Effect of Some Natural and Synthetic Antioxidants on Ground Beef Meat During Cold Storage

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ABSTRACT

Antioxidant efficiency of recommended optimum levels of rosemary (R) (0.10%), sage (S) (0.05%), mustard (M) (0.10%), clove (CL) (0.10%), fenugreek (FGK) (0.10%) and majorana hortansia (MH) (0.01%), were evaluated against mixed (1:1)butylated hydroxyanisole / butylated hydroxytoluene (BHA/BHT) (0.01%) and also were evaluated against a control (C) without antioxidant. Ground beef samples were treated then stored at 4C° for 0, 3, 6 and 9 days. Ground beef meat samples were anaylsed for lipid oxidation (peroxide value (PV),thiobarbituric acid reactive substances (TBARS) and free fatty acid (FFA), meat quality, pH, water holding capacity (WHC), drip loss, cooking loss, total volatile nitrogen (TVN), protein solubility, cholesterol content, myoglobin concentration and sensory attributes (flavour, juiciness, tenderness and overall palatability). Microbiological analysis (total plate count TPC and psychrophilic total count PTC) was also determined.

Addition of antioxidants decreased drip loss, cooking loss, TBARS, PV, FFA, cholesterol content, TVN, and reduced the rate of oxidation of myoglobin to metmyoglobin. On the other hand, WHC, protein solubility and the organoleptic characteristics were enhanced . Inhibition of microbial growth (TPC and PTC) were detected. Results showed that the addition of rosemary (R) significantly decreased the lipid oxidation of the ground beef during storage up to 9 days. It was found that (R) was more effective than BHA/BHT in preventing the increase of TBARS, PV, FFA values and significantly protected myoglobin from oxidation to metmyoglobin. Added R also markedly decreased the drip loss, cooking loss, TVN and cholesterol content. The WHC, protein solubility, sensory properties and activity against TPC and PTC bacteria were significantly higher with addition of R. This study pointed out natural antioxidants especially, rosemary were more effective than synthetic antioxidants when used to enhance quality and shelf life of ground beef.

Key words: natural antioxidant, BHA/BHT, physical, chemical, sensory characteristics, ground beef meat

INTRODUCTION

Preventing rancidity is an important factor in processing, preparation and storage of many foods. Food products which contain fats are susceptible to rancidity and often need antioxidants to increase products stability (Abramovič & Abram, 2006). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are effective antioxidants (Verhagen *et al*., 1990), Many studies showed that these antioxidants have oxidative characteristics and metabolites of which may contribute to carcinogenicity or tumorigenicity and mutagenic activity(Ito *et al*.,1985, VanEsh,1986, Fasoyiro *et al*., 2001). However, the same reactions may combat oxidative stress, there is evidence that certain persons may have difficulty in metabolizing BHA and BHT, resulting in health and behaviour changes (Ito *et al*., 1985,VanEsh,1986) .The plant kingdom offers a range of natural phenolic compounds found in spices and herbs such as red chili, cinnamon leaf, clove, rosemary, sage and mustard (Fasoyiro *et al*., 2001). Spices have been known to enhance the flavour and colour attributes. Moreover, these materials have bacteriostatic activity and antioxidant activity (Yanishlieva & Marinova, 2001, Sagdic & Özcan, 2003, Baydar *et al*., 2004). Natural antioxidants are readily acceptable by consumers as they are considered to be safer as compared with synthetic substences. Rosemary and sage have been shown to prevent oxidation and colour loss as well as, lowering microbial load of red meat packaged under modified atmosphere (Sanchez- Escalante *et al*., 2001, Djenane *et al*., 2002). Rosemary and sage are rich sources of potent antioxidants, rosmarinic acid, carnosic acid, rosmanol, carnosol, epirosmanol isorosmanol and their derivatives (Cuvelier *et al*.,1996, Wang *et al*., 1998, Lu *et al*., 2002, Bors *et al*., 2002).

The objective of the present study was to compare the effect of six tested powders, namely rosemary (R), sage (S), mustard (M), clove (CL), fenugreek (FGK) and majorana hortansia (MH) against synthetic antioxidants butylated hydroxyanisole/ butylated hydroxytoluene (BHA/BHT) on the quality of ground beef meat under cold storage.

MATERIALS AND METHODS

Samples preparation. Samples of meat were obtained from top round of local beef, minced through a 0.8 cm steel plate using electric grinder (National), and divided into eight portions of 1kg each. The tested antioxidant powder was added except for the control (C) as follows: rosemary (R) 0.10% , sage (S) 0.05% , mustard (M) 0.10%, clove (CL) 0.10%, fenugreek (FGK) 0.10%, majorana hortansia (MH) 0.01% and butylated hydroxyanisole/ butylated hydroxytoluene (BHA/BHT) 0.01%. The samples were immediately fine ground to pass through opening with a 0.5 cm, placed onto trays, overraped with oxygen permeable sheet, and kept under refrigeration at 4°C and fluorescent light for up to 9 days. The optimum concentrations were recommended in previous studies (McCarthy *et al*., 2001).

Microbial count. Samples (25g each) of the ground beef meat were aseptically removed from each package, mixed with 225ml sterilized 0.1%peptone solution and blended for 30 sec. with stomacher. Total plate counts and psychrophilic counts were determined following the method used by Lin & Lin (2002) . All microbial counts were reported as colony forming units per gram (CFU/g) of meat.

Physicochemical analysis. The pH of the meat samples was measured according to Xiong *et al*. (1993). Water holding capacity (WHC) and the solubility of myofibrillar protein were determined according to the procedure of DenHertog-Meischke *et al*. (1997). Total volatile nitrogen (TVN), peroxide value (PV) and free fatty acids (FFA) were determined according to Pearson *et al*. (1981). Thiobarbituric acid reactive substances (TBARS) was measured according to the method described by Balentine *et al*. (2006). Cholesterol content was determined as outlined by Reiser (1975). Myoglobin concentration was measured according to Zessin *et al*. (1961). Drip loss and cooking loss were determined according to Honikel (1998), and Purchas and Barton (1976), respectively.

Sensory evaluation. A trained sensory panel of eight members was used to evaluate: flavour, juiciness, tenderness and overall palatability of cooked ground beef meat samples (Caporaso *et al*.,1978). A judging scale was used as follows on a 8-point scale: 8= extremely desirable, extremely juicy, extremely tender, dark brown, extremely desirable and 1= extremely undesirable, extremely dry, extremely tough, very dark red and extremely undesirable, respectively.

Statistical analysis. A 8×4 factorial design including two factors, antioxidant and storage time, with three replications was analyzed by the analysis of variance (SAS, 2001). Significance between means was tested by Duncanś Multiple Range Test.

RESULTS AND DISCUSSION

The interaction effect of natural antioxidants on the pH values and WHC of ground beef meat at 4°C for 0, 3,6 and 9 days is given in Table (1).The pH value of all antioxidant treatments increased during the refrigerated storage than the control treatment but the pH values of R were found significantly higher ($P<0.01$) than all the other treatments. Antioxidant treatments positively affected the WHC values. WHC values of R were significantly ($P<0.01$) higher than S, M, CL, FGK, MH, BHA/BHT while the control (C) recorded the least WHC at all the storage periods. Addition of rosemary was intended to raise the pH of meat and to improve moisture binding by the meat, both actions accounted for increased WHC (Al-Rubeii *et al*., 2008). All treatments showed decrease in WHC as storage period was extended (Table 1). These results are in agreement with results of Saleh (2007) who used vitamins E and C and their combinations as well as grape seed extract and grape juice concentrate in ground meat. These additions improved the water holding capacity as compared with control after 90 days of frozen storage .

The results presented in Table (2) show that all the antioxidant treatments exhibited low ($P<0.01$) drip loss and cooking loss percentages as compared with control treatment at all storage times .That is probably due to the mode of action of natural antioxidant in increasing moisture bind, pH and WHC, and hence increase ability of meat tissue to retain water and reduce moisture loss during storage and cooking (Al-Rubeii *et al*., 2008). the percentage of drip loss and cooking loss in ground beef could be

Table 1: Influence of natural antioxidants on pH value and water holding capacity (WHC) during refrigerated storage of ground beef meat at 4°C

Treatment / Conc. %	pH				WHC			
	Storage time (days)				Storage time (days)			
	0	3	6	9	0	3	6	9
C0.0	5.59±0.01^{lm}	5.72±0.03^{jk}	5.83±0.03^{hij}	5.80±0.02^{ij}	53.14±0.14^{i}	47.44±0.18^{q}	43.15±0.15^{v}	38.21±0.18^{y}
R0.10	5.91±0.01^{defg}	6.00±0.01^{cde}	6.20±0.02^{a}	6.13±0.03^{ab}	65.30±0.20^{a}	61.80±0.21^{c}	56.33±0.17^{gh}	50.10±0.20^{m}
S0.05	5.80±0.04^{hij}	5.90±0.03^{efgh}	6.10±0.05^{abc}	6.00±0.04^{cde}	62.90±0.05^{b}	58.22±0.25^{e}	51.50±0.20^{k}	46.70±0.13^{r}
M0.10	5.86±0.03^{fghi}	5.91±0.02^{defg}	6.12±0.02^{ab}	6.07±0.02^{bcd}	62.00±0.00^{c}	52.39±0.10^{j}	47.90±0.13^{p}	45.40±0.10^{s}
CL0.10	5.65±0.05^{kl}	5.80±0.00^{ij}	5.97±0.02^{def}	5.91±0.02^{efgh}	56.51±0.10^{g}	51.67±0.09^{k}	48.29±0.08^{o}	41.29±0.07^{w}
FGK0.10	5.71±0.01^{jk}	5.85±0.05^{ghi}	6.02±0.02^{cde}	5.97±0.02^{def}	60.35±0.10^{d}	50.11±0.18^{m}	46.75±0.15^{r}	44.05±0.02^{u}
MH0.01	5.60±0.10^{lm}	5.70±0.05^{jk}	5.93±0.03^{defg}	5.90±0.00^{efgh}	56.01±0.10^{h}	49.05±0.05^{n}	44.80±0.05^{t}	40.52±0.02^{x}
BHA/BHT0.01	5.85±0.05^{ghi}	5.87±0.02^{fghi}	6.03±0.03^{bcd}	5.99±0.01^{cdef}	57.12±0.08^{f}	50.70±0.10^{l}	47.09±0.09^{qr}	40.90±0.13^{x}

Means ± SE within the column for the same test item having different letters (a-y)are significantly different among treatments ($P<0.05$). rosemary (R), sage (S), mustard (M), clove (CL), fenugreek (FGK) and majorana hortansia (MH) to syenthetic antioxidants butylated hydroxyanisole / butylated hydroxytoluene (BHA/BHT) and control.

Table 2: Influence of natural powders as antioxidants on drip loss and cooking loss during refrigerated storage of ground beef meat at 4°C

Treatment / Conc. %	Drip loss (%)				Cooking loss (%)			
	Storage time (days)				Storage time (days)			
	0	3	6	9	0	3	6	9
C0.0	1.85±0.05^{lmn}	2.54±0.06^{g}	3.80±0.05^{abc}	4.02±0.08^{a}	29.30±0.20^{a}	28.62±0.13^{b}	27.07±0.17^{cd}	27.50±0.15^{c}
R0.10	1.45±0.07^{q}	1.66±0.05^{mnop}	1.80±0.10^{lmno}	1.90±0.06^{klm}	22.67±0.03^{m}	20.12±0.17^{p}	19.70±0.15^{pq}	18.40±0.11^{r}
S0.05	1.52±0.03^{pq}	1.70±0.04^{mno}	1.91±0.09^{jklm}	2.15±0.05^{ij}	23.75±0.15^{l}	22.17±0.13^{mn}	20.81±0.10^{o}	19.33±0.17^{q}
M0.10	1.58±0.02^{pq}	1.87±0.03^{klm}	2.13±0.05^{ijk}	2.41±0.05^{ij}	23.96±0.19^{l}	22.60±0.07^{m}	21.79±0.14^{n}	19.82±0.23^{p}
CL0.10	1.70±0.05^{lmno}	2.47±0.08^{gh}	3.18±0.04^{e}	3.75±0.05^{bc}	26.35±0.14^{e}	25.59±0.22^{fg}	24.52±0.15^{jk}	22.25±0.15^{mn}
FGK0.10	1.60±0.08^{opq}	1.96±0.06^{jkl}	2.55±0.15^{g}	2.93±0.07^{f}	25.11±0.20^{hi}	24.80±0.18^{ij}	22.39±0.15^{m}	21.05±0.05^{o}
MH0.01	1.77±0.03^{lmno}	2.35±0.15^{ghi}	3.61±0.11^{cd}	3.90±0.11^{ab}	27.54±0.06^{c}	26.02±0.13^{ef}	25.83±0.17^{f}	24.90±0.22^{hij}
BHA/BHT0.01	1.63±0.10^{nopq}	2.25±0.08^{hi}	2.90±0.05^{f}	3.49±0.03^{d}	26.92±0.15^{d}	25.30±0.11^{gh}	24.10±0.11^{kl}	24.71±0.32^{ij}

Means ± SE within the column for the same test item having different letters (a-r)are significantly different among treatments (P<0.05). rosemary (R), sage (S), mustard (M), clove (CL), fenugreek (FGK) and majorana hortansia (MH) to syenthetic antioxidants butylated hydroxyanisole / butylated hydroxytoluene (BHA/BHT) and control.

ranked in descending order as folow: control > MH > BHA/BHT > CL > FGK > M > S > R.

Changes of TBARS and PV of ground beef meat during storage are given in Table (3). Statistical analysis indicated that TBARS and PV were affected significantly (P<0.01) by storage period and addition of antioxidants. Addition of natural antioxidants showed lower (P<0.01) TBARS and PV values than the control at 0, 3, 6 and 9 days. Highest (P<0.01) TBARS and PV values were found in control while the lowest were found with R. These results are similar to those of Djenane *et al*. (2002) and Sanchez-Escalante *et al*. (2003) who found that rosemary was able to retard lipid oxidation for more than six days.Rosemary contains many compounds with antioxidant properties: Rosmarinic acid, rosmanol, carnosic acid, rosmaridiquinone, carnosol and rosmaridiphenol were identified as phenolic type compounds which probably function as free radical scavengers similar to BHA and BHT (Senoranse *et al*., 2000, Djenane *et al*., 2002, Abramovič & Abram, 2006). Moreover, the Rosemary reduced TBARS and PV values formation more than BHA/BHT. Similar results were reported by McCarthy *et al*. (2001) and Tang *et al*. (2001) who reported that the natural equivalent of synthetic antioxidants is important with regard to human health because some synthetic antioxidants have carcinogenic activity and their usage in the food industry is more than natural antioxidants. The results obtained in the present study is supported also by those reported by Balentine *et al*. (2006) who found that when rosemary was added to ground beef meat it showed lower TBARS values of 2.77 mg malonaldehyde/kg of meat at 4°C after 144hr of storage than control 3.75mg malonaldehyde/kg of meat. Karpiňska *et al*. (2000) found that the content of malonaldehyde in control meat sample and when rosemary extract was added in amounts of 1% and 1.5% it decreased malonaldehyde contents after three months of storage by 28% in sample containing 1% and by 40% in sample containing 1.5% rosemary extract. The results in the present study also indicate that rosemary lowered the lipid oxidation products .

Free fatty acids (FFA) values of ground beef meat with added antioxidants during refrigerated storage are given in Table (4). All antioxidants treatments particularly R treatment showed significant (P<0.01) decrease in FFA values as storage period was extended as compared with the control. The antioxidant properties of spices specially rosemary are related to their phenolic contents, their antioxidant action is similar to synthetic phenolic antioxidants. High antioxidant activity of rosemary extract was indicated by Korczak *et al*. (1990), whereas among ten herbal seasoning from Labiatae family, rosemary showed the strongest antioxidative properties. Despite, the aforementioned researches were studied on model system, yet they suggest that rosemary can act in similar manner in meat preservation. On the other hand, control treatment showed the highest (P<0.01) FFA values at all the storage time. At the end of storage period (6 and 9 days) the FFA values in the control treatment were almost 3.10 and 4.06 times higher than those before storage (0 day), that may be attributed to the action of lipolytic enzymes (lipase and phosphlipase). Lipids in meat particularly phospholipids components undergo degradation and produce a large number of compounds such as, hydroperoxides, aldehydes and ketones and increase the release of free fatty acids which are responsible for the development of undesirable aroma and deterioration in flavour (rancidity) during storage (Kerry *et al*., 2002). Such results are in agreement with those reported by Saleh (2007) and Al-Rubeii *et al*. (2008).

The effects of antioxidants treatment on the cholesterol concentration of ground beef are presented in Table (4). Ground meat treatment with addition of antioxidant exhibited the low (P<0.01) cholesterol concentration as storage period was extended as compared with control treatment. Similar findings were observed by King *et al*. (1998) and Saleh (2007) who showed that cholesterol concentration decreased in meat during refrigerated storage in the presence of antioxidants.

Total volatile nitrogen contents (TVN) of the meat treatments are shown in Table (5). All antioxidant treatments showed lower TVN at all the storage times than the control. TVN values among treatments followed a similar increasing (P<0.01) trend with extending the refrigerated storage period. That is possibly due to an increase of proteolytic reaction and protein hydrolysis to small peptides and increase accumulation of free nitrogen groups that might lead to high TVN value (Ageena, 2001). This also enhance the microbial growth .Similar results were reported by Mohamed *et al*. (2005) and Al-Rubeii *et al*. (2008). Therefore, using natural antioxidants is important to reduce (P<0.01) the TVN formation and to improve meat quality upon cold storage.

Table 3: Effect of natural antioxidants on the oxidative stability thiobarbituric acid reactive substances (TBARS) and peroxide values (PV) of ground beef meat during refrigerated storage at 4°C

Treatment / Conc. %	TBA (mg malonaldehyde/ kg meat)				PV (meq O_2/ Kg oil)			
	Storage time (days)				Storage time (days)			
	0	3	6	9	0	3	6	9
C0.0	0.15±0.03^{mnop}	0.97±0.03^{c}	1.26±0.06^{b}	1.65±0.10^{a}	1.82±0.08^{hijk}	2.48±0.05^{de}	3.67±0.04^{b}	4.40±0.10^{a}
R0.10	0.04±0.01^{q}	0.10±0.00^{opq}	0.18±0.01^{lmn}	0.27±0.02^{gkl}	0.92±0.02^{r}	1.27±0.02^{pq}	1.40±0.10^{op}	1.52±0.03^{mno}
S0.05	0.08±0.01^{pq}	0.18±0.02^{lmn}	0.25±0.05^{klm}	0.39±0.04^{hij}	1.07±0.02^{r}	1.43±0.02^{nop}	1.75±0.05^{jikl}	1.93±0.03^{ghij}
M0.10	0.08±0.01^{pq}	0.22±0.02^{klmn}	0.40±0.01^{ghi}	0.55±0.05^{def}	1.05±0.05^{r}	1.38±0.02^{op}	1.60±0.10^{lmn}	1.89±0.05^{ghij}
CL0.10	0.11±0.01^{nopq}	0.34±0.01^{hijk}	0.51±0.01^{egf}	0.66±0.06^{d}	1.35±0.03^{op}	1.76±0.09^{jikl}	2.03±0.03^{fg}	2.55±0.05^{d}
FGK0.10	0.10±0.00^{opq}	0.28±0.05^{ghi}	0.44±0.04^{gfh}	0.60±0.05^{de}	1.21±0.00^{pq}	1.50±0.10^{mno}	1.90±0.05^{ghij}	2.37±0.03^{e}
MH0.01	0.12±0.02^{nopq}	0.40±0.05^{ghi}	0.59±0.04^{de}	0.93±0.03^{c}	1.50±0.00^{mno}	1.95±0.05^{ghi}	2.15±0.05^{f}	2.86±0.11^{c}
BHA/BHT0.01	0.07±0.02^{pq}	0.15±0.02^{mnop}	0.23±0.03^{klmn}	0.31±0.01^{ijk}	1.10±0.05^{qr}	1.39±0.03^{op}	1.67±0.02^{klm}	1.99±0.01^{fgh}

Means ± SE within the column for the same test item having different letters (a-r) are significantly different among treatments (P<0.05). rosemary (R), sage (S), mustard (M), clove (CL), fenugreek (FGK) and majorana hortansia (MH) to syenthetic antioxidants butylated hydroxyanisole / butylated hydroxytoluene (BHA/BHT) and control.

Table 4: Changes of free fatty acid (FFA) and cholesterol content in ground beef meat with addition of natural powders as antioxidants during refrigerated storage at 4°C

Treatment / Conc. %	FFA (%)				Cholesterol (mg/ 100g meat)			
	Storage time (days)				Storage time (days)			
	0	3	6	9	0	3	6	9
C0.0	0.30±0.05^{igkl}	0.69±0.03^{c}	0.93±0.03^{b}	1.22±0.04^{a}	66.70±0.15^{a}	65.10±0.10^{b}	64.73±0.07^{c}	63.40±0.10^{f}
R0.10	0.07±0.01^{o}	0.16±0.02^{mno}	0.21±0.01^{klmn}	0.29±0.02^{ijkl}	61.25±0.05^{k}	58.30±0.05^{o}	56.61±0.11^{s}	53.43±0.09^{u}
S0.05	0.11±0.01^{no}	0.25±0.05^{jklm}	0.33±0.03^{jijk}	0.39±0.03^{fghi}	62.00±0.30^{ij}	60.51±0.16^{l}	57.85±0.05^{q}	55.12±0.13^{t}
M0.10	0.10±0.00^{no}	0.20±0.05^{klmn}	0.27±0.02^{jklm}	0.34±0.01^{hijk}	63.97±0.03^{e}	62.36±0.08^{h}	58.84±0.09^{n}	57.19±0.03^{r}
CL0.10	0.20±0.03^{klmn}	0.37±0.02^{ghij}	0.50±0.05^{de}	0.61±0.06^{cd}	64.77±0.08^{bc}	63.49±0.10^{f}	60.59±0.11^{l}	58.68±0.04^{n}
FGK0.10	0.18±0.02^{lmn}	0.35±0.05^{ghij}	0.41±0.01^{efgh}	0.50±0.06^{de}	64.39±0.11^{d}	62.89±0.05^{g}	59.44±0.05^{m}	57.92±0.08^{pq}
MH0.01	0.26±0.01^{jklm}	0.45±0.05^{ef}	0.60±0.05^{cd}	0.88±0.02^{b}	65.11±0.21^{b}	64.05±0.15^{n}	62.22±0.09^{t}	61.90±0.11^{ij}
BHA/BHT0.01	0.13±0.02^{mno}	0.31±0.01^{ijkl}	0.38±0.02^{fghi}	0.42±0.03^{efg}	63.45±0.05^{f}	61.70±0.10^{j}	58.20±0.20^{op}	56.63±0.07^{s}

Means ± SE within the column for the same test item having different letters (a-t)are significantly different among treatments (P<0.05). rosemary (R), sage (S), mustard (M), clove (CL), fenugreek (FGK) and majorana hortansia (MH) to syenthetic antioxidants butylated hydroxyanisole / butylated hydroxytoluene (BHA/BHT) and control.

Table 5: Effect of natural antioxidants on total volatile nitrogen (TVN) and protein solubility of ground beef meat during refrigerated storage at 4°C

Treatment / Conc. %	TVN (mg N/ 100 meat)				Protein solubility (mg/g meat)			
	Storage time (days)				Storage time (days)			
	0	3	6	9	0	3	6	9
C0.0	4.66±0.04^{a}	8.29±0.06^{hi}	12.80±0.20^{c}	16.45±0.05^{a}	52.40±0.20^{u}	56.44±0.19^{p}	58.19±0.26^{m}	61.77±0.23^{h}
R0.10	2.75±0.05^{s}	4.60±0.07^{o}	6.90±0.10^{l}	8.05±0.05^{ij}	57.90±0.15^{nm}	61.20±0.21^{i}	65.42±0.13^{c}	68.81±0.04^{a}
S0.05	2.97±0.03^{rs}	5.37±0.04^{n}	8.13±0.03^{hij}	9.89±0.04^{f}	55.88±0.13^{a}	58.86±0.15^{e}	63.93±0.18^{e}	67.40±0.15^{b}
M0.10	3.15±0.10^{rs}	6.10±0.10^{m}	8.70±0.05^{gh}	10.25±0.05^{ef}	55.15±0.15^{r}	58.01±0.10^{mn}	62.77±0.13^{fg}	65.60±0.30^{c}
CL0.10	3.70±0.11^{pq}	7.32±0.08^{kl}	9.90±0.10^{f}	12.75±0.05^{c}	53.41±0.21^{t}	57.09±0.11^{mn}	59.84±0.26^{k}	63.71±0.14^{e}
FGK0.10	3.39±0.09^{qr}	6.91±0.11^{l}	9.05±0.05^{g}	10.65±0.02^{de}	54.29±0.11^{s}	57.95±0.05^{mn}	61.29±0.11^{i}	64.49±0.17^{d}
MH0.01	3.99±0.04^{p}	7.95±0.05^{ijk}	11.06±0.04^{d}	14.07±0.05^{b}	53.01±0.11^{t}	56.97±0.22^{o}	59.00±0.20^{l}	62.30±0.20^{g}
BHA/BHT0.01	3.67±0.06^{pq}	7.35±0.10^{kl}	10.10±0.05^{ef}	12.40±0.10^{c}	53.92±0.13^{s}	57.60±0.15^{n}	60.45±0.07^{j}	63.11±0.14^{e}

Means ± SE within the column for the same test item having different letters (a-u)are significantly different among treatments ($P<0.05$). rosemary (R), sage (S), mustard (M), clove (CL), fenugreek (FGK) and majorana hortansia (MH) to syenthetic antioxidants butylated hydroxyanisole / butylated hydroxytoluene (BHA/BHT) and control.

The results of protein solubility values of ground beef meat treated with antioxidants and stored at 4°C for 0, 3, 6 and 9 days are shown in Table (5). Statistical analysis indicated that protein solubility was affected significantly (P<0.01) by addition of antioxidants. All the treatments showed increase in protein solubility values with extending storage period. Addition of natural antioxidant increased (P<0.01) the protein solubility. Presence of rosemary exhibited the highest (P<0.01) protein solubility values as storage period was proceeded as compared with the other treatments. These results agree with the findings of Korczak *et al*. (1998) who reported that addition of natural antioxidant, such as rosemary extract (0.05%) and soy protein hydrolyzate inhibited significantly degradation of meat protein, increase the stability, and keep the biological value of protein in meat products. Estévez & Cava (2006) also, found that rosemary essential oil successfully inhibited oxymyoglobin oxidation in frankfurters produced with tissue from Iberian pigs.

The results of using antioxidant treatments on myoglobin concentration of ground beef under cold storage are presented in Table (6). All antioxidant treatments showed significant (P<0.01) increase in myoglobin concentration than the control at all storage periods. The R treatment had the highest myoglobin concentration (5.28, 5.02, 4.83 and 4.61mg/g) than S, BHA/BHT, M, CL, FGK, MH and C for days 0, 3, 6 and 9, respectively. Rosemary treatment was highly effective in inhibiting both metmyoglobin formation and lipid oxidation for 9 days. Chan *et al*. (1997) showed that lipid autoxidation products can increase the oxidation of oxymyoglobin to metmyoglobin. The present results are in agreement with the results of Chen *et al*. (1992), Banks *et al*. (1998) and Sanchez-Escalante *et al*. (2003) who found that rosemary extract was able to retard lipid oxidation for more than six days. All treatments showed significant (P<0.01) decrease in myoglobin concentration as storage period was extended, due to oxidation of myoglobin pigment to metmyoglobin during storage .

The sensory panel evaluation scores were given in Table (7). The organoleptic scores for flavour, juiciness, tenderness and overall palatability of cooked ground beef meat were affected significantly (P<0.01) by addition of antioxidants. Antioxidant treatments were superior (P<0.01) to the C treatment in overall sensory scores. This was attributed to the addition of these antioxidants effective in preventing lipid oxidation, reduced TBARS and PV and prevent the development of warmed over flavour (WOF) which affects overall palatability of cooked ground beef. The R treatment had the highest organoleptic scores for flavour, juiciness, tenderness and overall palatability of all other treatments.On the other hand, C treatment recorded the least organoleptic scores than other treatments at all the storage periods. The results in the present study support those reported by Huisman *et al*. (1994) who found that addition of rosemary in a sensory acceptable amount of 0.05% depressed the development of WOF of precooked pork meat balls during storage by 20%. Al-Rubeii *et al*. (2008) found that rosemary powder (0.1%) when added to ground poultry meat was markedly effective on preventing lipid oxidation with practically complete elimination of rancidity (TBARS and PV) and delaying myoglobin oxidation which reflected on improvement of organoleptic scores for flavour, juiciness, tenderness and overall palatability. The order of acceptability in the present work was found to be R>S>M>FGK>CL>BHA/BHT>MH>control. These results indicte that natural antioxidants are recommended to improve the overall organoleptic properties of ground beef during refrigerated storage.

The number of total plate count (TPC) for ground beef stored under refrigeration for 0, 3, 6 and 9 days are presented in Figure (1). All antioxidant treatments recorded significant (P<0.01) decrease in TPC at storage as compared with C treatment. The rosemary treatment (R) had higher effect (P<0.01) on TPC for ground beef meat stored under refrigeration condition than C treatment probably due to that rosemary have numerous phenolic compounds including carnosic acid, carnosol, epirosmanol, isorosmanol, rosmaridiphenol, rosmanol and rosmarinic acid (Zheng & Wang, 2001). The mechanism of inhibitory effect of these phenolic antioxidants on bacteria growth is believed to their effect on the function and composition of bacterial cellular membrane, the synthesis of DNA, RNA, proteins and lipids,and the function of the mitochondrion (Raccach,1984). Fernández-lopez *et al*. (2005) found that rosemary extract had effective activity against lactic acid bacteria and Listeria in cooked Swedish-style meat balls. The aforemen-

Table 6: Effect of natural antioxidants on myoglobin concentration of ground beef meat during refrigerated storage at 4°C

Treatment / Conc. %	Myoglobin concentration (mg/ g meat)			
	Storage time (days)			
	0	3	6	9
C0.0	3.67±0.10^{kl}	3.31±0.06^{n}	3.04±0.06^{o}	2.91±0.04^{o}
R0.10	5.28±0.07^{a}	5.02±0.02^{b}	4.83±0.070.07	4.61±0.06^{cde}
S0.05	4.85±0.05^{cb}	4.70±0.10^{cd}	4.52±0.03^{def}	4.14±0.04^{ghi}
M0.10	4.72±0.02^{cd}	4.51±0.11^{def}	4.16±0.04^{ghi}	3.76±0.09^{jkl}
CL0.10	4.66±0.04^{cd}	4.27±0.05^{gfh}	3.96±0.04^{ji}	3.60±0.10^{lm}
FGK0.10	4.59±0.03^{cde}	3.94±0.05^{ji}	3.78±0.04^{cde}	3.52±0.08^{lmn}
MH0.01	3.92±0.08^{jik}	3.62±0.08^{lm}	3.41±0.04^{mn}	3.29±0.01^{n}
BHA/BHT0.01	4.80±0.10^{cb}	4.61±0.06^{cde}	4.39±0.28^{efg}	4.12±0.07^{hi}

Means ± SE within the column for the same test item having different letters (a-o)are significantly different among treatments ($P<0.05$). rosemary (R), sage (S), mustard (M), clove (CL), fenugreek (FGK) and majorana hortansia (MH) to syenthetic antioxidants butylated hydroxyanisole / butylated hydroxytoluene (BHA/BHT) and control.

Table 7: Influence of natural antioxidants on sensery evaluation of ground beef meat during refrigerated storage at 4°C

Storage time (day)		Teatment Conc. (%)							
		C 0.0	R 0.10	S 0.05	M 0.10	CL 0.10	FGH 0.10	MH 0.01	BHA/BHT
Flavour	0	5.60±0.05^{h}	6.50±0.00^{a}	6.35±0.05^{abc}	6.40±0.00^{ab}	6.20±0.10^{cde}	6.45±0.05^{a}	6.10±0.10^{ed}	6.25±0.05^{bcd}
	3	5.55±0.05^{h}	6.25±0.05^{bcd}	6.20±0.10^{cde}	6.20±0.00^{cde}	6.10±0.10^{ed}	6.25±0.05^{bcd}	6.00±0.00^{ef}	6.10±0.05^{cde}
	6	5.30±0.00^{i}	6.15±0.05^{cde}	6.10±0.10^{ed}	6.05±0.05^{edf}	6.05±0.05^{edf}	6.10±0.05^{ed}	5.70±0.10^{gh}	6.00±0.00^{ef}
	9	5.10±0.05^{j}	6.10±0.05^{ed}	5.85±0.05^{fg}	6.00±0.00^{ef}	5.80±0.10^{g}	6.00±0.00^{ef}	5.55±0.05^{h}	5.70±0.10^{gh}
Juciness	0	5.80±0.10^{hij}	6.50±0.00^{a}	6.35±0.05^{abc}	6.25±0.10^{cde}	6.15±0.05^{cde}	6.20±0.05^{cde}	6.10±0.10^{def}	6.10±0.05^{def}
	3	5.60±0.05^{klm}	6.40±0.05^{ab}	6.30±0.10^{abc}	6.10±0.05^{def}	6.00±0.00^{ghi}	6.15±0.05^{cde}	6.00±0.00^{ghi}	6.00±0.05^{ghi}
	6	5.45±0.05^{m}	6.22±0.02^{cde}	6.10±0.05^{def}	6.00±0.10^{ghi}	5.90±0.10^{hij}	6.05±0.05^{efg}	5.70±0.10^{jik}	5.80±0.10^{hij}
	9	5.20±0.05^{n}	6.00±0.00^{ghi}	6.00±0.00^{ghi}	5.80±0.05^{ij}	5.70±0.05^{jik}	6.00±0.10^{ghi}	5.50±0.00^{lm}	5.55±0.05^{lm}
Tenderness	0	5.60±0.10^{ji}	6.50±0.00^{a}	6.30±0.10^{bc}	6.20±0.05^{cd}	6.10±0.05^{def}	6.20±0.10^{cd}	5.75±0.05^{gh}	6.10±0.05^{def}
	3	5.52±0.02^{jk}	6.40±0.05^{ab}	6.15±0.05^{cde}	6.12±0.02^{def}	6.02±0.02^{def}	6.15±0.05^{cde}	5.40±0.05^{kl}	6.00±0.00^{ef}
	6	5.30±0.10 ^{l}	6.20±0.05^{cd}	6.00±0.00^{ef}	6.07±0.02^{def}	5.95±0.05^{fg}	6.05±0.05^{def}	5.35±0.05^{kl}	5.60±0.10^{ji}
	9	5.10±0.00^{m}	6.00±0.10^{ef}	5.95±0.02^{fg}	6.00±0.00^{ef}	5.80±0.10^{gh}	6.00±0.00^{ef}	5.25±0.02 ^{l}	5.50±0.00^{jk}
Palatability	0	5.75±0.05^{hij}	6.60±0.10^{a}	6.55±0.05^{ab}	6.40±0.10^{abc}	6.20±0.05^{def}	6.25±0.10^{cde}	6.00±0.00^{ghi}	6.15±0.05^{efg}
	3	5.50±0.00^{k}	6.45±0.05^{abc}	6.35±0.05^{abc}	6.25±0.05^{cde}	6.10±0.05^{fgh}	6.20±0.02^{def}	5.95±0.05 ^{i}	6.00±0.00^{ghi}
	6	5.20±0.10 ^{l}	6.25±0.05^{cde}	6.10±0.05^{fgh}	6.18±0.03^{def}	6.00±0.00^{ghi}	6.10±0.05^{fgh}	5.60±0.10^{jk}	5.90±0.10^{hi}
	9	5.00±0.00 ^{l}	6.15±0.05^{efg}	6.00±0.00^{ghi}	6.00±0.00^{ghi}	5.80±0.10^{ghi}	6.00±0.10^{ghi}	5.50±0.00^{k}	5.75±0.05^{hij}

Means ± SE within the column for the same test item having different letters (a-n)are significantly different among treatments (P<0.05). rosemary (R), sage (S), mustard (M), clove (CL), fenugreek (FGK) and majorana hortansia (MH) to syenthetic antioxidants butylated hydroxyanisole / butylated hydroxytoluene (BHA/BHT) and control.

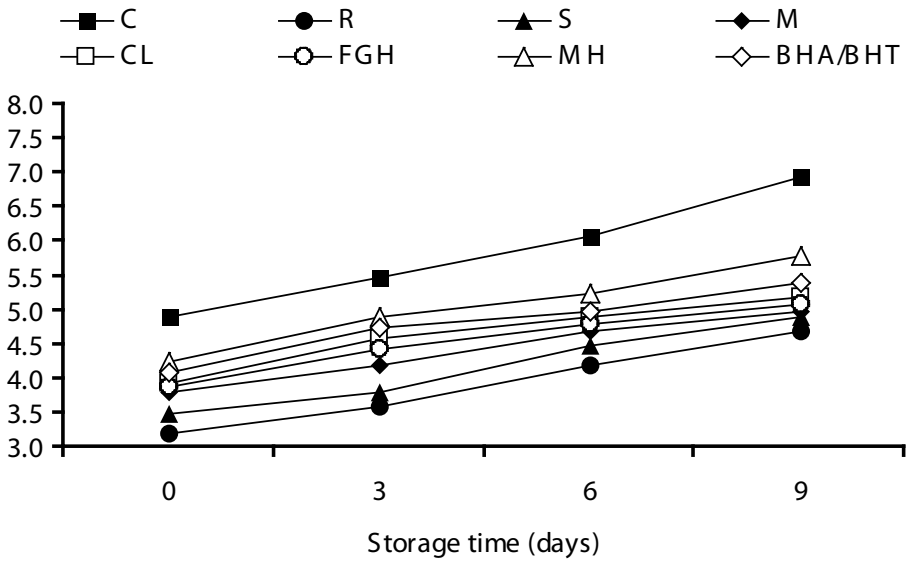

Fig. 1: Effect of natural antioxidants on the total plate count (TPC) of ground beef meat during refrigerated storage at 4°C

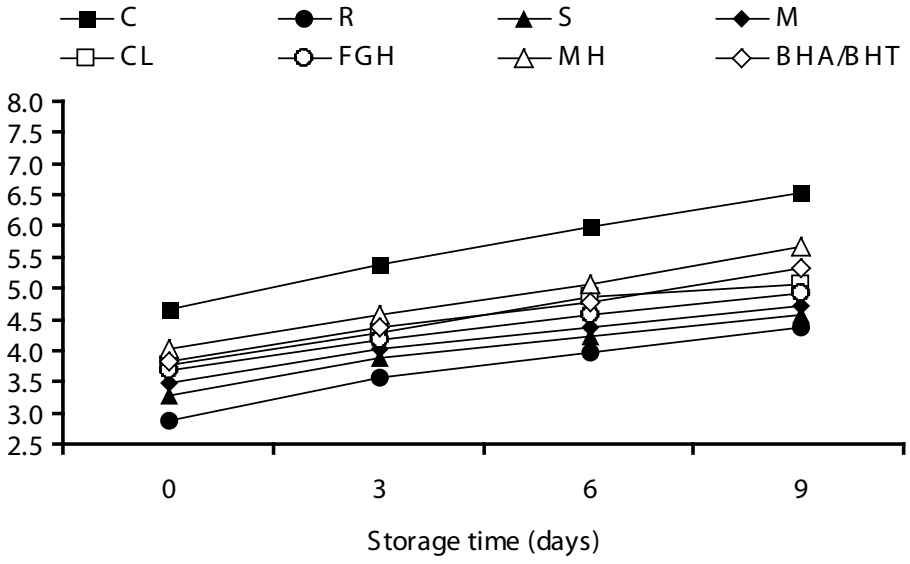

Fig. 2: Effect of natural antioxidants on psychrophilic count of ground beef meat during refrigerated storage at 4°C

tioned results are in agreement with the finding of Al-Rubeii *et al*. (2008) who found significant (P<0.01) decrease in TPC of treated poultry meat with rosemary powder (0.1%) during refrigerated storage for 9 days.

The results of adding antioxidant treatments on psychrophilic count (PTC) in ground beef meat stored under refrigeration are presented in Figure (2) .A significant differences (P<0.01) among antioxidant treatments were recorded. Microbial growth followed similer trends in all antioxidant treatments, R treatment reduced (P<0.01) PTC at all storage periods. While the C treatment recorded the higher (P<0.01) in PTC than other treatments. Shelef *et al*. (1980) found that rosemary was more effective in inhibition activity against the gram–positive rather than gram-negative bacteria. Farbood *et al*. (1976) showed that a 1.0 %concentration of rosemary extract had reduced *Styphimurium aerogenes* and *Staphylococcus aureus* growth by 43.2% and 99.9%, respectively in meats.

CONCLUSION

It could be concluded that direct addition of natural antioxidants is useful and significantly improve the quality characteristics of ground beef meat during storage at 4°C for 9 days. Meanwhile, the present study indicated that the most effective antioxidant was found to be rosemary powder (R) as compared with BHA/BHT, in which R showed lower TBARS, PV, FFA, cholesterol concentration, total volatile nitrogen, and reducing myoglobin oxidation. Moreover, R treatment reduced the percentage of drip loss and cooking loss. On the other hand, the R treatment had higher water holding capacity values, protein solubility, sensory characteristics and inhibitory effect on microbial growth (TPC and PTC) in ground beef meat during storage at 4°C for 9 days. The application of natural antioxidants at certain levels is recommended to replace of synthetic antioxidants (BHA/BHT).

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تأثير بعض مضادات الأكسدة الطبيعية والمشيده على مفروم اللحم البقري خلال التخزين المبرد

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[١]وزارة الزراعة، [٢]مركز سلامة الغذاء ، وزارة العلوم والتكنولوجيا، بغداد – العراق

تم تقيم المستوى الأفضل لكفاءة مضادات الأكسدة الطبيعية من مطحون الحصالبان (٠,١٠٪) (R)، المرمية ((S ٠,٠٥٪))، الخردل (٠,١٠٪) (M)، القرنفل (٠,١٠٪) (CL)،الحلبة (٠,١٠) (FGK ٪) والبردقوش (٠,٠١٪) (MH) مقارنة مع مخلوط (١:١) مضادات الأكسدة الصناعية ٠,٠١) BHA/BHT ٪) وجميع هذه المضادات قورنت مع الكونترول (C) في لحم الأبقار المفروم المخزن بالتبريد عند درجة حرارة °٤م لمدة صفر، ٣، ٦، ٩ أيام. تم تقديرالرقم البيروكسيدي PV، حامض الثيوباربيتيوريك TBA، الأحماض الدهنية الحرة FFA في لحم الأبقار المفروم مع قياس الأس الهيدروجيني pH، قابلية الإحتفاظ بالماء WHC، ذائبية البروتين، نسبة النتروجين الكلي المتطاير، (TVN) محتوى الكوليسترول، تركيز الميوجلوبين في حين تضمنت الصفات الحسية: النكهة، العصيرية، الطراوة، اللون والتقبل العام، أما التحليل الميكروبي تضمن أعداد البكتريا الكلية والمحبة للبرودة.

أدت إضافة مضادات الأكسدة إلى خفض نسبة السائل الناضح، الفقد أثناء الطبخ وكلا من قيم TBA ، PV ، FFA كما إنخفض محتوى الكوليسترول وقيمة TVN وتقليل معدل أكسدة الميوجلوبين إلى الميتاميوجلوبين من ناحية أخرى أرتفعت قيم WHC وذائبية البروتين وتحسنت الصفات الحسية وحدث نقص في معدل النمو الميكروبي (PTC، TPC) . وأشارت النتائج إلى أن إضافة الحصالبان (R) حققت إنخفاضاً معنوياً في أكسدة الدهون في لحم الأبقار المفروم خلال التخزين على درجة ٤م° لمدة صفر، ٣، ٦، ٩ يوم. وقد وجد أن R أكثر كفاءة بالمقارنة مع BHA/BHT في منع زيادة قيم مؤشرات أكسدة الدهون (TBA، PV، FFA) ونجح في حماية الميوجلوبين من الأكسدة إلى الميتاميوجلوبين وهذا قلل بصورة معنوية من تكوين الميتاميوجلوبين .كما أشارت النتائج أن إضافة R أدى إلى أنخفاض نسبة السائل الناضح والفقد أثناء الطبخ وقيمة TVN ومحتوى الكوليسترول، وأن كلا من WHC وذائبية البروتين والصفات الحسية والفاعلية ضد البكتريا الكلية والمحبة للبرودة أرتفعت معنوياً مع إضافة R.

أشارت هذه الدراسة إلى أن مضادات الأكسدة الطبيعية وبصورة خاصة الحصالبان كان أكثر فعالية بالمقارنة مع مضادات الأكسدة الصناعية . إضافة إلى أن استخدام مضادات الأكسدة الطبيعية هذه بالمستويات المضافة حسن نوعية ومدة صلاحية لحم البقر المفروم.