J. Agric. Sci. Mansoura Univ., 29 (6): 3339 - 3360, 2004

EFFECT OF MARJORAM ESSENTIAL OIL ON THE QUALITY OF GROUND BEEF PATTIES DURING REFRIGERATED STORAGE

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ABSTRACT

Salt addition, cooking and refrigerated storage are well known to accelerate oxidative deterioration and cause certain unfavorable changes in meat quality. Therefore our objective was to evaluate the effects of marjoram essential oil (MEO) and /or sodium chloride (NaCl) on the quality attributes of raw and cooked ground beef patties during refrigerated storage. The results indicated that addition of 1% NaCl to patty formula strongly accelerated pigment and lipid oxidation and showed a poorer quality characteristic as compared to control or marjoram-treated patties.

Addition of 500 mg MEO to Kg^{-1} minced meat was very effective in improving lipid stability, color measurements, sensory characteristics and microbial shelf – life in marjoram-treated patties as shown by the consistently lower values of thiobarbituric acid reactive substances (TBARS), total volatile basic nitrogen (TVBN), pH, total plate counts (TPCs) and the intensity of negative flavor notes associated with lipid oxidation, initially and during the course of chilling study , in comparison to control or salt-treated patties. The results also demonstrated that no remarkable benefit effect in patty qualities was detected from doubling marjoram level (1000 mg kg^{-1} minced meat). In view of the possible health benefits from sweet marjoram use, MEO may have potential as natural antioxidant, antimicrobial and flavor enhancer source for food use.

Keywords: Sweet marjoram – essential oil – natural antioxidant – beef patties – quality attributes.

INTRODUCTION

Lipid oxidation in muscle foods, which occurs during raw material storage, processing, heat treatment and further storage of final products, is one of the basic processes causing rancidity in food products, leading to their deterioration in many quality characteristics such as flavor, color, texture, nutritive value and safety, and limits acceptability of these products *(Buckley et al., 1995 and Karpinska, et al., 2001)*. Lipid oxidation in meat seems to be initiated by meat pigments, tissue enzymes, non-heme iron, trace elements and sodium chloride added to processed meat products *(Skibsted et al., 1998)*. Furthermore, ground meat tends to become brown and rancid more rapidly than whole muscle retail cuts. Grinding not only exposes more surfaces to air and microbial contamination, but also accelerates loss of intracellular reductants, such as reduced nicotinamide-adenine dinucleotide, which minimizes metmyoglobin formation *(Ledward and macfarlane, 1971)*. In recent years, demand for precooked ready-to-eat meats has increased. However, pre-cooked meat is even more susceptible to lipid oxidation and development of warmed over flavor (WOF) than raw meat during refrigerated

carcinogenic effects to humans *(Gardner, 1979; Frankel, 1991)*. Products of lipid oxidation also influence other food constituents; e.g. they interfere with the absorption of protein or folic acid, inhibit activity of enzymes and increase the content of cholesterol and peroxides in blood serum *(Ames, 1983)*. Considering the possibility of undesirable influences of oxidized lipids on the human organism, it seems to be of essential importance to prevent or at least minimize the content of lipid oxidation products in food *(Gray, et al., 1996)*. Synthetic phenolic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ) have been reported to be effective at a level of 200 ppm in reduction of lipid oxidation *(Shahidi et al., 1987)*. However, use of these types of antioxidants is controlled due to their carcinogenic potential *(Chen et al., 1992 and Sun and Fukuhara, 1997)*, as well as general rejection of synthetic food additives by consumers.

The in vitro addition of tocopherol and ascorbic acid derivatives that are being used as alternatives to BHA, BHT, PG and TBHQ to minced meat during processing was not very effective in controlling lipid oxidation *(Higgins et al., 1998)*. Therefore, the development and utilization of more effective antioxidants of natural origin are desired. Natural antioxidants extracted from plants such as rosemary, sage, paprika, carnosine, soybean, citrus peel, sesame seed, olives and grapes can be used as alternative to the synthetic antioxidants because of their equivalent or greater effect on inhibition of lipid oxidation *(O'Neill et al., 1999 and Aguirrezabal et al., 2000)*. However, such antioxidants are not widely applied industrially and commercially either for their limited availability, high costs or color and flavor problems *(Hettiarachchy et al., 1996;Tang et al., 2001)*. Hence, investigations directed towards finding a suitable, low cost, versatile, and effective antioxidant source are needed. Sweet marjoram (Origanum magorana) is a herbaceous and perennial plant native to southern Europe and the Mediterranean. Marjoram belongs to the Labiatae family like rosemary, thyme, Dittany and oregano, but has a less penetrating taste than these spices. Plants of Labiatae family are well known for their antioxidant properties *(M'Carthy et al., 2001)*. For food uses, the highly aromatic leaves and flowering tops of marjoram plant are applied fresh, dried, and ground to flavor sausages, meats, soups and salads *(Novak et al., 2000)*. Moreover, marjoram is also used for health-treatment and in cosmetics *(Jun et al., 2001)*. Marjoram, a low cost food ingredient traditionally consumed in Egypt, Europe, United States and Asia countries without reported ill effects, was tested as a possible source of natural antioxidants. Moreover, little information is available on the effect of marjoram essential oil on the quality of ground beef patties. Therefore, our objective was to study the effectiveness of marjoram essential oil at two levels with or without sodium chloride in preventing / minimizing lipid oxidation in raw and cooked ground beef patties during refrigerated storage. The effects of these additives on physico- chemical, organoliptical and microbiological qualities of ground beef patties are also investigated.

MATERIALS AND METHODS

Chemicals:

All chemicals used for analysis were " AnalaR" grade.

Preparation of Marjoram Essential Oil (MEO):

Dried marjoram (Origanum majorana) leaves (3kg) were obtained from Al-Dahlia Company, Nasr city, Cairo, Egypt. Portions of 500 g of marjoram leaves with 1 5L. distilled water were placed in a flask (2L.).
A Continuous steam distillation extraction head was attached to the flask. After steam distillation ca. 3h the essential oil was collected and dried using anhydrous sodium sulphate, then stored in dark glass bottles at 4±1°C until usage *(Tassou et al., 1995)*.

Patty samples preparation:

Fresh beef lean (from the round position) and fat (from beef trim) were purchased from a local market, bone, skin and connective tissues were removed, then cut into small pieces (2 5 cm^3) and minced individually through a 8-mm plates (coarse). The minced meat and fat portions needed to formulate 3 kg. patties from each group (Table 1) were mixed together in a meat blender for 1 min. to assure uniform fat level, then 3% potato starch (as a binding agent) was added and minced through a 3-mm holes (fine), reminced twice with 10 % water (as ice flakes), aiming to keep the mixtures smooth as well as to minimize temperature rise and microbial contamination, then tempered in a freezer for 10 min. (Podolak *et al.*, 1997) before treatments. Meat mixture of each group was passed through a plate with 3-mm holes, then treated with either 1% NaCl (S) or 500 mg MEO kg^{-1} minced meat (M1) or 1% NaCl Plus 500 mg MEO Kg^{-1} minced meat (M1S) or 1% NaCl plus 1000 mg MEO Kg^{-1} minced meat (M2S). Control minced meat contained neither NaCl nor MEO. First MEO was dispersed in about 2 ml of distilled water before adding to the formulation in a similar way to the method of (*Hettiarachchy et al., 1996)*. Ground meat was blended with salt and/or antioxidant (MEO) at speed 1 for 30 second to thoroughly mix all ingredients, then increased to speed 2 for another 2 minutes and chilled again for 10 minutes, then ground beef patties approximate (75 g weight, 7 cm in diameter and 1.2 cm thickness) were formed according to the method of *Andersen and Skibsted (1991)*, using a Moulinex burger machine.

Experimental design and analysis:

1- Raw ground beef patties (20 patties from each treatment and control) were packed in polyethylene bags (2patties/bag) and stored at 4±1°C for 15 days with no exposure to light.
2- 20 patties from each treatment and control were cooked in a multipurpose electric oven (Olympic–Hana) pre-heated to 175°C to an internal temperature of 75°C. (as indicated by Thermo- meter), according to the method of Tang *et al.*(2001).Cooked patties were cooled to room

temperature, packed in polyethylene bags (2 patties /bag) and stored at 4 ± 1°C for 9 days with no exposure to light.

Raw patties were evaluated at 0, 3, 6, 9, 12 and 15 days for pH, total volatile basic nitrogen, color measurements, total plate counts (TPCs), TBARS and oxidative stability, while cooked patties were evaluated at 0, 1, 3, 5, 7 and 9 days for the above mentioned parameters as well as sensory evaluation.

Table 1: Formulas for ground beef patties per kg emulsion:

Treatments / Ingredients	Control (c)		Salt (S)		M1		M1 S		M2 S	
	%	G	%	G	%	G	%	G	%	G
Minced lean beef	72	720	71	710	71.95	719.50	70.95	709.50	70.90	709.0
Minced beef fat	15	150	15	150	15	150	15	150	15	150
Potato starch	3	30	3	30	3	30	3	30	3	30
Water (as ice flakes)	10	100	10	100	10	100	10	100	10	100
Salt (NaCl)	/	/	1	10	/	/	1	10	1	10
Marjoram essential oil (MEO)	/	/	/	/	500 ppm	0.5	500 ppm	0.5	1000 ppm	1000

1- Control (C) : No, additives.
2- Salt (S) : 1% NaCl.
3- M1 : 0.5g MEO kg^{-1} minced meat.
4- M1S : 0.5g MEO kg^{-1} minced meat plus 1% NaCl.
5- M2S : 1.0 g MEO kg^{-1} minced meat plus 1% NaCl.

Analytical Methods:

At a given storage time two raw and/or cooked ground beef patties from each treatment and control were homogenized in a food processor to ensure a homogeneous and representive sample for chemical analysis. All analyses were performed at least in duplicate.

Proximate composition was determined according to the methods recommended by the A.O.A.C. (1995).Total volatile basic nitrogen (TVBN) as mg nitrogen per 100 g flesh was measured according to *Pearson (1981)*. Thiobarbituric acid reactive substances (TBARS) as mg malonaldehyde per kg meat (mg MDA/kg), were performed according to *Vyncke(1975)*.The sum of TBARS values for untreated and treated patties samples were used to calculate the inhibition (+) or promotion (-) of lipid oxidation as a percentage of control samples, which (%) = (control – treatment) ÷ control x 100), according to *Tang et al.,(2001)*. pH values were recorded as per *De-Fritas et al.,(1997)*. Color evaluation of raw and cooked patties was determined using a Hunter Lab Scan XE colorimeter (Hunter Laboratory Inc. Restonva), patties were scanned at three different locations to determine L, a and b values. Microbiological quality was assessed by measuring total plate counts (TPCs) following the method recommended by *Harrigan and Margaret,(1966)*.Sensory evaluation of cooked beef patties was performed only on edible patty samples from each group according to the method of Kim and Marshall (1999) by 10 panelists. Prior to testing patty samples were

reheated in a microwave oven (Gold Star, 980 w) for 30 sec., and serving hot to panelists. Flavor and appearance of cooked treated and control patty samples were evaluated during refrigerated storage at 4±1°C till noticeable deterioration in chemical indices (TBARS and TVBN) was observed.

RESULTS AND DISCUSSION

Proximate analyses:

Proximate chemical composition of raw and cooked ground beef patties either control or treated with salt and / or MEO are given in Table 2. Display of data revealed that raw fresh control samples had 60.24% moisture, 17.3% protein, 17.15% fat and 2.16% ash (on fresh weight basis). In this respect, Kulshrestha and Rhee (1996) reported that raw fresh beef patties contained 60.25% moisture and 18.23% fat (on fresh weight basis). While Saleh and Ahmed (1998) found that raw fresh control beef patties showed 69.1% moisture, 19.7% protein, 6.0% fat and 2.7% ash (on fresh weight basis). Such a high degree of variability in chemical composition of different patty types could be explained in the basis that formula ingredients, fat level as well as method of processing affect to a great extent the proximate composition and quality attributes of formulated patties (Krichner *et al.*, 2000). From the same given results of Table 2, it could be observed that the addition of MEO and/ or NaCl to raw fresh patties samples had little effect on their proximate composition, the values for moisture, protein, fat and ash contents in treated patties were consistent between treatments, indicating that all treatments were similar in composition (Table 1) except for salt and / or antioxidant used. Table 2 further shows that cooking process caused evaporation of a certain amount of water in all patty samples; moisture content was reduced to 51.8-53.6% in cooked patties. Such reduction in moisture content could be due to protein denaturation and consequently the decrease in water holding capacity, which led to loss in moisture content. On the other hand, the reduction of moisture content in cooked patties leads to slight apparent increase in their protein, fat and ash contents (Table 2). Similar trend of these changes was observed in beef patty samples during various thermal processes (Saleh and Ahmed, 1998; Kirchner *et al.*, 2000 and Karpinsha *et al.*, 2001).

Cooking yields and cooking losses of different formulated beef patties are also presented in Table 2. The obtained results indicated that cooking loss of control patty samples was 24.6%, which means that cooking yield in control samples was 75.4%. These values are in harmony with those obtained by Kirchner *et al.*, (2000) who reported that cooking yields of beef patties was 75.6%, also Kulshrestha and Rhee (1996) found that beef patties samples showed 23 4% cooking loss. The discrepancy in cooking yields may be due to differences in fat levels, cooking end-point temperature, cooking method as well as degree of doneness (Kirchner *et al.*, 2000). Concerning cooking yield, it is apparent from the results in Table 2 that incorporation of MEO in patties formulas reduced the cooking losses in treated samples as compared to control ones. Additionally, as the concentration of MEO

increased in the formula the cooking loss percentages decreased. This could be due to the effect of essential oil in reducing the rendering rate of juice during cooking and hence decreased cooking losses. Similar results were recorded in cooked beef sausages treated with cardamom essential oil by Hassan (1997).

Table 2: Proximate chemical composition and cooking yields of ground beef patties (on fresh weight basis)

Constituents / Treatments	Moistare %		Protein %		Fat %		Ash %		Cooking %	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Control (C)	60.24	51.80	17.30	19.76	17.15	18.0	2.16	2.47	24.6	75.4
Salt (S)	61.40	53.25	16.76	19.07	16.60	17.48	2.18	2.50	23.3	76.7
MEO (M1)	60.32	52.14	17.41	20.1	17.06	18.71	2.29	2.63	23.1	76.9
(M1S)	61.0	53.0	17.04	19.30	16.90	18.15	2.32	2.68	22.9	77.1
(M2S)	61.20	53.60	16.98	19.65	16.84	18.47	2.35	2.70	22.2	77.8

1- Control (C) : no additives.
2- Salt (S) : 1% NaCl.
3- M1 : 0.5g MEO kg^{-1} minced meat.
4- M1S : 0.5g MEO kg^{-1}+1% NaCl.
5- M2S : 1.0 g MEO kg^{-1} + 1% NaCl.
6- MEO : Marjoram Essential oil.

Lipid stability during refrigerated study:

Evaluation TBARS values could be useful to determine whether an additive was potentially antioxidative or prooxidative (Tang *et al.*, 2001).

Data illustrated in Fig 1 showed the changes that took place in TBARS values of raw ground beef patties during refrigerated storage for 15 days. Data indicated that salt(S) treated raw patties exhibit the highest TBARS values at any given time of chilling study as compared to control(C) or marjoram containing patties (M1, M1S, M2S). Conversely, marjoram treated (M1) samples had the greatest oxidative stability as shown by the consistently lower TBARS values initially and during the refrigerated study.

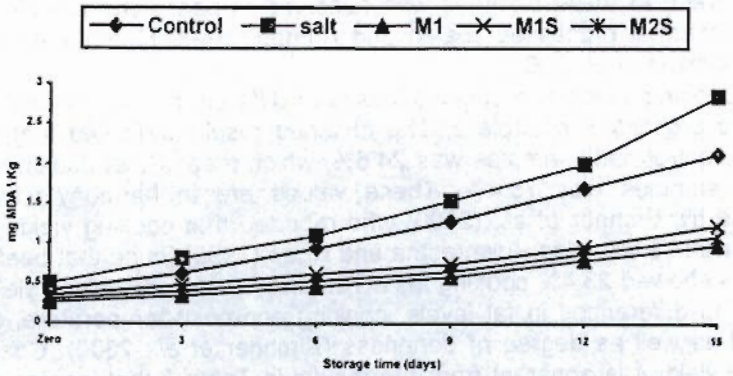


Fig. (1): TBARS values of raw (uncooked) ground beef patties during refrigerated storage at 4±1°C for 15 days.

Fig.1 further shows that the storage stability of raw ground beef patties was improved by inclusion of marjoram essential oil in the formula. In addition, the effectiveness of MEO increased as its concentration in the formula increased. These results confirmed the findings obtained by (Hettiarachchy *et al* ,1996 and Hassan, 1997)on their framework on meat products treated with natural antioxidants.

As shown in Fig. 1 TBARS values increased over time in all raw (uncooked) patties, with the salt treated(S) patties oxidizing most rapidly and to the greatest extent as compared to control samples, these results indicated that addition of 1% NaCl to patty formula promoted lipid oxidation. Numerous studies have demonstrated the prooxidant properties of NaCl in muscle foods (O'Neill *et al.*, 1999; Agguirrezabal, *et al.*, 2000). However, the prooxidant properties of NaCl could be due to its ability to reduce antioxidant enzyme activities (Lee *et al.*, 1997), and also due to its ability to displace ionic iron from myoglobin, providing free iron ions for the catalysis of lipid oxidation (Kanner *et al.*, 1991).

It is clear from the results in Fig.1 that incorporation of marjoram essential oil in raw patties, alone or in combination with 1% NaCl (M1, M1S, M2S) had lower and acceptable TBARS values (less than 0.9 mg MDA kg^{-1} meat) as required by the Egyptian standards for meat products (ES – 1688/ 1991) compared to control(C) or salt(S) treated patties. Moreover, marjoram containing patties still showed good quality even after 12 days of refrigerated storage versus to only six days for control samples (Fig. 1). These results are in agreement with the findings achieved by Karpinska *et al.*, (2001) who reported that marjoram addition retarded lipid oxidation in minced meat balls, and it is advised that marjoram essential oil could be used as an antioxidant additive in industrial processing of food. However, the accumulation of malonaldehyde in patty samples during refrigerated storage could be due to hydrolytic and oxidative processes in the lipid fraction (Brake and Fennema, 1999), as well as to the increase in "free" iron ions during refrigerated storage of meat (Kanner *et al.*, 1991).On the other hand, the high efficiencies found in marjoram essential oil were closely related to the high content of phenolic compounds, confirming the key role of phenolic compounds as scavengers of free radicals and as primary, chain breaking antioxidants (Jun *et al.*, 2001; Hamed 2003).

TBARS values of cooked ground beef patties as affected by treatments and chilling storage were illustrated in Fig 2. From which it is apparent that directly after cooking, the content of malonaldehyde in all patty samples increased with a higher rate in salt(S) treated and control(C) samples than marjoram treated samples (M1, M1S, M2S). Moreover, during refrigerated storage of cooked patties further increases in. TBARS were observed in all samples under investigation. In this concern, M'Carthy *et al.*, (2001) and Karpinska *et al.*, (2001) reported that cooking resulted in a four – fold increase in TBARS over raw patties. Recently Tang *et al.*, (2001) found that TBARS of cooked control patties increased from1.5 to 16.83 mg MDA kg^{-1} meat during 10 days at 4°C. They also reported that salt treated patties showed higher TBARS values than control samples initially and during

refrigerated storage. However the wide variety of TBARS values could be due to differences in meat species, pH values, cooking methods, pigments concentrations, methods for the TBARS assay, total lipid and PUFA concentrations as well as to differences in catalase activity and H_2O_2 remaining in the muscle tissue (Asghar *et al.*, 1988; Rhee *et al.*, 1996 and Tang *et al.*, 2001).

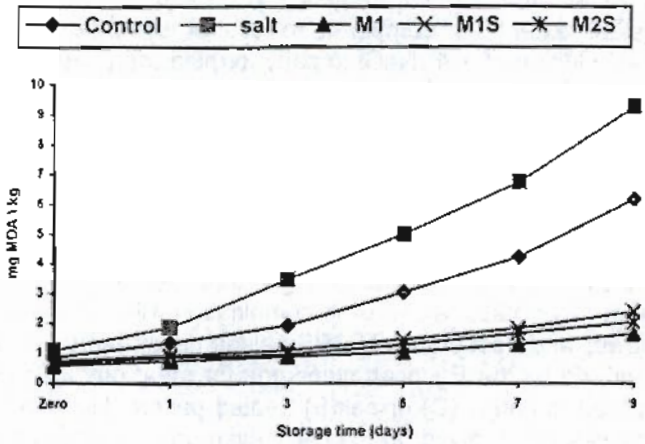


Fig. (2):TBARS Values of cooked ground beef patties during refrigerated storage at 4±1°C for 9 days.

Generally, the results illustrated in Figs. 1 and 2 supported the findings of Kingston *et al.*, (1998) who reported that cooked meat is more susceptible to lipid peroxidation than raw meat during refrigerated storage, possible due to the fact that cooking inactivates catalase, denatures the membrane and release phospholipids (the major source of poly unsaturated fatty acids) bringing reactants and catalysts closer (Rhee, 1988), also cooking decreased the activation energy for oxidation, breaking down pre-formed hydroperoxides that propagate lipid peroxidation (Kanner *et al.*,1991).

By examining data in Fig. 2 it is obvious that all cooked marjoram containing patties (M1, M1S, M2S) exhibit quite low and well within the range (< 2.0) generally considered acceptable for cooked meat, initially and during 7 days of chilling study, indicating that the addition of marjoram essential oil at a concentration of 500 mg kg^{-1} minced meat offered the most effective protection against salt- catalyzed oxidation. In this concern it is worth mentioning that, the threshold of TBARS values for oxidized flavor in cooked beef was between 0.6 – 2.0 mg MDA/kg meat (Greene and Cummuze, 1982; Tang *et al.*, 2001). With this threshold as an indicator, marjoram essential oil almost totally controlled the development of lipid oxidation in cooked beef patties and prolonged the shelf-life of cooked treated patties to 7 days compared to only 3 days for cooked control patties at 4± 1°C. These results confirmed the findings obtained by Tang *et al.*, (2001), who reported that pre-

cooked beef patties treated with tea catchins (as a natural antioxidant) at a concentration of 300 mg kg^{-1} minced meat, alone or in combination with 1% NaCl, showed good quality after 10 days at 4°C compared to only 3 days for control patty samples. Regarding the antioxidative capacity of marjoram essential oil (MEO), it is worth mentioning that antioxidant compounds from sweet marjoram exhibited the strongest O_2^- scavenging ability as compared to tea catechins (EGCG and EC), BHA, α - tocopheral, ascorbic acid and BHT in a decreasing order. Its activity was approximately five times greater than that of α -tocopherol, which is a natural O_2^- scavenger with high efficiency (Jun *et al.*, 2001).

Oxidative stability during refrigerated study:

Inhibition (+) rate of lipid oxidation by MEO or promotion (-) rate by NaCl added to patty formula during refrigerated storage at 4 ± 1 °C are shown in Table (3) from which it is evident that NaCl addition promoted lipid oxidation for patty samples under investigation. However, the promotion rate was increased in cooked patties (-56.05) than in corresponding raw patties (-24.29%) as compared to control samples.From the same given results of Table 3 it is apparent that addition of marjoram essential oil at a level of 500 mg kg^{-1} minced meat (M_1 samples) had the greatest oxidative stability (+51.69%) as compared to other patties containing marjoram, which exhibit + 38.14% and +45.62% inhibition rate for M_1S and M_2S raw samples; respectively. Marjoram contains phenolic compounds (Hamed, 2003) and this acts as an antioxidant by interrupting the free radical chain in the propagation step of the oxidative process (Farag *et al.*, 1989).Table 3 further shows that the inhibition rate due to addition of marjoram essential oil were found to increase in cooked samples than in the corresponding raw patties, the M1, M1S and M2S cooked patties exhibit +64.81, +51.79 and 57.36% ; respectively. These results suggest that marjoram essential oil (MEO) should receive attention as a new naturally occurring antioxidant.

Table (3): inhibition (+) and promotion (-) rates of lipid oxidation during refrigerated storage.

Treatment	Refrigerated storage at 4 ± 1°C	
	Raw	Cooked
Salt	-24.29 %	-56.05 %
M1	+51.69 %	+64.81 %
M1S	+38.14 %	+51.79 %
M2S	+45.62 %	+57.36 %

pH changes:

Meat pH is considered as one of the most important technological properties as it alters pigment and lipid stability (Jermiah and Gibson, 2001). Table 4 shows the pH values of raw ground beef patties during refrigerated storage at 4 ± 1°C for 15 days. From which it is apparent that pH value of raw

fresh control patty samples at zero time storage was 5.80. This result is in agreement with the finding of Warren *et al.*, (1996) who reported that raw fresh beef patties had pH value of 5.75, while Kulshertha and Rhee (1996) reported that raw fresh beef patties exhibit 5.84 for pH. M'Carthy *et al.*, (2001) found that pH value of raw fresh pork patties was 5.90. Table 4 further shows that pH value of salt treated patty samples was 5.70, indicating that addition of 1% NaCl to patty formula decreased the pH value by 0.10 units. These results confirmed the findings of Andersen and Skibsted (1991) in their framework in beef patties treated with sodium chloride and/or polyphosphates. From the same given results in Table 4 it is clear that inclusion of marjoram essential oil in patty formula (M_1, M_1S, M_2S) had almost no effect on their initial pH values.

Data presented in Table 4 also indicated that during refrigerated storage pH values of raw patty samples tended to decrease up to 6 days, and then gradually increased till the end of chilling period. Similar trend of these changes was observed by M'Carthy *et al.*, (2001). However, the decline in pH values could be due to the formation of carbonic and lactic acids (Jermiah and Gibson, 2001), while the increase in pH values in the late period of refrigerated storage could possibly due to proteolysis, leading to the increase in alkaline groups and ammonia produced by microbial development in patty samples (Pearson, 1981).

Table 4 : pH values of raw (uncooked) ground beef patties during refrigerated storage at 4 ± 1°C for 15 days.

Treatments	Zero	3	6	9	12	15
Control	5.80	5.76	5.71	5.90	6.13	6.29
Salt	5.70	5.68	5.74	6.00	6.24	6.38
M1	5.81	5.79	5.73	5.84	5.93	5.98
M1S	5.76	5.72	5.80	5.87	5.95	6.05
M2S	5.78	5.80	5.75	5.82	5.90	6.00

pH values of cooked ground beef patties during refrigerated storage at 4 ± 1°C for 9 days are presented in Table 5. Data indicated that cooking process increased the pH values of ground beef patties under investigation. In this respect, Warren *et al.*, (1996) reported that cooking increased the pH of beef patties from 5.75 to 5.84, Kulshrestha and Rhee (1996) found that cooking beef patties raised the pH values form 5.84 to 5.97, also M'Carthy *et al.*, (2001) came to the conclusion that cooked patties had higher pH (6.17) values than raw patties (5.90). However, the increase in pH values after cooking could be explained in the basis that heating increased the formation of ester linkages of protein molecules and cleavage of hydrogen sulfide (Hassan , 1997).

From the same given results in tables 4 and 5 it is evident that at any given time of refrigerated storage cooked samples exhibit higher pH values than their corresponding raw pH values. In addition, control(C) and salt (S) treated samples exhibit higher pH values than samples containing marjoram essential oil, this could be explained in the basis that the essential oil posses antimicrobial properties, and hence reduced the accumulation of basic

substances produced by microbial development in beef patties. Similar results were achieved by Hassan (1997) and M'Carthy *et al.*, (2001) in their framework on meat products treated with natural antioxidants. Data present in Tables 4 and 5 further shows that the pH values of raw and cooked beef patties were below the critical limit value of 7.0 (Pearson, 1981) during the course of chilling study.

Table 5 : pH values of Cooked gound beef patties during refrigerated storage at 4 ±1°C for 9 days.

Treatments	Zero	1	3	5	7	9
Control	5.91	5.82	5.80	5.94	6.20	6.35
Salt	5.82	5.74	5.90	6.12	6.33	6.45
M1	5.90	5.84	5.89	5.95	6.05	6.12
M1S	5.85	5.80	5.88	6.08	6.17	6.21
M2S	5.89	5.82	5.90	5.93	6.10	6.15

Total Volatile basic Nitrogen (TVBN):

TVBN is considered the most commonly used biochemical methods for assessing meat spoilage (Pearson, 1981). TVBN of raw (uncooked) patties during refrigerated storage at 4 ± 1°C for 15 days are illustrated in Fig. 3. From which it is clear that TVBN of raw fresh control samples was 12.9 mg N/100 g flesh. These values are indicative of the good quality of the raw material used in this assay and they are similar to those found by other authors (Hassan, 1997; Moawad and Mohamed, 2002). From the same given results in Fig 3, it is apparent that raw salt treated patties exhibit the highest TVBN (13.0 mg N/100 g flesh) as compared to other patty samples initially and also during the course of chilling study.

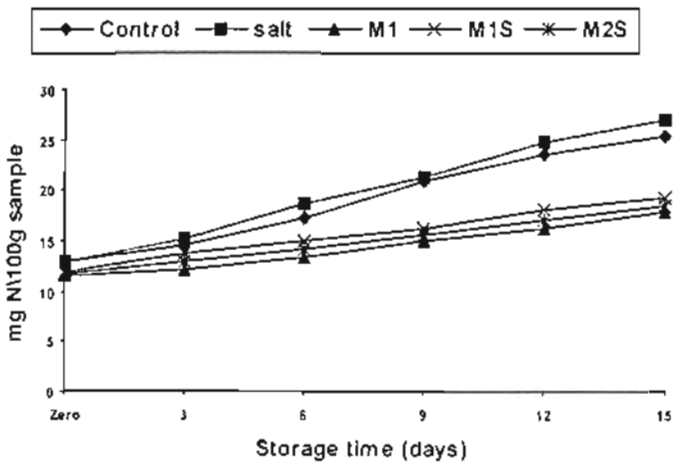


Fig. (3): TVBN of raw (uncooked) ground beef patties during refrigerated storage at 4±1°C for 15 days.

Conversely, marjoram treated samples (M1) had the lowest TVBN (11.6 mg N / 100 g flesh), followed by M2S and M1S, indicating that as the concentration of marjoram essential oil increased the accumulation of basic volatile nitrogen in the patties decreased. Similar results were achieved by Hassan (1997) in meat sausages treated with cardamom essential oil. Fig. 3 further shows that TVBN of all patty samples increased progressively throughout the storage with a higher rate in salt (S) and control(C) samples than marjoram-treated samples. However, TVBN in patty samples treated with marjoram essential oil alone or in combination with 1% NaCl were well below the legal limits set for this index at 20 mg N/100 g flesh (ES – 1688/1991) even after 15 days of chilling storage compared to less than 9 days for control (C) or salt(S) treated samples, which probably concides with the onset of spoilage and logarithmic phase of microbial growth (Figs. 5, 6). However, the increase in TVBN reflects the decomposition of meat protein by microorganisms (Pearson, 1981) on the other hand, the low levels of TVBN detected in marjoram containing samples could be due to the antimicrobial properties of natural essential oil from plants belong to Labiatae family such as sweet marjoram, rosemary and dittany (Farag *et al.*, 1989).

TVBN of cooked patty samples during refrigerated storage at 4 ± 1°C for 9 days are illustrated in Fig. 4. From which it is clear that immediately after cooking (zero time storage), cooked patties exhibit low levels of TVBN compared to their corresponding raw values, indicating that cooking reduced TVBN. This could be due to losses occurring via volatilization during heating. Similar results were achieved by Hassan (1997) in meat sausages. Referring to Fig 4, it is apparent that TVBN of cooked patty samples increased progressively during refrigerated study due to post-cooking contamination, with the salt(S) treated samples rejected most rapidly compared to control or marjoram containing samples.

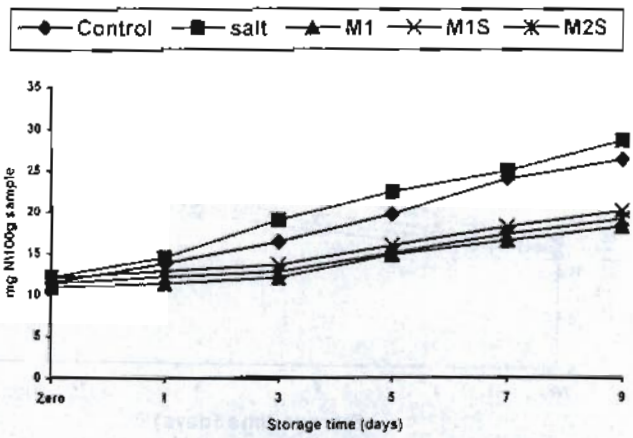


Fig. (4): TVBN of cooked ground beef patties during refrigerated storage at 4±1°C for 9 days.

Generally the changes of TVBN in raw or cooked patties during refrigerated study followed similar trend of changes as in total plate counts assay at each analytical period during the course of chilling study; indicating good correlation (R^2 =0.9666) between TPCs and TVBN values in patty samples under investigation.

Color measurements:

The main factor determining consumer acceptance in the selection of meat purchased is meat color (Jeremiah and Gibson, 2001). Consequently, desirable color must be maintained through out the storage of meat and meat products. Hunter L, a and b values of raw ground beef patties are shown in Table 6. From which it is obvious that raw fresh control patty samples exhibit 41.80, 14.10 and 12.40 for lightness(L), redness(a) and yellowness(b); respectively. In this concern, Maca *et al.*, (1997) reported that raw fresh ground beef patties had 40.50, 14.22 and 11.64 for Hunter L, a and b values; respectively. Also, Eckert *et al.*, (1997) reported that Hunter L, a and b values of raw fresh ground beef patties were 42.20, 9.12 and 15.61; respectively. However, the differences in color components (L, a and b values) could be explained in the basis that, content and state of myoglobin, pH values, light, temperature and length of storage, addition of salt and packaging affect to a great extent color measurements of ground meat (Hettiarachehy *et al.*, 1996; Jeremiah and Gibson, 2001).

Table 6: Hunter color L*,a* and b* values of raw (uncooked) ground beef patties during refrigerated storage at 4 ± 1°C for 15 days.

Treatments	Measurement	Zero	3	6	9	12	15
Control (C)	L	41.8	41.6	41.26	41.05	39.70	38.50
	a	14.10	13.92	13.40	12.64	11.00	10.10
	b	12.40	142.18	11.65	10.68	9.04	8.06
Salt (S)	L	42.2	41.36	40.18	39.00	37.60	36.85
	a	13.00	11.20	10.84	9.73	8.90	8.10
	b	12.70	10.35	9.60	8.06	7.10	6.30
M1	L	39.8	39.24	38.70	38.26	37.91	37.48
	a	15.30	15.00	14.82	14.15	13.60	12.90
	b	12.00	11.72	11.53	10.80	10.14	9.64
M1S	L	40.9	40.65	40.10	39.74	39.25	38.40
	a	14.70	14.28	13.90	13.35	12.89	12.00
	b	12.20	11.62	11.17	10.58	9.93	9.11
M2S	L	40.40	40.00	39.80	39.65	38.42	38.00
	A	15.00	14.80	14.48	13.92	13.10	12.54
	b	12.10	11.75	11.32	10.76	9.87	9.28

As shown in Table 6 beef patties with added NaCl (Salt-treated samples). showed, however, a poorer color stability with respect to meat redness(a) giving initiall lower values and higher Land b values than the control patties. This indicated less bright red, which was related to the brown / yellow discoloration caused by salt addition (Mikkelsen *et al.*, 1991). Table 6 further shows that the most effective ingredient in terms of meat redness (a) was MEO. All marjoram treated patties (M1, M1S, M2S) had a higher initial

Hunter a values than control (C) or salt (S) treated samples. In addition as the concentration of MEO increased the Hunter a values also increased. On the other hand, MEO was found to decrease lightness (L) and yellowness (b) values in patty samples. Similar trends of these changes were achieved by Eckert *et al.*,(1997); Lee *et al.*, (1998) and M'carthy *et al.*,(2001) in Patty samples treated with salts of organic acids or natural antioxidants. The results in Table 6 also indicated that, over storage time a values of all patties decreased, indicating that samples were becoming less red or brown due to metmyoglabin formation. Lightness (L) values steadily decreased as expected to day 15. The yellowness (b) values followed similar trend decreasing to day 15. This indicated increased graying over storage days particulary in control (C) and salt (S) treated samples. Microbial spoilage and breakdown of myoglobin was most likely responsible for the overall graying effects seen over storage time. Similar trends of these changes were observed by Eckert *et al.*, (1997), who reported that L, a and b values of ground beef patties were found to decrease as the time of refrigerated storage increased.

It can be deduced from the results obtained in Table 6 that, addition of marjoram essential oil has protected patties color by retarding pigment oxidation and subsequently reduced metmyoglobin formation, which confirms the findings of TBARS (Figs 1, 2). Several studies were reported on the color stability of meat products as affected by natural antioxidants (Sprouls and Brewer, 1995; Hettiarachehy *et al.*, 1996; M'carthy *et al.*, 2001).

Effect of cooking on color components (L, a, and b values) of beef patty samples during refrigerated storage at 4 ±1°C for 9 days are presented in Table 7. From which it is apparent that cooking process decreased Hunter (a) values and increased Hunter (L) and (b) values. These results supported the finding reported by Hassan (1997) and Lee *et al.*, (1998) on color changes which took place in meat products after cooking process. From the same given results of Table 7, it could be noticed that salt treated samples exhibited the lowest (a) values, while marjoram treated samples (M1) showed the highest values for Hunter (a), initially and during refrigerated storage, indicating that MEO inhibited lipid oxidation catalysts and hydrogen peroxide-activated metmyoglobin. Similar results were obtained by Higgins *et al.*, (1998) in their framework on Turkey breast patties treated with α-tocopherol.

Display of data demonstrated in Table 7 revealed that during refrigerated storage of patty samples Hunter L, a and b values were found to decrease as the time of storage progressed, with a higher rate in salt (S) treated and control (C) samples than in patties treated with MEO in any combination. Similarly, Eckert *et al.*, (1997) reported that color measurements of patties samples were found to decrease during refrigerated storage. It is generally accepted that, the oxidation of lipid and oxymyoglobin in meat appear linked (Mikkelsen *et al.*, 1991). This is supported by observations that products of both lipid oxidation (TBARS) and oxymyoglobin oxidation (i.e.

metmyoglobin) increase during storage, and that the addition of antioxidants can result in a reduction of both of these deteriorative processes.

Table 7: Hunter color L*,a* and b* values of cooked ground beef patties during refrigerated storage at 4 ± 1°C for 15 days.

Treatments	Measurement	Zero	1	3	5	7	9
Control (C)	L	44.10	43.80	43.62	42.75	42.10	41.84
	a	11.90	10.40	9.15	8.34	7.40	7.00
	b	12.50	10.18	9.25	8.28	7.05	6.50
Salt (S)	L	45.30	45.00	44 70	44.35	43.65	43.00
	a	10.80	9.30	8.45	7.60	6.90	5.84
	b	12.80	10.18	9 00	7.90	6.95	5.64
M1	L	42.60	42.20	41.92	41.45	40.87	40.25
	a	13.90	12.70	12.00	11.40	10.70	10.12
	b	12.50	10.71	10.00	9.32	8.56	7.95
M1S	L	43.40	43.00	42.85	42.50	41.30	41.00
	a	13.10	12.00	10.90	10.32	9.85	9.20
	b	12.60	10.84	9.68	8.97	8.50	7.72
M2S	L	43.00	42.70	42.53	42.00	41.15	40.56
	a	13.50	12.40	11.60	11.00	10.16	9.70
	b	12.25	10.89	9.94	9.23	8.30	7.83

Microbiological quality:

Chemical indices, together with microbiological evaluation, have been used extensively to asses the quality and shelf – life of meat products (Kim and Marshall, 1999).

Fig.5 shows total plate counts (TPCs) in raw tested patty samples. The results indicated that, TPCs of raw fresh control patty samples was 5.6 $\log_{10}$ $CFUg^{-1}$ samples. In this respect, Maca *et al.*, (1997) reported that TPCs of raw fresh beef patties was 5.2 $\log_{10}$ $CFUg^{-1}$ sample, while Eckert *et al.*, (1997) found that raw fresh control beef patties exhibit 4.0 $\log_{10}$ CFU g^{-1} samples for aerobic plate counts. However, microbial quality of meat patties depends on several factors including initial microbial quality of raw materials, storage conditions, processing temperature, microbial quality of additives, and post processing conditions (Hettiarachchy *et al.*, 1996).

Fig. 5 also demonstrates that incorporation of marjoram essential oil in raw patty formula (M1 samples) was effective in lowering the initial TPCs compared to control or other treatments. Within marjoram treatments (M1S and M2S), TPCs tended to decrease with increasing marjoram level in the formula. Similar results were obntained in beef sausages treated with cardamom essential oil by Hassan (1997). Fig.5 further shows that TPCs for control and salt treated patty samples markedly increased during the course of refrigerated study reaching 8.0 and 8.2 $\log_{10}$ $CFUg^{-1}$ samples; respectively after 12 days at 4±1°C. On the other hand, all marjoram treated patties exhibit quite low and acceptable TPCs up to 12 days as compared to control or salt treated samples. After 12 days of storage TPCs for marjoram treated samples were nearly 2 – 2.4 logs lower than other treatments.

Concerning the shelf – life of meat products, Cox *et al.*, (1998) reported that spoilage of meat generally occurs when microbial counts exceed 7 log_{10} CFU g^{-1}. On the other hand, the Egyptian Standards (ES – 1688 / 1991) determined that, total plate counts in meat products should not exceed 6.0 log_{10} CFU g^{-1}. Thus microbiological shelf-life of beef patties treated with marjoram essential oil (M1, M1S, M 2S) were 12 days at 4 ±1°C compared to the untreated control shelf – life of 6 days (Fig .5). Marjoram essential oil not only caused a decrease in the initial numbers of aerobic microorganisms but also decreased the growth rate as reflected by increasing shelf – lifes (Fig. 5). These results are consistent with studies on the effectiveness of natural essential oils on microbial shelf – life of raw and cooked meat products (Hassan, 1997; Tang *et al.*, 2001).

Total plate counts of cooked patty samples during refrigerated storage at 4 ± 1 °C for 9 days are illustrated in Fig 6, from which it is clear that, immediately after cooking the TPCs markedly decreased in all samples, the values were not different due to treatments in day zero. Similar results were achieved in cooked meat products by Eckert *et al.*, (1997); and Hassan, (1997). Results in Fig. 6 also revealed that TPCs of cooked control and cooked salt treated samples markedly increased during refrigerated storage reaching 5.8 and 5.6 log_{10} CFU g^{-1} samples; respectively after 4 days, while treated samples containing MEO showed good microbiological quality (less than 10^6 $CFUg^{-1}$ samples) even after 7 days of refrigerated study as required by (ES – 1688/1991). However, the inclusion of marjoram essential oil in patty formula improved the shelf – life due to the presence of phenolic compounds (Hamed, 2003). In this concern, Farag *et al.*, (1989) reported that, the active antimicrobial compounds of essential oils are terpenes, which are phenolic in nature. The results of microbiological quality confirmed the results of TBARS and TVBN previously discussed in both raw and cooked beef patties and indicated that marjoram essential oil added at a level of 500 mg kg^{-1} minced meat alone or in combination with 1%. NaCl increased shelf-life of raw patties to 12 days compared to only 6 days for raw control samples. Cooked samples containing marjoram essential oil also showed good quality after 7 days of refrigerated storage compared to only 3 days for cooked control samples.

Sensory evaluation:

Sensory quality of meat product reflects physico – chemical changes which take place during cooking and storage. Flavor (taste and aroma) of the cooked product can be the criteria for rejection of any kind of food if they differ greatly from what is expected by the consumer; also product appearance has become a major factor in evaluation of meat quality (Kim and Marshall, 1999). Sensory data of cooked ground beef patties are shown in table 8. From which it is apparent that at zero time of storage the score was very high, which matches the initial pH, TVBN and TBARS values. However, salt treated samples exhibit higher scores for flavor and appearance at zero

time storge than control sample, indicating that addition of 1% NaCl improved the flavor and appearance characteristics of ground beef patties.

Fig. (5): Total plate counts (TPCs as log_{10} $CFUg^{-1}$) of raw (uncooked) ground beef patties during refrigerated storage at 4±1°C for 15 days.

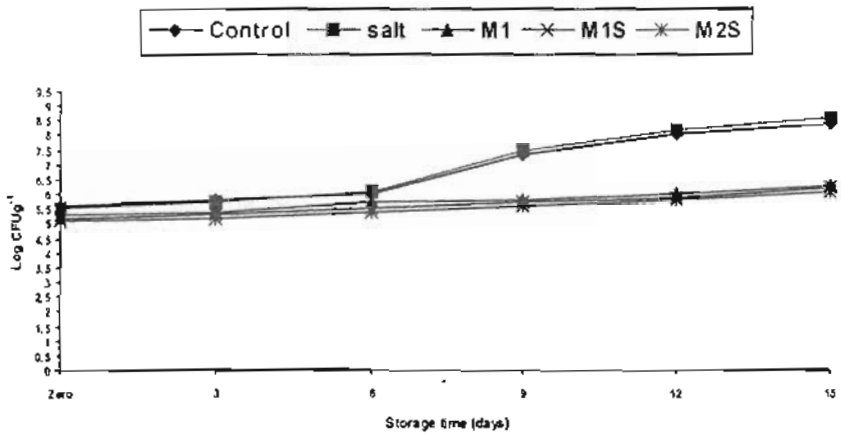
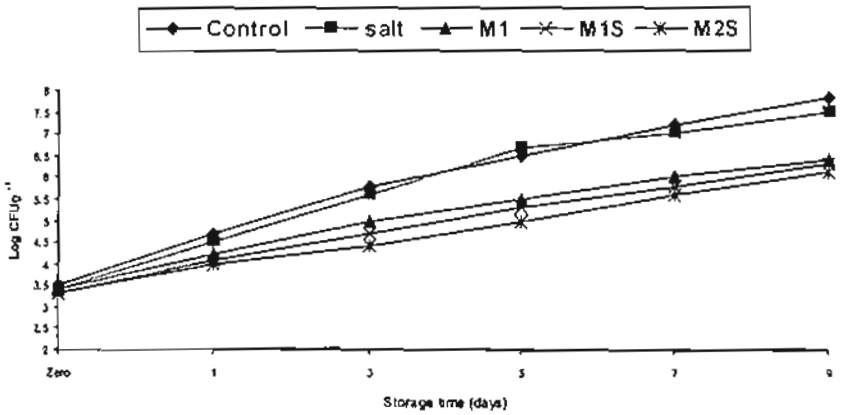


Fig. (6):Total plate counts (TPCs as log_{10} $CFUg^{-1}$) of cooked ground beef patties during refrigerated storage at 4±1°C for 9 days.



These results confirmed the findings of Eckert *et al.*, (1997), who reported that salt addition improved flavor and texture scores of ground beef patties. From the same given results of table 8, it is clear that cooked samples containing marjoram essential oil (M1, M1S, M2S) exhibit higher flavor and appearance scores than control samples initially (zero time storage) and during refrigerated storage, indicating that marjoram incorporation improved flavor intensity and appearance scores, the natural oil did not impart an off – flavors to patty samples, even when used at a concentration 5-fold (1000 ppm) that of synthetic antioxidant (200 ppm). Similar results were observed

by Karpinska et al (2001) who reported that minced meat balls made with addition of marjoram exhibit higher flavor (aroma and taste) profiles and lower rancid off-flacor than control samples. However, the results of sensory quality confirmed the findings of. Novak *et al.*, (2000) who reported that marjoram is employed to flavor sausages meats, salads and soups. Data presented in Table 8 further shows that the quality of cooked salt treated and cooked control patties sharply decreased as the time of refrigerated storage increased as shown by the consistently lower flavor and appearance scores. However, marked increases in intensity of rancid flavor were detected in these samples after 2 and 3 days of refrigerated storage; respectively. On the other hand, addition of MEO alone or in combination with sodium chloride increased shelf-life of the ground beef patties by decreasing negative flavor associated with lipid oxidation, addition of MEO gave the most desirable product after 7 days of storage period in comparison to 3 days for control samples. The protective effect of MEO again was demonstrated by the results of sensory evaluation at each analytical period probably as a result of the antioxidant and antimicrobial activity of compounds present in marjoram (Jun *et al.*, 2001). A strong correlation between flavor scores and TBARS values (R^2 = 0.997) was observed. Sensory characteristics remained in agreement with intensity of hydrolytic and oxidative changes in lipid fraction of patties. Similar results were observed by Karpinska *et al.*, (2001) who reported that natural antioxidant improved sensory scores and prolonged shelf-life of minced meat balls.

Table 8: Sensory characteristics of cooked beef patties during refrigerated storage at 4±1°C (Means ± SD, n = 10).

Treatment	Measurements	Refrigerated Storage (days)					
		Zero	1	3	5	7	9
C	Flavor	8.4 ± 0.66	7.0 ± 0.75	5.6 ± 0.74	R	R	R
	Appearance	8.6 ± 0.67	7.3 ± 0.48	6.2 ± 0.63	R	R	R
S	Flavor	8.7 ± 0.63	6.4 ± 0.99	R	R	R	R
	Appearance	9.0 ± 0.75	7.0 ± 0.78	R	R	R	R
M1	Flavor	9.0 ± 0.75	8.1 ± 0.78	7.4 ± 0.66	7.0 ± 0.78	6.0 ± 0.67	R
	Appearance	8.8 ± 0.42	8.1 ± 0.79	7.3 ± 0.54	6.8 ± 0.54	5.7 ± 0.53	R
M1S	Flavor	9.2 ± 0.68	8.2 ± 0.48	7.0 ± 0.58	6.5 ± 0.5	5.4 ± 0.57	R
	Appearance	9.0 ± 0.75	8.1 ± 0.79	6.9 ± 0.54	6.2 ± 6.63	5.1 ± 0.39	R
M2S	Flavor	9.3 ± 0.68	9.2 ± 0.42	7.2 ± 0.43	6.8 ± 0.54	5.7 ± 0.63	R
	Appearance	9.1 ± 0.81	8.0 ± 0.53	7.0 ± 0.58	6.5 ± 0.78	5.3 ± 0.63	R

C : Control (no additives)
S : Salt 1% NaCL.
M1 : 500 mg MEO kg^{-1} minced meat
M1S : 500mg MEOkg-1 minced meat + 1 % NaCl
M2S : 1000mg MEO kg-1 minced meat + 1% NaCl.
R : Rejected

CONCLUSION

Based on the above physico- chemical, sensory and microbiological quality parameters, it is concluded that marjoram essential oil added at a level of 500 mg kg^{-1} minced meat alone or in combination of 1% NaCl was

effective in decreasing microbial growth and improving lipid and pigment stability of treated patties compared to control samples during refrigerated storage. However, marjoram, a natural food ingredient without any known toxic effects, may be a promising source of natural antioxidants and antimicrobial for extending shelf-life of refrigerated consumer products.

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تأثير زيت البردقوش العطرى على جودة باتيه اللحم البقرى أثناء التخزين بالتبريد

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إضافة الملح والطبخ والتخزين بالتبريد تسرع من التدهورات المصاحبة للأكسدة وتسبب عديد من التغيرات غير المرغوبة فى صفات جودة اللحوم لذا أجريت هذه الدراسة لتقييم تأثير اضافة زيت البردقوش العطرى أو ملح الطعام أو اضافتهما معاً على صفات جودة باتيه اللحم البقرى الطازج والمطبوخ أثناء التخزين بالتبريد. أكدت النتائج أن إضافة ١% من ملح الطعام فى خلطة الباتيه أسرعت بشدة من عمليات أكسدة الدهون واللون وأظهرت أقل درجة جودة مقارنة بعينات الكنترول أو عينات الباتيه المعاملة بزيت البردقوش العطرى.

وعلى جانب آخر فإن اضافة ٥٠٠مجم من زيت البردقوش العطرى لكل كجم من خلطة الباتيه لها اثر جيد في تحسين ثبات الدهون وقياسات اللون المختلفة ودرجات التقييم الحسى ومدة حفظ الباتيه من الناحية الميكروبية، كما هو واضح من انخفاض قيم نواتج حمض الثيوباربتيوريك والقواعد النيتروجينية الكلية الطيارة وقيم الأس الأيدروجينى والعد الكلى للبكتريا وأيضا انخفاض النكهة غير المرغوبة المصاحبة لأكسدة الدهن فى العينات المعاملة بزيت البردقوش مقارنة بعينات الكنترول والعينات المعاملة بالملح فقط. أظهرت النتائج أيضا أنه لم يستدل على زيادة ملحوظة فى التأثيرات المحسنة لصفات الجودة عند مضاعفة تركيز زيت البردقوش (١٠٠٠مجم/كجم) فى خلطة الباتيه. ومن المنظور الصحى للفائدة المرجوة من استخدام البردقوش فأن زيت البردقوش العطرى من الممكن أن يكون مصدراً طبيعياً كمضاد للميكروبات والأكسدة ومحسن للنكهة فى الاستخدام الغذائى.