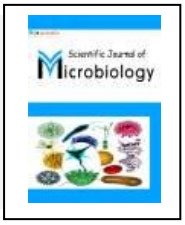
Sjournals
Scientific Journals
Scientific Journal of Microbiology (2013) 2(5) 95-103
ISSN 2322-2948

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com



Original article

The use of some herbs for improving the refrigerated storage stability of minced camel meat

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ARTICLE INFO

Article history:
Received 03 May 2013
Accepted 18 May 2013
Available online 30 May 2013

Keywords:
Natural antioxidant
Phenolic compounds
Meat quality

ABSTRACT

Improving the quality of minced camel meat by adding 3% w/w of marjoram, rosemary and sage powder were investigated during refrigerated storage. Minced camel meat stored at 5°C and analyzed periodically for thiobarbituric acid reactive substances (TBARS), the count of total aerobic bacteria, total coliform bacteria, psychrotrophic bacteria, yeast and moulds. The sensory attributes during 12 days of storage period were also investigated. Total phenols in three herbs were also determined. Marjoram and rosemary were found to have higher content of total phenols (4143 and 4104 mg /100g, respectively) than sage (3404 mg /100g). All tested herbs significantly reduced the TBARS values compared to control after 5 and 12 days with acceptable values (less than 1.0 mg maloaldhyde/100g) and enhanced the sensory attributes. Marjoram reduced the total aerobic bacteria by one log cycle compared to rosemary and sage. The count of yeast and moulds attained the lowest count in marjoram than other treated samples all over the storage period. Marjoram showed the most antimicrobial activity than other tested herbs and may offer a promising choice in food safety and preservation.

1. Introduction

Although camel meat is not universally consumed, it might be a potential alternative for beef particularly in arid/semi-arid regions where camels are usually bred (Rashed, 2002).In recent years the potential of the camel as a meat source has received increased recognition due to health reasons, as they produce carcasses with less fat as well as having less cholesterol and relatively high polyunsaturated fatty acids than other meat animals This is an important factor in reducing the risk of cardiovascular disease (Giese 1992 & Jouki and Khazaei, 2012). The iron content and its bioavailability are much higher in camel meat and may resolve the iron-deficiency problem in poor communities. (Rashed, 2002 & Soltanizadeh *et al*.2010). Quality characteristics affected in meat by lipid oxidation include flavour, colour, texture and its nutritional value. The development of rancidity in meat by lipid oxidation begins at the time of slaughter and continues during storage. Storing meat at low temperature retards the rapid development of rancidity. However, oxidation of lipids may continue even during frozen storage (Weber *et al.,* 2007). Meat and meat products provide excellent growth media for a variety of micro flora (bacteria, yeasts and molds) and has a short shelf-life unless preservation methods are used (Olaoye and Onilude, 2010 & Jay *et al*., 2005). Spoilage by microbial growth is the most important factor in relation to the keeping quality of meat (Lambert *et al.,* 1991). Some of the microorganisms originate from the animal's intestinal tract as well as from the environment (Koutsoumanis and Sofos, 2004). Other organisms, including psychrotrophic bacteria, are recovered from the stages of processing (Gill, 2005). Many varieties of preservation techniques are employed in improving its keeping quality and shelf life. In good hygienic conditions, after slaughter and evisceration, the optimal way to preserve meat is under refrigeration at temperatures around 4°C. The aims of preservation methods are to inhibit the microbial spoilage and to minimize the oxidation and enzymatic spoilage. The addition of antioxidants to processed meats is often carried out to counteract the negative effects of processing aids. However, may be desirable to replace these conventional antioxidants with natural antioxidative substances (Chen *et al.*, 1992). Some studies have demonstrated that shelf-life and meat quality can be improved by using natural antioxidants in some stages of meat production to reduce microbial growth and lipid oxidation during storage (Velasco and Williams, 2011).

Rosemary (*Rosmarinus officinalis L*.) can inhibit lipid oxidation, chelate metals and scavenge superoxide radicals. The antioxidant activity of carnosic acid, the main antioxidant constituent of rosemary, is found to be than twice that of any other phenolic diterpene. It has several times the antioxidative capacity of BHT and BHA (Richheimer *et al.,* 1996).

Marjoram (*Origanum majorana L*.) essential oil inhibits formation of initial compounds during the oxidation of unsaturated fatty acids by 50% and the generation of secondary oxidation products by 80% in a model system (Schmidt *et al.*, 2008). Mansour *et al.,* (2006) reported that adding marjoram as well as rosemary to a minced beef system reduced secondary oxidation products during refrigerated storage. A purified component isolated from marjoram,T3b, is found to be a better superoxide anion radical scavenger than BHT, BHA, α-tocopherol, ascorbic acid and a variety of polyphenolic flavonoids (Jun *et al.,* 2001).

Sage (*Salvia officinalis L*.) contains a variety of antioxidative substances including carnosol, rosmanol, rosemadiol, epirosmanol, isorosmanol, galdosol and carnosic acid (Nakatani, 2003& Miura *et al.*, 2002& Deans *et al.,* 2000 & Cuvelier *et al*., 1994). The essential oils of sage can reduce oxidation in beef. The ethanolic extract of sage can reduce both peroxide oxygen (POV; chemical measure of oxidation) and TBARS values. Sage added to a cooked beef homogenate inhibited lipid oxidation by 47% during refrigerated and frozen storage (Wong *et al*., 1995 & El-Alim *et al*., 1999).

The current study aims to improve the storage stability of minced camel meat and increase its shelf- life by using some herbs (as natural preservatives) e.g., marjoram (*Origanum majorana L*.) rosemary (*Rosmarinus officinalis L*.), and sage (*Salvia officinalis L*.).

2. Materials and methods

2.1. Samples preparation

The camel meat (deep part of *Longissimus dorsi*) was purchased from local butchery in the day of slaughter and rapidly under complete aseptic conditions transported to the laboratory in an icebox. The meat was minced through 3 mm plate by using meat grinder (Sirman- TC 32 CHICA 60in\USA). Herbs (marjoram, rosemary and sage)

purchased from local market. The herbs were milled fine by miller (Brabender- 880804\ Germany) and stored in sealed poly ethylene bags until use. The minced meat was divided into four portions, first portion without treatment (as a control sample), the other three portions were treated with marjoram, rosemary and sage (3%, w/w) then mixed for blender to ensure uniform distribution of herbs added. The minced meat treatments were individually packaged in poly ethylene bags. The treatments were stored in refrigerator and analysis after 5 and 12days.

2.2. Chemical analysis

2.2.1. Determination of total content of phenolic compounds

The total content of phenolic compounds (TPC) in rosmary, marjoram and sage powde was extracted with methanol and determined according to the method reported by Boyer and Hai Liu **(**2004). One ml of extract was mixed with 5 ml of 10 % Folin-Ciocalteu reagent in distilled water and 4 ml of 7.5 % sodium carbonate solution. After incubation of samples with reagent at room temperature for 30 min with periodical mixing, the absorbance at 765 nm was measured. The calibration curve was constructed within the concentration range 0.075−0.6 mg/ml of Gallic acid. Mean values were calculated from as gallic acid equivalents (GAE) in mg/100 g of dry plant material using the following equation:

$C = a \times \gamma \times (V/m) \times 100$,

Where: C = total amount of phenolic compounds, mg/100g as gallic acid; a = dilution number; γ = concentration obtained from calibration curve (mg/ml); V = volume of aqueous ethanol used for extraction; m = weight of sample (g).

2.2.2. Determination of thiobarbituric acid reactive substances

All the camel meat samples were analyzed for thiobarbituric acid reactive substances (TBARS) according to the method of Du and Ahn (2002) as follow: Five grams of meat were homogenized with 15 ml of deionized distilled water. One milliliter of the meat homogenate was transferred to a test tube and 50 µL of butylated hydroxyl toluene (7.2%) and 2 ml of thiobarbituric acid (TBA)–trichloroacetic acid (TCA) (15 mM TBA–15% TCA) were added. The mixture was vortexed and then incubated in a boiling water bath for 15 min to develop color. Then samples were cooled in ice water for 10 min, vortexed again, and centrifuged for 15 min at 2500×g. The absorbance of the resulting supernatant solution was determined at 531 nm (UVIKON 930, Kontron Instruments, Italy) against a blank containing 1 ml of deionized water and 2 ml of TBA–TCA solutions. The amounts of TBARS were expressed as milligrams of malonaldehyde per kilogram of meat.

2.3. Microbiological analysis

Ten grams for each camel meat treatment from each bag were transferred aseptically into 90 ml peptone water solution (Oxoid, CM0009) in sterile bag and homogenized thoroughly for 1 min using Lab Blender (400\UK). Serial dilutions (10^1-10^5) were performed and 1 ml aliquots of the appropriate dilution were inculated in triplicate on universal and selective media. Plate count agar (Oxoid CM0325) incubated at 7°C for 7 days for enumeration of total psychrotrophic bacteria. Nutrient agar (Oxoid CM0003) incubated at 30°C for 72h for enumeration of total aerobic mesophilic bacteria. Violet red bile agar (VRBA) (Oxiod CM 0107) incubated anaerobically at 37°C for 24 h for enumeration of coliform bacteria, Round, purple-red colonies (0.5–2mm diameter) surrounded by purple-red haloes on VRBA plates were counted as coliforms.. Acidified potato dextrose agar (PDA) (Oxoid CM0139) incubated at 30°C for 48h for enumeration of yeasts and moulds, (0.1 ml of the appropriate dilution spread plated on this medium).

2.4. Sensory attributes

A sensory evaluation of raw samples was performed according to the method described by Djenane *et al*., (2002). Scores from the evaluation were recorded by ten trained panelists under the suitable conditions of light and temperature. “Odor” scores assigned to the intensity of spices flavor and off odors associated to meat spoilage: 1 = none, 2 = slight, 3 = smell, 4 = moderate and 5 = extreme. Discoloration of uncooked meat scores referred to percentage of discolored surface: 1 = none, 2 = 0 - 10 %, 3 = 11 - 20 %, 4 = 21 - 60 %, and 5 = 61 - 100 %. Results were expressed as the predominant score given by panelists.

2.5. Statistical analysis

A multiple comparison statistical procedure using Fisher's least significant difference test (SAS software, version 6.11) was used at significance levels of *(P*≤ 0.05).

3. Results and discussion

3.1. Total phenols content

The total phenols content (as mg gallic acid /100g) of the selected herbs was determined in this study. Marjoram and rosemary were found to have higher total phenol content (4143 and 4104 mg /100g, respectively) compared to sage (3404 mg /100g). These findings are in accordance with Chrpova *et al*., (2010).

3.2. Thiobarbituric acid reactive substances (TBARS)

The effect of herbal addition to the minced camel meat on the lipid oxidation during refrigerated period was tabulated in Table (1). In spite of that camel meat have less fat compared to other meat animals it have relatively high polyunsaturated fatty acids which may play an important role in increasing the rate of lipid oxidation (Jouki and Khazaei, 2012) The TBARS values of control sample increased significantly from 0.79 to 1.10 and 1.29 mg malonaldehyde/kg after 5 and 12 days of storage, respectively. This sample was unacceptable according to Chang *et al*.,(1961) who stated that the judges were able to detect the undesirable odors and flavors when the values of the TBARS in meat sample were between 0.5-1.0 mg malonaldehyde/kg. The incorporation of marjoram, rosemary and sage in minced camel meat resulted in about 63.6% reduction of TBARS values after 5days of storage compared to control sample. Moreover, at the end of the storage TBARS values recorded about 86% decrease compared to the untreated meat. The TBARS values of all treated samples were found to be under the value of 1.0 mg malonaldehyde/kg through the12 days of the storage (0.170 - 0. 70 mg malonaldehyde/Kg). No significant (*P < 0.05*) difference was observed after 5 and 10 days between the three herbs. This reduction or delaying of lipid oxidation may due to the presence of the phenolic compounds of the selected herbs and essential oil. Phenoilc compounds have free radical scavenger activity similar to the synthetic antioxidants and may also chelate metal ions, such as Fe2, result in the reduction of the formation rate of activated oxygen. Different phenolic compounds have been identified from rosemary and marjoram by different authors (Fecka *et al*., 2002; Löliger, 1991; Sellami *et al*., 2009). Our results are in agreement with Mohamed *et al*., (2011) who stated that herbal extracts of marjoram, rosemary and sage have antioxidant effects and can be used to minimize lipid oxidation and decrease irradiation odor production in irradiated ground beef. The antioxidative activity of these herbs is due to carnosol (an odourless and tasteless phenolic diterpen), carnosic acid, rosmanol, epirosmanol, isorosmanol and rosmarinic acid (Madsen and Bertelsen 1995). Sahar *et al*., (2007) studied the anti-oxidative effect of rosemary on refrigerated storage burger at concentration of 1 and 2% and found that the retarding effect of antioxidants on the development of oxidation depended on the level and type of antioxidants.

3.3. Total aerobic mesopholic bacterial count

The count of total aerobic mesopholic bacteria in minced camel meat treated with some herbs during refrigerated period are shown in Table (2). After addition of all herbs, total count of aerobic mesopholic bacteria were reduced compared to control. After 5 days, all treatments reduced the total aerobic mesopholic bacterial counts by 1-2 log cycle compared to zero time control. Increasing the storage period to 12 days, did not influence the count of the mesopholic bacteria in samples treated with rosemary and sage. Meanwhile the sample treated with marjoram recorded 66.08 % reduction in the count after 12days compared to that after 5days. This may be due to that marjoram contains some aromatic components that have antimicrobial properties (Busatta *et al*., 2008). This results agree with Mohamed *et al.*, (2011) how stated that ethanol Marjoram showed strong exhibited antimicrobial activity against Gram positive, Gram negative bacteria and fungi of sausage during 2 and 7days of storage at 5 ºC. These findings are also in a line with Ezzeddine *et al.*, (2001) who found that among several essential oils marjoram oil (*Origanum majorana L*.) have the greatest potential antimicrobial effect. It is worthy to mention that, herbal treated and control camel meat was under the acceptable level of bacterial count allowed during storage (10^7 cfu.cm^{-2}) as described by Borch *et al*., (1996). Antibacterial activity of natural antioxidants has been determined through in vitro assays against *Escherichia coli* 0157:H7, *Salmonella typhimurium* (Helander *et al*.,

1998 & Elgayyar *et al.,* 2001), *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Elgayyar *et al.,* 2001 & Lambert *et al.,* 2001).

3.4. Coliform bacterial count

The total coliform bacterial count was not detected in either control sample or treated samples during refrigerated period. The meat sample used in this study was deep part of *Longissimus dorsi* this part is less susceptible to the intestinal contamination. This may indicates the performance of good hygiene practice during slaughtering animal and trading.

3.5. Psychrotrophic bacterial count

The count of psychrotrophic bacterial in minced camel meat treated with some herbs during refrigerated period are illustrated in Table (3). The present results indicated that the microbial counts were sufficiently low during the storage period in minced meat treated with natural herbs. After herbal addition to camel meat the psychrotrophic bacterial counts significantly decreased from 16.8×10^2 in control to 5.0×10^2, 12.4×10^2 and10.1×10^2 cfu/g in marjoram, rosemary and sage, respectively. After 5days, marjoram and rosemary treatments showed the lowest count (5.0×10^2 and 7.3×10^2). All psychrotrophic bacterial counts in all samples were under the microbiological standard for this type of raw meat products in Egypt (Mohamed and Mansour 2012) that psychrotrophic counts should not be more than 106cfu/g meat. These findings may due to the performance of hygienic condition during slaughtering animal, grounding, packaging and sampling. The data in a line with Abou-Arab and Abou-Salem (2010) who studied the effect of ascorbic acid, α-tocopherol and rosemary on the stability of ostrich meat during storage and they found that the bacterial counts of untreated samples (control) were higher than those in the antioxidant treated samples. However, Mohamed and Mansour (2012) found that addition of antioxidants did not reduce the psychrotrophic counts in beef patties.

3.6. Yeasts and moulds count

The effect of adding herbs to the minced camel meat on the yeasts and moulds count during refrigerated period was tabulated in Table (4). Marjoram and rosemary treated samples induced marked decrease in yeast and moulds count compared to control and sage treated samples. The same inhibitory activity of spices and their derivatives on the growth of bacteria, yeasts, fungi and microbial toxin synthesis has been reported (Notermans and Hoogenboon-Verdegaal 1992 & Sagdiç *et al.,* 2003). This finding was in contrast with Karapinar (1985) who was reported that various powder concentrations of sage significantly inhibited the growth of some fungi.

3.7. Sensory evaluation

It could be noticed from table (5) that the panelists were able to clearly detect the differences in aroma quality, off odor and discoloration of minced camel meat treated with selected herbs, compared with untreated samples (control) after 5 and 12 days of storage at 5 °C. The presence of selected herbs extended the changes in off odor of meat with score (1) to (2) even after 12 days of storage. The extended changes in the discoloration of treated samples were also noticed. No to slight discoloration were recorded throughout the storage period in treated samples. All tested herbs enhanced the flavor of minced camel meat and scored 4.3 in sage treated samples to 4.7 in rosemary treated samples. Meanwhile, untreated samples reached the highest value corresponding to off odor (score 5) and 21 - 60 % of discoloration (score 4), after 12 days. These results were in agreement with those obtained by Barbut *et al.* (1985) who found that rosemary inhibited undesirable odor appearance in Turkey sausage stored at 4 °C. Also, these results were in harmony with those recorded by Escalante *et al.* (2001) who showed that rosemary alone or with ascorbic acid extended the fresh beef patty odor during storage time.

4. Conclusion

The results of this study support many recommendations for using natural herbs in preserving meat. The present study reveals potential application of rosemary, marjoram and sage as a good natural source of antioxidants that seemed to be effective in minimizing the lipid oxidation in refrigerated minced camel meat. Marjoram reduced the total aerobic bacterial counts, psychrotrophic bacterial count and the count of yeast and

moulds during chilling storage for 12 days. Marjoram possesses strong antimicrobial as well as the antioxidant effect leads to a promising alternative for preservation of camel meat.

Table 1
The effect of adding herbs to the minced camel meat on the thiobarbituric acid reactive substances (TBARS) during refrigerated period.

Treatments	TBARS mg malonaldehyde/Kg		
	Storage time (day)		
	0	5	12
Control	0.79 ± 0.05^{b}	1.10 ± 0.05^{ab}	1.29 ± 0.06^{a}
Meat + Marjoram	0.70 ± 0.05^{bc}	0.40 ± 0.05^{bcd}	0.18 ± 0.01^{d}
Meat + Rosemary	0.51 ± 0.01^{bcd}	0.41 ± 0.04^{bcd}	0.19 ± 0.02^{d}
Meat + Sage	0.63 ± 0.04^{bc}	0.40 ± 0.03^{bcd}	0.17 ± 0.04^{d}

Different superscript small characters mean significant differences (P<0.05)

Table 2
The effect of adding herbs to the minced camel meat on the total aerobic mesopholic bacterial count during refrigerated period.

Treatments	Total aerobic mesopholic bacterial count (cfu/g)		
	Storage time (day)		
	0	5	12
Control	76.0×10^{5}	48.0×10^{4}	14.5×10^{4}
Meat + Marjoram	63.4×10^{5}	56.0×10^{3}	19.0×10^{3}
Meat + Rosemary	75.5×10^{5}	11.1×10^{4}	15.1×10^{4}
Meat + Sage	40.5×10^{5}	10.7×10^{4}	30.0×10^{4}

Table 3
The effect of adding herbs to the minced camel meat on the psychrotrophic bacterial count during refrigerated period.

Treatments	Psychrotrophic bacterial count (cfu/g)		
	Storage time (day)		
	0	5	12
Control	16.8×10^{2}	18.2×10^{2}	40×10^{2}
Meat + Marjoram	5.0×10^{2}	7.3×10^{2}	22×10^{2}
Meat + Rosemary	12.4×10^{2}	9.5×10^{2}	28×10^{2}
Meat + Sage	10.1×10^{2}	15.3×10^{2}	34×10^{2}

Table 4
The effect of adding herbs to the minced camel meat on the yeasts and moulds count during refrigerated period.

Treatments	Yeasts and moulds count (cfu/g)		
	Storage time (day)		
	0	5	12
Control	165	150	139
Meat + Marjoram	172	50	19
Meat + Rosemary	180	82	51
Meat + Sage	193	190	182

Table 5
Sensory evaluation for minced camel meat incorporated with selected herbs.

Treatments	Storage time (day)		
	0	5	12
Discoloration			
Control	1.0[b]	2.1[b]	4.2[a]
Meat+ Marjoram	1.0[b]	1.2[b]	2.3[b]
Meat+ Rosemary	1.0[b]	1.2[b]	1.3[b]
Meat + Sage	2.0[b]	2.1[b]	2.1[b]
Off odor			
Control	1.0[c]	3.2[b]	5.0[a]
Meat + Marjoram	1.0[c]	1.1[c]	1.2[c]
Meat + Rosemary	1.0[c]	1.0[c]	1.1[c]
Meat + Sage	1.0[c]	1.5[c]	2.1[c]
Flavor			
Control	1.0[c]	1.0[c]	1.0[c]
Meat + Marjoram	4.7[a]	4.1[a]	3.9[b]
Meat + Rosemary	4.6[a]	4.1[a]	4.0[b]
Meat + Sage	4.3[a]	3.9[a]	3.9[b]

Different superscript small characters mean significant differences ($P<0.05$)

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