

Antioxidant and antimicrobial effects of rosemary and ginger extract added to Karadi yearling lamb patties

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Abstract

The purpose of this investigation was to study the effect of adding natural extracts (rosemary and ginger) on the oxidative rancidity and microbial growth in lamb patties. Lamb patties were blended with 0.05%, 0.075% and 0.100% rosemary extract and 0.50%, 0.75%, and 1.00% ginger extract and untreated (control) group at 4°C for seven days. The results revealed that lipid oxidation of lamb patties started to increase after four days of refrigeration and increase rapidly to reach the maximum after seven days of storage. Extract of rosemary and ginger apparently retarded significantly ($P<0.01$) oxidative rancidity of lamb patties. TBA (Thiobarbituric acid) values demonstrated that level of 0.05% rosemary extract and 0.50% ginger extract added to lamb patties were more effective compared with other concentrations of each of rosemary or ginger added to patties. Total plate count, psychrophilic count and coliform decreased significantly ($P<0.01$) with the addition of both extracts during storage. It was concluded that adding of natural antioxidant and antimicrobial extract (rosemary, ginger) led to retarded oxidative rancidity and microbial growth during refrigerated storage of lamb patties.

Keywords: Extract Of Rosemary, Ginger, Lipid Oxidation, Microbial Count, Lamb Patties

Introduction

Ultimately, food quality is defined in terms of consumer acceptability: taste, aroma and appearance characteristics. The increasing demand for convenient food has led to rapid growth in the ready-to-eat product category (Hofstrand, 2008). Many of the food ingredients contain unsaturated fatty acids that are quite susceptible to quality deterioration, especially under oxidative stress. For this reason, efforts to reduce oxidation have increased. Most often, the best strategy is the addition of antioxidants (Brewer, 2011). Moreover, lipid oxidation and microbial growth in meat products may be controlled or at least minimized by using either synthetic or natural food additives commonly used in the meat industry (Gray et al., 1996; Lee et al., 1997; Mielnik et al., 2003; Sallam et al., 2004; Estevez and Cava, 2006).

The natural antioxidants found in plants have gained considerable interest for their role in preventing the auto-oxidation of fats, oils and fat containing food

products (Sherwin, 1978). The antioxidant properties of herbs, spices, plant and other food extracts are apparently related to their phenolic content, suggesting that antioxidant action is similar to that of synthetic phenolic antioxidants (Lai et al., 1991).

The rhizome of the popular ginger species (*Zinger officinal*) is widely used as a spice and food seasoning due to its sweet aroma and pungent taste. It is well known to have antioxidant activity (Zia-ur-Rehman et al., 2003) and effective antimicrobial agents (James et al., 1999; Ibrahim et al., 2011). Also, rosemary (*R. officinalis*) has antioxidant and antimicrobial activity (Hra et al., 2000; Wang et al., 2008; Hernandez-Hernandez et al., 2009).

The objective of the present study was to establish the optimum concentrations of rosemary and ginger as sources of natural antioxidants and/or antimicrobial agents to be added to Karadi yearling lamb patties in order to diminish oxidative and microbiological deterioration.

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Material and Methods

Rosemary (*R. officinalis*) and ginger rhizomes (*Zingiber officinal*) were purchased from the local market in Duhok. The dried rosemary leaves were ground and passed through 80 mesh screen. Batches of one hundred grams of ground rosemary were extracted with 1 liter of 95% ethanol overnight in a closed conical flask at room temperature in the dark, and then filtered through cheese cloth. The process was repeated three times. The combined filtrate was concentrated in a vacuum oven at 40°C (Pokorný et al., 1997). The extract was stored in containers in the freezer until use.

The active compounds of rosemary extract were analyzed by High Performance Liquid Chromatography (HPLC) according to the method described by Thorsen and Hildebrandt (2003). The active compounds found are Carnosol, Carnosic acid, Rosmanol, Cirsimaritin, and Genkwanin (Table 1). Similarly, it was found that the most active antioxidative constituent of rosemary are phenolic diterpenes (Carnosol, Carnosic acid, Rosmanol, rosmadial, 12-methoxycarnosic acid, epi-andiso-rosmanol) and phenolic acids (rosmarinic and caffeic) as reported by Brewer (2011).

Ginger rhizomes were washed, dried, ground and passed through a 50 mesh screen. One hundred grams of ground ginger were extracted with 1 liter of 95% ethanol in a closed conical flask for 24 h at room temperature in the dark. The extract was filtered through cheese cloth and the residue was re-extracted three times using the same solvent. The combined filtrate was evaporated in a vacuum oven at 40°C (Rehman et al., 2003). The obtained aqueous extract was frozen until use.

The active compounds of ginger extract were analyzed by HPLC as described by Xuesong (1997). The active compounds found were Gingerol, Zingiberene, α -farnesne, β -sesquiphellanderene, and α -Curcumene (Table 2). Also, Singh et al. (2005) indicated that analysis of fresh rhizome essential oil of ginger showed the presence of 69 components and the major component was α -Zingiberene.

Preparation of patties

Steaks from longissimus dorsi muscle were removed from Karadi yearling lamb carcasses. After 24 h of chilled storage (4°C) the excess fat and connective tissue were trimmed from the meat, then chopped and minced through 0.8 cm plate in a meat grinder. Salt (70% NaCl and 30% KCl) was added at a rate of 1.5% to the minced meat. The minced meat was subdivided into seven treatments (1 kg each): control (not added plant extract). Three treatments were blended with 0.050, 0.075 and 0.100% rosemary extracts, the other three treatments were blended with 0.50, 0.75 and 1.00% of ginger extract. They then mixed well and

formed into patties (100g) using a meat former. Patties were placed on plastic foam meat trays, wrapped with polyethylene film and kept in a refrigerator at 4°C for seven days. They were used to evaluate for TBA and microbial count at 1, 4 and 7 days of storage time.

Table 1: The active compounds of rosemary extract

The active compounds of rosemary extract	Concentration mg/100gm rosemary extract
Carnosol	38.90
Carnosic acid	27.17
Rosmanol	18.25
Cirsimaritin	92.32
Genkwanin	64.93

Table 2: The active compounds of ginger extract

the active compounds of ginger extract	Concentration mg/100gm ginger extract
Gingerol	38.03
Zingiberene	182.84
β -bisabolene	80.06
α -farnesne	127.13
β -sesquiphellanderene	162.40
α -Curcumene	147.12

TBA

The TBA values were determined according to the method described by Witte et al. (1970). Twenty grams of the minced meat were blended with 50 mL of cold solution containing 20% trichloroacetic acids in 2 M phosphoric acids. The resulting slurry was then transferred into a 100 mL with distilled water, homogenized by shaking and filtered through Whatman no.1 filter paper. Five mL of the filtrate was then pipette into a test tube while another 5 mL of fresh chilled 2-thiobarbituric acid (0.005 M in distilled water) was added. The test tube was shaken well and placed in the dark at room temperature (25°C) for 15- 17 h to develop the color reaction. The resulting color was measured in spectrophotometer (6400-JENWAY) at 530 nm to calculate the TBA values.

The TBA value was expressed as mg MDA/kg meat, which was calculated by multiplying the absorbance by 5.2 factors as follows:

$$\text{TBA (mg (MDA)/kg meat)} = A_{530} \times 5.2$$

Microbial count

Microbial count was determined as recommended by the American Public Health Association for food stuff examination (APHA, 1992). Total plate count (TPC) was determined on nutrient agar medium (LAB), and the plates of different dilutions were incubated at 37°C for 48 h. The average number of colonies per countable plate as well as the total number of colonies per gram (CFU/g) was determined. MacConky agar medium (LAB) was used for determination of coliform bacteria, and the inoculated plates were incubated at 37°C for 48 h, and the number of dark red colonies was

calculated. Psychrophilic bacteria were determined by using LAB for plating, and the inoculated plates were inoculated at 7°C for 10 days.

Sensory evaluation

The investigated samples were evaluated using a panel test according to Cross et al. (1978), at day seven of storage at 4°C.

Statistical analysis

Data on the effects of addition natural antioxidants extracts and storage period were subjected to analysis of Variance (ANOVA) using SAS/STAT, (2002). Also, Duncan's multiple range tests were used to detect differences among mean within each factor.

Results and Discussion

TBA

The TBA values for the yearling lamb patties containing different levels of each of rosemary and ginger extracts during storage at 4°C for seven days is presented in Table 3. The analysis of variance for the TBA data indicates that the values were significantly ($P<0.01$) affected by the both extract treatments and the storage period. Hence, the TBA values for all extract samples were significantly lower ($P<0.01$) than those for the control.

During storage at 4°C lipid oxidation of patties in the control batch started to increase from 0.88 mg (Day one) to 1.18 mg (Day 4) and increased rapidly after 7 days of storage to reach 2.89 mg MDA/kg meat. While the meat patties contained extract of rosemary or ginger apparently retarded oxidative rancidity during storage at 4 °c as compared to control (Table 3). Moreover, TBA values demonstrated that the level of 0.05% rosemary extract and 0.5% ginger extract added to meat patties was more effective in comparison with other concentrations of each of rosemary and ginger added to patties.

TBA value is routinely used as an index of lipid oxidation in meat products in store and the rancid flavor is initially detected in meat products between TBA values of 0.5 and 2.0 (Raharjo and Safos, 1993). In the current work, data of TBA indicated that all the tested of rosemary and ginger added to meat patties showed a good antioxidant properties. Similarly, Chen et al. (1992) reported that rosemary extract could be delayed the oxidation of deboned poultry meat, processed beef gels, pork fat and chicken sausage. Also, AbdEl-Hamied et al. (2009) demonstrated that addition of 0.1% rosemary extract to minced meat resulted in a significant decrease in TBA values as compared to control. Moreover, the effect of addition of synthetic BHA, BHT and rosemary extracts to α -tocopherol was studied by Formanek et al. (2001). They found that

rosemary extracts were as effective in reducing TBA as the combination of synthetic antioxidants BHA/BHT. Gordon (1990) reported that the rosemary extracts contain phenolic primary antioxidants which react with lipid or hydroxyl radicals and convert them into stable products. Also, Fang and Wada (1993) indicated that the rosemary extracts may chelate metal ions, such as Fe resulting in a reduced rate of formation of activated oxygen.

With regard to ginger extract, Ibrahim et al. (2011) reported that ginseng, jatrophia, jojoba and ginger extract used as antioxidants was effective against TBA formation when incorporated into lamb patties. Moreover, the polyphenolic extracts are excellent electron and proton donors, and their intermediate radicals are quite stable due to electron delocalization phenomena as well as owing to the lack of positions attackable by oxygen (Djenane et al., 2005). In the current study, since the natural extracts used in preparing lamb patties contained phenolic compounds, these substances could cause an inhibition of the chain reactions during lipid oxidation (El-Diwani et al., 2009). From a commercial standpoint, incorporation of rosemary or ginger in meat products can substantially suppress lipid oxidation and increase shelf life at refrigerated temperatures.

Microbial changes

Tables 4 and 5 shows the effects of different concentration of each of rosemary and ginger extracts to the prepared lamb patties stored at 4°C for seven days on total plate count (TPC), psychrophilic bacterial count (Psy) and coliform bacteria. A remarkable increase ($P<0.01$) was noticed in (TPC) throughout storage, especially in the control sample at days 4 and 7 (from 14.667 to 42.333×10^5) log CFU/g respectively. It has been reported by Insausti et al. (2001) that meat spoilage cannot be said to occur until total viable (TVC) counts reach to certain limit of microbiological acceptability. In general, a significant decrease was noticed for all treated patty samples with rosemary or ginger in their TPC count during the storage period. Thus, the results show that the TPC count decreased significantly with the addition of the natural extract during the storage at 4°C for seven days. Similarly, Jitoe et al. (1992), Zia-ur-Rehman et al. (2003) and Ibrahim et al. (2011) found that ginger has antioxidant activity and effective antimicrobial agent. Also, Jatosinska and Wilczak (2009) concluded that the extract from rosemary was characterized with the strongest activity against microbes, which points to the longest shelf life of the product examined.

Result of Psy (Tables 4 and 5) showed that addition of rosemary or ginger showed a significantly ($P<0.01$) higher inhibitory effect as compared to the control sample. Also, it seems that addition of ginger resulted

Table 3: Changes in TBA values of yearling lamb during storage at 4°C for 7 days

Natural antioxidant storage (days)	TBA (mg MDA/kg)		
	Day 1	Day 4	Day 7
Rosemary extract (%)			
Control	0.88 ± 0.08 ^c	1.18 ± 0.01 ^b	2.85 ± 0.06 ^a
Rosemary 0.05%	0.44 ± 0.00 ^c	0.50 ± 0.00 ^e	0.70 ± 0.00 ^d
Rosemary 0.075%	0.54 ± 0.04 ^c	0.53 ± 0.00 ^e	0.82 ± 0.00 ^c
Rosemary 0.100%	0.44 ± 0.02 ^c	0.68 ± 0.02 ^d	0.78 ± 0.00 ^{cd}
Ginger extract (%)			
Control	0.88 ± 0.08 ^d	1.18 ± 0.01 ^b	2.89 ± 0.06 ^a
Ginger 0.50%	0.41 ± 0.00 ^{fg}	0.34 ± 0.00 ^g	0.80 ± 0.00 ^d
Ginger 0.75%	0.41 ± 0.00 ^{fg}	0.45 ± 0.01 ^{ef}	0.88 ± 0.01 ^d
Ginger 1.00%	0.51 ± 0.03 ^c	0.45 ± 0.01 ^{ef}	0.98 ± 0.00 ^c

Means with different letters within each column and each row differed significantly (P<0.05)

Table 4: Effect of rosemary extracts on change in total plate count, psychrophilic bacteria count and coliform count of lamb patties stored at 4°C for 7 days

Treatment Rosemary Extract	Days		
	1	4	7
	T.P.C. X 10 ⁵		
Control	4.50 ± 0.28 ^d	14.66 ± 1.45 ^c	42.33 ± 1.76 ^a
Rosemary 0.05%	0.90 ± 0.02 ^e	2.63 ± 0.08 ^{de}	20.00 ± 0.57 ^b
Rosemary 0.075%	0.82 ± 0.03 ^e	2.13 ± 0.17 ^e	19.00 ± 0.57 ^b
Rosemary 0.100%	0.63 ± 0.03 ^e	1.93 ± 0.17 ^e	18.33 ± 0.17 ^b
Psy X 10 ⁵			
Control	2.63 ± 0.08 ^c	7.50 ± 0.28 ^b	26.00 ± 1.15 ^a
Rosemary 0.05%	0.06 ± 0.01 ^d	0.53 ± 0.06 ^d	8.13 ± 0.33 ^b
Rosemary 0.075%	0.09 ± 0.00 ^d	0.33 ± 0.08 ^d	8.00 ± 0.30 ^b
Rosemary 0.100%	0.05 ± 0.00 ^d	0.26 ± 0.03 ^d	7.93 ± 0.29 ^b
Choliform X 10 ³			
Control	8.13 ± 0.17 ^f	40.00 ± 1.15 ^b	56.66 ± 1.76 ^a
Rosemary 0.05%	4.20 ± 0.23 ^g	12.66 ± 0.88 ^e	20.00 ± 0.57 ^c
Rosemary 0.075%	4.20 ± 0.15 ^g	11.66 ± 0.88 ^e	17.66 ± 0.33 ^{cd}
Rosemary 0.100%	3.90 ± 0.10 ^g	11.33 ± 1.20 ^e	16.00 ± 1.15 ^d

Means with different letters within each column and each row differed significantly (P<0.05)

Table 5: Effect of ginger extracts on change in total plate count, psychrophilic bacteria count and coliform count of lamb patties stored at 4°C for 7 days.

Treatment Ginger Extract	Days		
	1	4	7
	T.P.C. X 10 ⁵		
Control	4.50 ± 6.28 ^c	14.66 ± 1.45 ^b	42.33 ± 1.76 ^a
Ginger 0.5%	0.45 ± 0.02 ^d	1.16 ± 0.16 ^d	16.00 ± 0.57 ^b
Ginger 0.75%	0.43 ± 0.01 ^d	0.83 ± 0.16 ^d	14.66 ± 0.88 ^b
Ginger 1.00%	0.41 ± 0.02 ^d	0.81 ± 0.03 ^d	14.00 ± 0.57 ^b
Psy X 10 ⁵			
Control	2.63 ± 0.08 ^c	7.50 ± 0.28 ^b	26.00 ± 1.15 ^a
Ginger 0.5%	0.11 ± 0.01 ^d	0.40 ± 0.05 ^d	7.33 ± 0.03 ^b
Ginger 0.75%	0.09 ± 0.00 ^d	0.30 ± 0.05 ^d	7.20 ± 0.05 ^b
Ginger 1.00%	0.09 ± 0.00 ^d	0.26 ± 0.03 ^d	6.86 ± 0.17 ^b
Choliform X 10 ³			
Control	8.13 ± 0.17 ^c	40.00 ± 1.15 ^b	56.66 ± 1.76 ^a
Ginger 0.5%	4.16 ± 0.38 ^d	8.93 ± 0.58 ^c	10.00 ± 1.15 ^c
Ginger 0.75%	4.06 ± 0.17 ^d	8.73 ± 0.72 ^c	9.16 ± 0.93 ^c
Ginger 1.00%	3.76 ± 0.03 ^d	8.30 ± 0.62 ^c	8.40 ± 0.83 ^c

Means with different letters within each column and each row differed significantly (P<0.05)

in a population of Psy content lower than those samples treated with rosemary, during storage at 4°C for 10 days. These results are in agreement with those reported by Oke et al. (2009) who stated that plant extracts and

essential oils constitute a natural source of antimicrobial mixtures or pure compounds for centuries. Essential oils and purified components are used as natural products that prevent the growth of

food-borne bacteria and molds in food systems, as well as extend the shelf-life of processed foods. Also, Abdel-Hamied et al. (2009) reported that addition of rosemary, sage and their combination resulted in a higher inhibitory effect against Psy count as compared to the control sample.

Coliform counts of the prepared lamb patties containing different concentration of the tested natural antioxidants during storage for seven days at 4°C are shown in tables 4 and 5, respectively. It was observed that total coliform count increased significantly ($P<0.01$) with the storage time for all groups. However, bacteria in the control group grew significantly ($P<0.01$) more quickly than did treated groups, indicating antibacterial effects of rosemary and ginger on lamb patties. Similarly, it has been reported that extract of rosemary and ginger showed an inhibitory activity against coliform bacteria (Malu et al., 2009; Kenar et al., 2010).

Sensory characteristics of cooked patties as affected by different concentration of each of rosemary and ginger are presented in Table 6. Result of analysis of variance revealed that added rosemary and ginger at a rate of 0.050 and 0.75% to lamb patties had significantly better overall acceptability compared to untreated or other treated groups. However, tenderness was not affected significantly with the addition of both natural antioxidants.

It could be recommended the addition of natural extracts of rosemary and ginger retarded the oxidative rancidity and microbial growth of the storage lamb patties.

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