 2nd day. But the other groups especially treated with Marjoram oil (0.3% Vol/W) showed decrease in accumulation of basic volatile nitrogen reaching to 18.13 mg/100g at the end of 7th days storage followed by samples treated with Marjoram oil (0.2% Vol/W), Cumin oil (0.3% Vol/W), Cumin oil (0.2% Vol/W) and Mixture of both oils (0.2% Vol/W), respectively. reached to 16.9±0.4 mg /100g, 19.1±0.06 mg /100g ,19.13±0.29 mg /100g and 20.17±0.5 mg /100g in 6th,5th,4th and 5th days, respectively.

The present data in table (5) showed that in control samples, the mean value (log.cfu/g) of APC increased from 5.75 ±0.04cfu/g at zero day to 6.11±0.11cfu/g at 2nd day. While, Marjoram oil (0.2% Vol/W and 0.3% Vol/W) reduced it to 5.5 ±0.02cfu/g and 5.3 ±0.4cfu/g at zero day, respectively. In comparison, Cumin oil (0.2% Vol/W and 0.3% Vol/W) reduced APC to 5.51 ±0.04cfu/g and 5.4 ±0.02cfu/g at zero day, respectively. Finally, the Mixture of both oils reached (0.2% Vol/W) to 5.45 ±0.03cfu/g at zero day. It is evident from the present investigation that Staphylococci Count in table (6) of treated and untreated minced meat groups showed significant differences during cold storage (4±1˚C). The treated groups with Cumin oil (0.3% Vol/W) followed by Mixture of both oils (0.2%Vol/W) and Cumin oil at (0 .2% Vol/W) showed the lowest counts in this parameter at 4th day 2.72±0.13, 2.89±0.03 and 2.94±0.04 cfu/g. And in 5th days decreased to 2.36±0.23 and 2.97±0.001 cfu/g in Cumin oil (0.3% Vol/W) and Mixture of both oils (0.2% Vol/W) groups, respectively. On the other hand, it could be observed that the control group had the highest count of Coliform at all days of cold storage compared to other treated groups. It is clear that Marjoram oil (0.3% Vol/W) have strong effect against the growth of Coliform especially at 4th and 5th days as count of Coliform decreased to 2.38±0.04 and 2.58±0.2 cfu/g, respectively. But Cumin oil at (0.3% Vol/W) and (0.2% Vol/W) had no decreasing effect on Coliform Count (table 7).

The results illustrated in table (8) showed that the highest reduction percentage of APC, Staphylococci and Coliform Counts were in Marjoram oil treated sample (0.3% Vol/W) in 5th day of storage (87%), in Cumin oil treated sample (0.3% Vol/W) in 5th day of storage (78%) and in Marjoram oil treated sample (0.3% Vol/W) in 4th day of storage (89%), respectively.

Table (1) The effects of various concentrations of essential oils on overall acceptability of the examined minced meat samples stored at 4±1 ºC

Days	Control sample	Marjoram (0.2%)	Marjoram (0.3%)	Cumin (0.2%)	Cumin (0.3%)	Mixture (0.2%)
zero	5	5	5	5	5	5
2nd	3	4	5	4	3	4
4th	2	3	3	3	3	3
5th	1	3	3	2	3	3
6th	1	3	3	1	2	2
7th	1	2	3	1	1	1
8th	1	1	2	1	1	1

(5) Very acceptable, (4) Acceptable, (3) Middle, (2) Unacceptable, (1) Rejected

Table (2): The effects of various concentrations of essential oils on pH of the examined minced meat samples stored at 4±1 ºC. The values represent mean±SD of three experiments.

Days	*Control*	*Marjoram (0.2%)*	*Marjoram (0.3%)*	*Cumin (0.2%)*	*Cumin (0.3%)*	*Mixture* (0.2%)
Zero	5.57±0.08^{a}	5.3±0.03b	5.1±0.05^{c}	5.55±0.07 ^{ab}	5.54±0.07^{ab}	5.48±0.06^{ab}
2nd	5.9±0.02^{a}	5.4±0.03^{c}	5.2±0.05^{d}	5.70±0.01^{b}	5.66±0.05^{b}	5.63±0.01^{b}
4th	Spoiled	5.7±0.03^{b}	5.36±0.03^{c}	5.86±0.01^{a}	5.80±0.03^{a}	5.83±0.03^{ab}
5th	-	6.0±.05^{a}	5.7±0.05^{b}	Spoiled	6.02±0.09^{a}	6.1±0.05^{a}
6th	-	6.1 ±0.6	5.91±0.1	-	Spoiled	Spoiled
7th	-	Spoiled	6.11±0.2	-	-	-
8th	-	-	Spoiled	-	-	-

Means within a row followed by different letters are significantly different ($P < 0.05$)

Table (3): The effects of various concentrations of essential oils on TBA of the examined minced meat samples during cold storage at 4±1 ºC

Days	*Control*	*Marjoram (0.2%)*	*Marjoram (0.3%)*	*Cumin (0.2%)*	*Cumin (0.3%)*	*Mixture* (0.2%)
Zero	0.03±0.01^{a}	0.03±0.01	0.03±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}
2nd	0.50±0.02^{a}	0.27±0.01^{c}	0.26±0.01^{c}	0.36±0.02^{b}	0.29±0.01^{c}	0.29±0.01^{c}
4th	Spoiled	0.43±0.01^{b}	0.41±0.01^{b}	0.48±0.01^{a}	0.43±0.01^{b}	0.46±0.01^{a}
5th	-	0.54±0.02^{b}	0.46±0.01^{c}	Spoiled	0.62±0.01^{a}	0.62±0.01^{a}
6th	-	0.63±0.01	0.54±0.07	-	Spoiled	Spoiled
7th	-	Spoiled	0.58±0.05	-	-	
8th	-	-	Spoiled	-	-	-

Table (4): The effects of various concentrations of essential oils on TVN of the examined minced meat samples during cold storage at 4±1 ºC

Days	*Control*	*Marjoram (0.2%)*	*Marjoram (0.3%)*	*Cumin (0.2%)*	*Cumin (0.3%)*	*Mixture* (0.2%)
Zero	6.43±0.08^{a}	6.43±0.08^{a}	6.33±0.11^{a}	6.43±0.08^{a}	6.43±0.08^{a}	6.43±0.08^{a}
2nd	19.6±0.86^{a}	8.0±0.20^{d}	7.83±0.17^{d}	12.7±0.23^{b}	9.43±0.23^{c}	10.4±0.10^{c}
4th	Spoiled	8.9±0.17^{d}	8.33±0.08^{e}	19.13±0.29^{a}	14.5±0.02^{c}	15.31±0.06^{b}
5th	-	13.0±0.2^{c}	9.31±0.15^{d}	Spoiled	19.1±0.06^{b}	20.17±0.50^{a}
6th	-	16.9±0.4	12.8±0.4	-	Spoiled	Spoiled
7th	-	Spoiled	18.13±0.4	-	-	-
8th	-	-	Spoiled	-	-	-

Table (5): The effects of various concentrations of essential oils on APC of the examined minced meat samples during cold storage at 4ºC

Days	*Control*	*Marjoram (0.2%)*	*Marjoram (0.3%)*	*Cumin (0.2%)*	*Cumin (0.3%)*	*Mixture* (0.2%)
Zero	5.8±0.04^{a*}	5.5±0.02^{b*}	5.3±0.4^{d}*	5.51±0.04^{b}*	5.4±0.02^{c}*	5.45±0.03^{bc}*
2nd	6.1±0.13^{a}*	5.3±0.9^{b}*	5.1±0.7^{c}*	5.3±0.02^{b}*	5.1±0.04^{b}*	5.38±0.04^{b}*
4th	Spoiled	5.0±0.3^{d}*	4.8±0.4^{e}*	5.8±0.01^{a}*	5.5±0.02^{b}*	5.20±0.1^{c}*
5th	-	4.8±5.6^{c}*	4.6±0.5^{d}*	Spoiled	5.9±0.02^{a}*	5.70±0.03^{b*}
6th	-	5.9±0.6*	5.3±0.1*	-	Spoiled	Spoiled
7th	-	Spoiled	5.8±0.2*	-	-	-
8th	-	-	Spoiled	-	-	-

Table (6): The effects of various concentrations of essential oils on Staphylococci Count of the examined minced meat samples during cold storage at 4±1 ºC

Days	*Control*	*Marjoram. (0.2%)*	*Marjoram (0.3%)*	*Cumin (0.2%)*	*Cumin (0.3%)*	*Mixture* (0.2%)
Zero	3.36±0.03^{a}	3.34±0.04^{a}	3.30±0.05^{ab}	3.24±0.08^{ab}	3.11±0.08^{b}	3.21±0.09^{ab}
2nd	3.65±0.1^{a}	3.32±0.02^{b}	3.25±0.04^{b}	3.16±0.9^{b}	2.89±0.7^{c}	3.13±0.04^{bc}
4th	Spoiled	3.26±0.06^{a}	3.03±0.03^{b}	2.94±0.04^{b}	2.72±0.13^{c}	2.89±0.03^{bc}
5th	-	3.62±0.6^{a}	3.41±0.18^{a}	Spoiled	2.36±0.23^{b}	2.79±0.001^{b}
6th	-	3.92±0.7	3.47±0.1	-	Spoiled	Spoiled
7th	-	Spoiled	3.75±0.2	-	-	-
8th	-	-	Spoiled	-	-	-

Table (7): The effects of various concentrations of essential oils on Coliform Count of the examined minced meat samples during cold storage at 4±1 ºC

Days	*Control*	*Marjoram (0.2%)*	*Marjoram (0.3%)*	*Cumin (0.2%)*	*Cumin (0.3%)*	*Mixture* (0.2%)
Zero	3.39±0.03 [a]	3.39±0.02 [a]	3.35±0.02 [a]	3.39±0.03 [a]	3.39±0.03[a]	3.39±0.01 [a]
2nd	4.5±0.06[a]	3.37±0.02[b]	3.33±0.02[b]	3.40±0.02[b]	3.41±0.02[b]	3.36±0.02[b]
4th	Spoiled	3.35±0.01[a]	2.38±0.04[b]	3.42±0.02[a]	3.39±0.01[a]	3.34±0.2[a]
5th	-	3.31±0.01[a]	2.58±0.2[b]	Spoiled	3.42±0.002[a]	3.39±0.01[a]
6th	-	3.38±0.6	3.05±0.1	-	Spoiled	Spoiled
7th	-	Spoiled	3.33±0.2	-	-	-
8th	-	-	Spoiled	-	-	-

Table (8) Reduction percent of (APC-Staphylococci- Coliform) with different concentrations of essential oils. No reduction

M.O	Groups	zero	2nd	4th	5th	6th	7th
APC							
	Marjoram (0.2%)	44%	66%	82%	88%	-	-
	Marjoram (0.3%)	61%	79%	87%	92%	64%	-
	Cumin (0.2%)	41%	54%	-	-	-	-
	Cumin (0.3%)	53%	61%	44%	-	-	-
	Mixture	50%	57%	73%	-	-	-
Staphylococci							
	Marjoram (0.2%)	3%	7%	19%	-	-	
	Marjoram (0.3%)	12	22%	54%	-	-	
	Cumin (0.2%)	22%	35%	62%	-	-	
	Cumin (0.3%)	40%	65%	76%	87%	-	
	Mixture	26	39%	66%	73%	-	
Coliform							
	Marjoram (0.2%)	.7%	5%	11%	17%	3%	-
	Marjoram (0.3%)	9%	13%	90%	80%	55%	30%
	Cumin (0.2%)	-	-	-	-	-	-
	Cumin (0.3%)	-	3%	7%	-		-
	Mixture	1%	7%	12%	1%		-

4. DISCUSSION

As in all foods, the organoleptic tests are generally the final guide of the quality from the consumer's point of view. Thus, it is beneficial to make a comparison between sensory evaluation for untreated and treated minced meat with the studied oils. The current results (Table 1) are in agreement with El-Desouky et al., (2006); Chouliaraet al., (2007) and Darwish-Soumia et al., (2012**)** who stated that incorporating of lower concentrations of essential oils in meat products in combination with refrigeration also had positive effects on their sensory traits as well as improving the quality, safety and shelf-life stability.

On the other hand, changing in pH values may be due to the activation effect of microbial load which may cause protein hydrolysis with the appearance of alkaline groups. and due to endogenous enzymes, bacterial metabolites as hydrogen sulfides, organic sulfides, other volatile organic compounds like amines (Gill,1986). Similar trends of pH changes (Table 2) have been observed in meat, poultry and fish products treated with natural essential oils (El-Desouky et al., 2006 Keokamnerdet al., 2008; Shalaby et al., 2013).

Also, TBA value is routinely used as an index of lipid oxidation in meat products in store (Raharjo and Sofos, 1993). The results (Table 3) are in agreement with Mohamed et al., (2011) and Badee et al., (2013) who stated that herbal extract of Marjoram has antioxidant effects and can be used to minimize lipid oxidation. according to (E.O.S, 2005) the TBA value accepted till 0.9 mg Melanoaldehyde /Kg raw minced beef. This mean all in the experiment accepted.

The present results (Table 4) regarding TVN are in agreement with the findings achieved by El-

Desouky et al., (2006); Chouliara et al., (2007); Keokamnerd et al., (2008) and Badee et al., (2013) This may be attributed to the breakdown of proteins as a result of activity of microbial strains and proteolytic enzymes (Yassin - Nessrien, 2003). The treatments with Marjoram oil (0.3% Vol/W) was more effective in delaying the rate of TVN increase during the subsequent cold storage. This may be attributed to the role of such oil on microbial population and bacterial growth as antimicrobial agents (Sachindra et al., 2005). E.O.S. (2005) stated that 20 mg TVN/ 100 gm raw samples indicate the spoilage of minced meat, this appear in 5th day of storage in Mixture of both oils group was 20.17 mg TVN/ 100 gm thus, indicate meat spoilage.

Aerobic plate count is a general measure of the microbiological status of meat and is considered as an indicator of the overall degree of microbial contamination of meat. This results (Table 5) were agreed with Kamel, (2013) who stated that Marjoram oil reduced the total aerobic mesophilic bacterial counts by (1-2) log compared to zero-time control This may be due to that Marjoram contains some aromatic components that have antimicrobial properties (Busatta et al.,2008). Also in a line with (Ezzeddine et al.,2001) who found that among several essential oils Marjoram oil has the greatest potential antimicrobial effect. All samples were accepted except the control sample in 2nd day of storage according to E.O.S. (2005) as permissible limit (10^6). Concerning to (Table 6) the result were agreement with (Iacobellis et al., 2005) as they showed that Cumin-aldehyde in *Cuminum cyminum* essential oil has potent antibacterial effects against gram positive bacteria. Also (Rezai et al., 2014) studied the effect of Cumin essential oil which holds a great antimicrobial potential, thus it may be used as a substitute for chemical preservatives for controlling *S. aureus* caused poisoning resulting from consuming meat products. The control as well as all treated groups were unaccepted by (E.O.S., 2005) as permissible limit of Staphylococci in minced meat (10^2). The detection of Coliforms is widely used as a mean of measuring the effectiveness of decontamination (Lues and Van Tonder, 2007). Regarding to (table7) the examined essential oils are less active against Gram- negative bacteria. The better effectiveness of essential oils against Gram-positive bacteria than Gram negative bacteria may be due to volatile action of essential oils and due to absence of lipo-polysaccharide layer in Gram positive bacteria that might function as an effective barrier against any incoming biomolecule (Delaquis et al., 2002). There might be another possibility that essential oils may successfully inhibit microbial respiration and increase the plasma membrane permeability, which results in to death of bacterial cells after massive ion leakage (Walsh et al., 2003). It may also happen due to hydrophilic nature of bacterial cell wall. On the other hand, Gram- negative bacteria have intrinsic resistance against toxic components, since they have a permeability barrier in the outer cell envelope against toxic agents. Hydrophobic macromolecules, such as essential oil constituents, are unable to penetrate the barrier. On the other hand, essential oils usually express low aqueous solubility, which prevents them from reaching a toxic level in cellular membranes (Mann et al., 2000). In conclusion, Marjoram oil proved to be more efficient than Cumin oil. So, the use of Marjoram (0.3% Vol/W) as it is safe natural agent, is therefore recommended to improve safety and extend shelf life of meat product. Due to the limited number of studies and in order to optimize the synergy potential of mixtures of essential oils, scientific research should focus on additional in vivo studies and clinical trials to study the mechanism of action of the synergisms, additions or antagonisms in order to optimize and further evaluate the activity of essential oils in food preservation, medicine, cosmetics, pharmacological applications, animal models and veterinary or aquaculture field

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