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Antioxidative and antimicrobial effects of garlic in ground camel meat

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Abstract: The aim of this study was to investigate the antioxidative and antimicrobial effects of equivalent concentrations of garlic derivatives in ground camel meat during storage at 4 ± 1 °C. The addition of either garlic or butylated hydroxyanisole (BHA) (0.1 g/kg) significantly delayed lipid oxidation when compared with the control. The antioxidant activities of the various ingredients added followed the order of fresh garlic (FG), garlic powder (GP), BHA, and garlic oil (GO). After 14 days' storage, the aerobic plate count (APC) of both FG- and GP-formulated meat was significantly lower than that of either the control or BHA-formulated samples. However, the addition of GO or BHA resulted in no significant difference in APC when compared with the control. Gas chromatography analysis results showed that oxidation caused fatty acids degradation during storage and these degradations were higher in the control group. The results suggest that fresh garlic and garlic powder, through their combined antioxidant and antimicrobial effects, are potentially useful in preserving meat products.

Key words: Antioxidative, antimicrobial, garlic, camel meat

Introduction

Camels are a good source of meat in areas where the climate adversely affects the production efficiency of other animals. The demand for camel meat appears to be increasing, especially in arid regions. Camel meat is healthier because the carcass contains less fat and has lower levels of cholesterol in the fat than other meat animals. Camel meat is also relatively high in polyunsaturated fatty acid in comparison to beef (1,2). This is an important factor in reducing the risk of cardiovascular disease, which is related to saturated fat consumption. Camel meat is also used for remedial purposes for diseases such as hyperacidity, hypertension, pneumonia, and respiratory disease as well as an aphrodisiac (3).

The oxidation of oxymyoglobin and lipids, as well as microbial contaminations, is a serious concern for meat producers and consumers. Therefore, the application of the proper agent(s) with both antioxidant and antimicrobial activities may be useful to maintain the meat quality, extend shelf-life, and prevent economic loss. Either synthetic or natural food additives can be used for this purpose. However, consumers are concerned about the safety of synthetic food additives. This concern has aroused a great interest in natural additives (4). Natural agents possessing antioxidant and antimicrobial properties have the advantage of being readily accepted by consumers, as they are considered natural.

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Garlic (*Allium sativum* L.) belongs to the family Liliaceae and is a common food spice used widely in many parts of the world. For many centuries, various species of the genus *Allium* have been used as vegetables and spices, and as folk medicines for curing various diseases. The antioxidant potential of garlic *in vivo* and *in vitro* has been proved (5). Garlic is rich in selenium and organosulfur compounds, which have pronounced antioxidant activity (6). In addition to its antioxidant activity, it has antibacterial, antiviral, antifungal, and antiprotozoal properties and beneficial effects on the cardiovascular and immune systems (7). The antimicrobial activity of garlic and garlic-derived organosulfur compounds was widely investigated against both food spoilage bacteria and food-borne pathogens (8,9). Garlic-rich organosulfur compounds and their precursors (allicin, diallyl sulfide, and diallyl trisulfide) are thought to play a key role in these biological effects (10,11).

To date, most studies have focused independently on either the antioxidant or antimicrobial activities of garlic in meat products. The objective of the present study was to investigate the antioxidant as well as the antimicrobial effectiveness of 3 garlic preparations, i.e. fresh, powder, and oil at various concentrations, in preserving ground camel meat during refrigerated storage.

Materials and methods

Materials

Fresh garlic (FG) bulbs (*Allium sativum*, var., Chinese white garlic) were purchased from a local market. The dry skins of the bulb were removed before use and then the cloves were peeled and crushed finely, using a kitchen hand held grater. Garlic powder (GP) was purchased from the Pars Foods Company (Ghazvin, Iran). According to the manufacturer data, 3 kg of produced GP is weight equivalent to 10 kg of FG.

Garlic oil (GO) was prepared in the laboratory by steam distillation. 1 kg of peeled garlic cloves were blended with 2 L of distilled water using an Ultra Turrax T-50 (Janke & Kunkel IKA-Labortechnik, Staufen, Germany). The garlic oil was extracted by heating the homogenate for 3 h, using a vertical steam distillation unit. The extract was dried over

anhydrous sodium sulfate and then filtered through a nitrocellulose acetate membrane (12).

The oily product was designated GO. At the end of the extraction process, a final yield of 3 g of GO was obtained from 1 kg of garlic cloves, indicating that 0.03 g of the GO is weight equivalent to 10 g of the FG. The resulting GO was kept in a tightly closed opaque bottle at 4 °C until use.

Butylated hydroxyanisole (BHA) was purchased from the Merck Company (Darmstadt, Germany) and used as a reference antioxidant. It was added to the ground camel meat at a concentration of 0.1 g/kg.

Sample preparation

Biceps femoris muscles were obtained from 3 Iranian dromedary 1-humped camel carcasses, 2 days postmortem. All animals were mature and male. The meat samples were ground through a plate with 1.27-cm diameter holes, and reground through a plate with 0.32-cm holes. The ground meat was divided into 11 batches, which were formulated to contain either FG (10, 20, or 40 g/kg), GP (3, 6, or 12 g/kg), GO (0.03, 0.06, or 0.12 g/kg), BHA (0.1 g/kg), or no additives (control). These corresponding levels of the 3 garlic forms are weight equivalent. The meat samples were thoroughly mixed by hand, reground through a 0.32-cm grinder plate, and divided into 100-g samples. Each sample was packaged in a sterile polyethylene bag, labeled, and stored at 4 ± 1 °C. The ground camel meat was sampled at 0, 2, 7, and 14 days of storage for microbiological and chemical analyses. Analyses were conducted in triplicate; all reagents were of analytical grade. This work was done in May 2010.

Compositional analysis and pH measurement

Before storage, the ground meat was analyzed for its moisture, protein, and fat content, according to the methods of AOAC International (13). For determination of the pH, 20 g of ground meat was blended with 20 mL of distilled water for 1 min using an Ultra Turrax T-25 (Janke & Kunkel IKA-Labortechnik, Staufen, Germany). The homogenate was filtered and the pH value of the filtrate was determined using a Metrohm pH-meter (Herisan, Switzerland) at 20 °C, standardized at pH 4 and 7.

Assessment of lipid oxidation

Measurement of TBA value

The 2-thiobarbituric acid (TBA) assay was carried out according to the extraction method described by Vyncke (14) with a few modifications: the meat sample (1.50 g) was homogenized (Ultra Turrax T-25, Janke & Kunkel IKA-Labortechnik, Staufen, Germany) with 6 mL of a 7.5% trichloroacetic acid (TCA) solution including 0.1% propyl gallate (PG), 0.1% ethylenediaminetetraacetic acid, and disodium salt (EDTA) for 45 s at 13,500 rpm, and the homogenate was filtered through a filter paper, 589.3. The extract (2 mL) was mixed with 0.02 M thiobarbituric acid (2 mL), heated, and cooled, as described by Vyncke (14). The absorbance was measured at 532 and 600 nm using a CARY 3 UV-visible spectrophotometer (Varian Australia Pty Ltd), and the absorbance difference, $A_{532 \text{ nm}} - A_{600 \text{ nm}}$, was calculated with $A_{600 \text{ nm}}$ correcting for sample turbidity. Thiobarbituric Acid Reactive Substances (TBARS), expressed as micromole of malonaldehyde per kilogram of meat, was calculated using TEP/malonic aldehyde as a standard.

Measurement of peroxide value

The IDF standard method was used to determine the peroxide values of all samples (15). The extracted lipid of sample (≤ 0.01 -0.3 g) was mixed in a disposable glass tube with 9.8 mL of chloroform-methanol (7 + 3, v/v) on a vortex mixer for 2-4 s. Ammonium thiocyanate solution (50 μL) was added, and the sample was mixed on a vortex mixer for 2-4 s. Then 50 μL iron(II) solution was added, and the sample was mixed on a vortex mixer for 2-4 s. After a 5 min incubation at room temperature, the absorbance of the sample was determined at 500 nm, against a blank that contained all the reagents except the sample, using a spectrophotometer (CARY 3 UV-visible, Varian Australia Ltd).

Aerobic plate count

Meat samples of 25 g were aseptically removed from the bags and homogenized in 225 mL of sterile buffered peptone water (1 g/L, Merck, Darmstadt, Germany) for 1 min, using a Stomacher 400 Lab Blender (Seward Medical, London, UK). From this

homogenate, decimal serial dilutions were made in the same sterile peptone water and used for microbiological analysis. Aerobic plate counts (APC) were determined by inoculating 0.1 mL of the sample homogenate, at selected dilutions, onto sterile plates of plate count agar (Merck, Darmstadt, Germany) using the surface spread technique; then the plates were incubated for 48 h at 35 °C. Results were expressed in log 10 number of colony forming unit/g (log CFU/g). The lower limit detection was less than 1 log CFU/g.

Transesterification and fatty acid composition

Methyl esters were prepared by transmethylation according to the ISO 5509 (16) method, using KOH 2 mol L⁻¹ in methanol and n-heptane medium. The fatty acid methyl esters (FAME) were analyzed using a Shimadzu 14A (Japan) gas chromatograph equipped with a flame ionization detector and Varian (USA) fused silica capillary column (100 m \times 0.25 mm and 0.20 μm of bis-cyanopropyl polysiloxane, CP-Sil 88). The column temperature was programmed from 140 to 225 °C, at 10 °C min⁻¹.

The injection port and detector were maintained at 220 and 230 °C, respectively. Hydrogen, at 1.2 mL min⁻¹, was used as a carrier gas and nitrogen, at 30 mL min⁻¹, was used as the make-up gas in split mode 1:100. The identification of normal fatty acids was carried out comparing sample FAME peak relative retention times with those obtained for Sigma (USA) standards. The peak areas were determined by the CG-300 computing integrater program (CG Instruments, Brazil). Data were calculated as normalized area percentages of fatty acids. The palmitic, stearic, linoleic, and arachidonic acid contents at day 0 decreased from their values at day 14 in each treatment and the percentages of their decrease were calculated.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) of the General Linear Models procedure of the Statistical Analysis System software (17). Duncan's multiple range test was used to determine if significant differences existed among treatments. $P < 0.05$ was considered as the level of significance.

Results and discussion

Composition and pH value

The moisture, protein, and fat contents (g/100 g) and pH in the ground camel meat were 73.80 ± 1.15 , 19.17 ± 1.85 , 5.10 ± 0.62 , and 5.91 ± 0.29 , respectively. Our results were in agreement with previous references (2,18).

Antioxidant activity

Figure 1 shows the effect of different concentrations of FG, GP, and GO on TBA values in the ground camel meat during storage at 4 ± 1 °C. The TBA value increased over the storage time ($P < 0.05$). The averaged initial TBA value was 0.34, and after 14 days of storage, it ranged from 1.04 to 1.44 in FG-formulation, 1.24 to 1.67 in GP-formulation, 1.71 to 1.83 in GO-formulation, and averaged 1.62 in BHA-formulated samples. These values were significantly lower than that of the control samples (2.05) ($P < 0.05$). A significant difference was also noted between the FG- and GO-formulated samples and the TBA value in the BHA-formulated samples was significantly higher than in the FG-formulated samples (20 and 40 g/kg) ($P < 0.05$). The TBA value is routinely used as an index of lipid oxidation in meat products in stores (19), and the rancid flavor

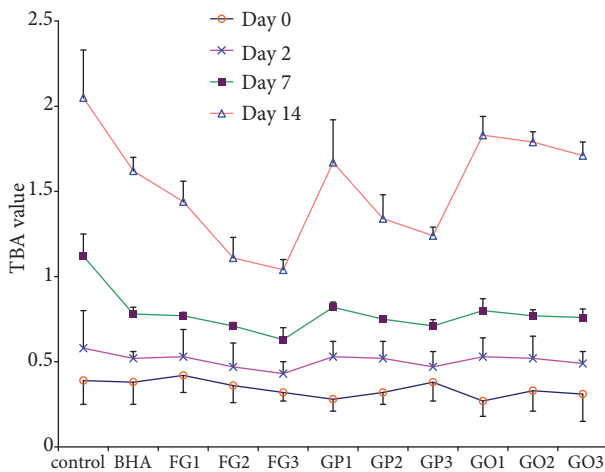


Figure 1. Effects of 3 equivalent concentrations of fresh garlic (FG1 10 g/kg, FG2 20 g/kg, and FG3 40 g/kg), garlic powder (GP1 3 g/kg, GP2 6 g/kg, and GP3 12 g/kg), and garlic oil (GO1 0.03 g/kg, GO2 0.06 g/kg, and GO3 0.12 g/kg), as well as butylated hydroxyanisole (BHA) (0.1 g/kg) on the 2-thiobarbituric acid (TBA) value in ground camel meat during 14 days' storage at 4 ± 1 °C. Values represent means \pm SD of 3 replicates.

is initially detected in meat products between TBA values of 0.5 and 2.0 (20).

Figure 2 shows changes in the peroxide value (POV) of garlic-formulated ground camel meat. The POV significantly increased between the 7th and 14th day ($P < 0.05$). The average initial POV was 0.046, however, after 14 days of storage, it ranged from 0.39 to 0.68 in the FG-formulated samples, 0.51 to 0.74 in the GP-formulations, and 0.88 to 0.92 in the GO-formulations, while it was 0.83 in the BHA-formulated samples. These values were significantly lower than that of the control (1.49) ($P < 0.05$).

Moreover, a significantly lower POV was noted in FG- and GP-formulated samples in comparison with GO formulations. The POV of the FG- (20 and 40 g/kg) and GP-formulated (6 and 12 g/kg) samples were also significantly lower than that of the BHA-formulated samples ($P < 0.05$).

It is difficult to provide a specific guideline relating peroxide value to rancidity. High peroxide values are a definite indication of a rancid fat, but moderate values may be the result of depletion of peroxides after reaching high concentrations. Low peroxide values may also be obtained for any extremely rancid products, because the peroxides initially formed have all undergone further oxidation reactions (21).

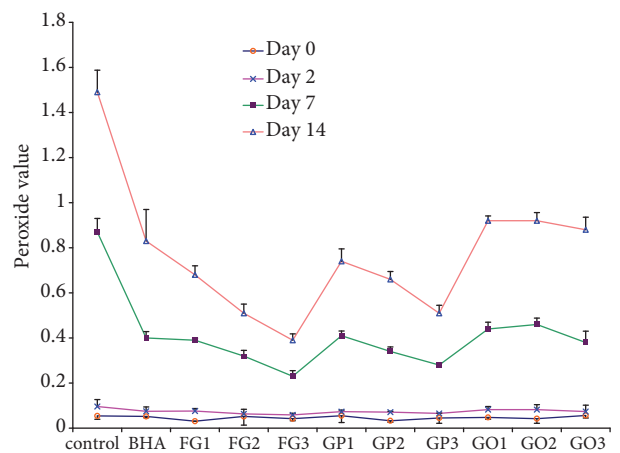


Figure 2. Effects of 3 equivalent concentrations of fresh garlic (FG1 10 g/kg, FG2 20 g/kg, and FG3 40 g/kg), garlic powder (GP1 3 g/kg, GP2 6 g/kg, and GP3 12 g/kg), and garlic oil (GO1 0.03 g/kg, GO2 0.06 g/kg, and GO3 0.12 g/kg), as well as butylated hydroxyanisole (BHA) (0.1 g/kg) on the peroxide value (POV) in ground camel meat during 14 days' storage at 4 ± 1 °C. Values represent means \pm SD of 3 replicates.

Lipid oxidation represented by TBA and POV (Figures 1 and 2) was reduced with higher concentrations of each of the 3 forms of garlic ($P < 0.05$). This result was in accordance with that of Yang et al. (22), who noted that the antioxidant activity of several compounds of garlic and garlic extracts was concentration dependent. The investigated antioxidant activity of the 4 materials added followed the order FG, GP, BHA, and GO. The low antioxidant activity of GO in comparison with that of FG or GP could be attributed to the losses of volatile sulfur compounds during steam distillation. Some authors reported that garlic oil or steam-distilled garlic did not contain a large amount of allicin, but did contain various products of allicin transformation, none of which appears to have much biological activity as either fresh garlic or garlic powder (23,24).

Aerobic plate count

As may be expected, the increase in storage time produced significant proliferations in APC, whatever the treatment conditions. The average initial aerobic plate count in the ground meat camel was $5.68 \log_{10}$ CFU/g (Figure 3), and during the first 7 days of storage, the APC in all of the meat formulations (except the control and BHA-formulated samples) remained below $7 \log_{10}$ CFU/g, which is the maximal permissible limit (MPL) for APC recommended

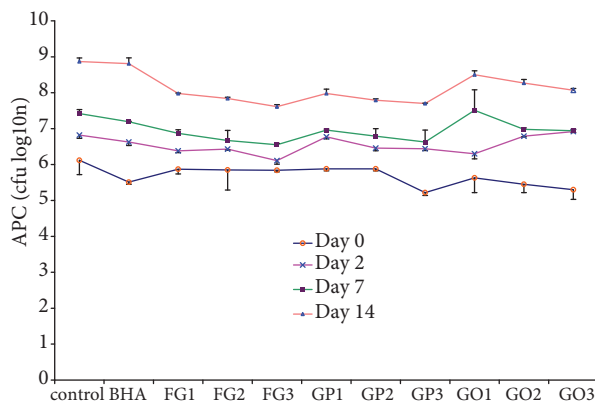


Figure 3. Effects of 3 equivalent concentrations of fresh garlic (FG1 10 g/kg, FG2 20 g/kg, and FG3 40 g/kg), garlic powder (GP1 3 g/kg, GP2 6 g/kg, and GP3 12 g/kg), and garlic oil (GO1 0.03 g/kg, GO2 0.06 g/kg, and GO3 0.12 g/kg), as well as butylated hydroxyanisole (BHA) (0.1 g/kg) on the aerobic plate count (APC) in ground camel meat during 14 days' storage at 4 ± 1 °C. Values represent means \pm SD of 3 replicates.

by the ICMSF (25). On the 14th day of storage, all samples exceeded the limit of $7 \log_{10}$ CFU/g.

After 14 days' storage, the APC of both FG- and GP-formulated meat were significantly lower than that of either the control or BHA-formulated samples. Moreover, FG-formulated samples showed significantly lower APC than those of the GO formulations ($P < 0.05$). However, the addition of garlic oil or BHA resulted in no significant difference in APC when compared with the control samples (Figure 3) ($P > 0.05$). The weak antimicrobial activity of the GO may be due to the losses of sulfur compounds as mentioned above, and also due to the nature of GO itself, which is volatile and hydrophobic. The order of antimicrobial activity of the different materials added was FG, GP, GO, and BHA. This observation supported the findings of Murray (26), who claimed that only fresh garlic preparations provide the full range of beneficial compounds. However, as an antimicrobial, we noted that GP showed activity close to FG. To date, although the antimicrobial activities of garlic and garlic-derived organosulfur compounds were widely reported in culture media, few reports are available on its effect in meat products (27,28).

Fatty acid changes

The saturated fatty acids in camel meat account for 51.5% of the total fatty acids, which is higher than the saturated fatty acids in the meat of true ruminants (like as sheep and cow). The major saturated fatty acid in camel meat is palmitic (26.0%). In the camel meat, 10 polyunsaturated fatty acids were diagnosed, and linoleic and arachidonic acids are the most important of them. In camel meat, the polyunsaturated to saturated fatty acid ratio is 0.36. This ratio in cow, sheep, and goat is 0.22, 0.26, and 0.36, respectively (29). The fatty acids in meat oxidize and degrade during storage. Decreasing percentages of palmitic, stearic, linoleic, and arachidonic acid during the 14 storage days are shown in the Table. Palmitic, stearic, and arachidonic acid values in garlic derivatives or BHA-formulated meat were significantly lower than that of the control. FG-, GP-, and BHA-formulated meat showed lower palmitic and arachidonic acids values in comparison with GO-formulated meat (except for GO 0.12 g/kg) ($P < 0.05$). The BHA or garlic derivatives' formulation had no significant effect on linoleic acid values ($P > 0.05$).

Table. Decreased percentages of palmitic, stearic, linoleic, and arachidonic acid in different treatments of ground camel meat during 14 days' storage at 4 ± 1 °C.

Group	Control	BHA (0.1 g/kg)		FG (10 g/kg)		FG (20 g/kg)		FG (40 g/kg)		GP (3 g/kg)		GP (6 g/kg)		GP (12 g/kg)		GO (0.03 g/kg)		GO (0.06 g/kg)		GO (0.12 g/kg)		
Fatty acids																						
Palmitic	17.83 ± 2.33a	4.31 ± 2.32de	5.64 ± 0.41de	2.77 ± 0.49e	2.36 ± 0.42e	8.11 ± 0.07cd	5.08 ± 0.02de	2.90 ± 1.50e	14.30 ± 3.40 ab	11.96 ± 2.82bc	7.60 ± 0.78d											
Stearic	20.21 ± 7.60a	3.61 ± 1.31b	3.13 ± 0.72b	1.35 ± 0.70b	0.59 ± 0.04b	6.22 ± 1.48b	2.61 ± 2.40b	0.75 ± 0.01b	8.20 ± 3.30b	3.51 ± 0.43b	1.70 ± 1.40b											
Linoleic	19.91 ± 8.49	13.24 ± 8.25	16.70 ± 8.25	10.44 ± 4.12	6.53 ± 4.58	16.17 ± 4.18	8.94 ± 10.48	6.58 ± 0.97	14.94 ± 0.23	11.60 ± 14.30	10.88 ± 9.72											
Arachidonic	82.42 ± 4.00a	17.47 ± 3.14cde	14.83 ± 3.13de	17.42 ± 2.78cde	3.30 ± 1.63e	39.57 ± 9.36bc	31.87 ± 0.75bcd	18.41 ± 4.18cde	54.31 ± 3.15b	47.38 ± 5.29b	38.57 ± 9.07bc											

Means ± SD in the same row followed by different small letter superscripts (a-e) are significantly different (P < 0.05).

Conclusion

This study concluded that fresh garlic, garlic powder, and garlic oil provide antioxidant and antimicrobial benefits to ground camel meat during cold storage (4 ± 1 °C) and the effects are concentration dependent. Among the garlic forms studied, fresh garlic at a concentration of 40 g/kg of meat demonstrated the most potent effect, but such a high concentration may not be acceptable by many people because of its strong flavor.

However, the addition of fresh garlic at 20 g/kg or garlic powder at 6 g/kg did not result in a strong flavor and, at the same time, produced significant antioxidant and antimicrobial effects and extended the shelf-life of the products. Therefore, it is suggested that garlic, as a natural herb, could be used to extend the shelf-life of meat products, providing the consumer with food containing natural additives, which might be seen as more healthy than those of synthetic origin.

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