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Effect of curing on camel meat lipid oxidation and enzymatic activity during refrigerated storage

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ABSTRACT

Fat oxidation is a major factor in decreasing food quality, especially in meat products. Our object was to study mixed curing effects on catalase and glutathione peroxidase (GSH-Px) activity and lipid oxidation indices in camel meat during refrigerated storage. Samples were selected from the *Longissimus dorsi* of 5 adult male camel after slaughter. After adding the curing salts, samples were stored at 4 °C for 0, 4, 8 and 12 days. Results showed no significant difference in catalase activity and peroxide value between the control and cured groups. However, during the storage time there was a decrease in GSH-Px activity and this decrease was greater in the control group. The content of vitamin E was higher in the cured samples than in the control group. The content of thiobarbituric acid (TBA) in the cured samples was lower than in the control group. In conclusion, curing of camel meat can decrease lipid oxidation rancidity and maintains more meat nutrients, such as vitamin E.

Key words: lipid oxidation, vitamin E, antioxidant enzymes, curing, camel meat

Introduction

Lipid peroxidation is one of the primary mechanisms of quality deterioration in stored foods. The changes in quality can be manifested by deterioration in flavor, color, texture, and nutritive value, and the production of toxic compounds (SCOLLAN et al., 2006; MOHAMED et al., 2008). Endogenous antioxidants in the muscle can control the oxidation in several ways (CHAN and DECKER, 1994). These include fat soluble α- tocopherol and Coenzyme Q_{10}, along with water soluble ascorbic acid and histidine-containing dipeptides. Superoxide dismutase, catalase and glutathione peroxidase (GSH-Px) are antioxidative enzymes which also contribute to oxidative defense (FRANKEL, 1998). GSH-Px is a selenium-containing enzyme, catalyzing the reduction of lipid and hydrogen peroxides to less harmful alcohols and water. The mammalian GSH-Px family consists of at least four

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selenoproteins: cellular, extracellular, phospholipids hydroperoxide, and gastrointestinal GSH-Px (ARTHUR, 2000). Catalase is a heme-containing enzyme that catalyzes the decomposition of H_2O_2 to give H_2O and O_2 (AEBI, 1983; CLAIBORNE, 1985). Removal of H_2O_2 by catalase inhibited oxymyoglobin oxidation in oxymyoglobin-liposome systems (CHAN et al., 1997), prevented the formation of H_2O_2-activated metmyoglobin, which is regarded as a major factor in lipid oxidation in stored meat (RHEE, 1988). Endogenous antioxidant enzymes, especially catalase and GSH-Px, could potentially delay the onset of oxidative rancidity in stored meat. However, information on factors influencing the activity of such enzymes in meat products is limited. Some commonly used non meat additives could compromise their antioxidative potential. Salt (NaCl) is one such additive.

The role of the camel as a meat producer is becoming more important, due to the versatile role it plays, than being a symbol of social prestige, which was the role it used to play but this has since greatly diminished (DAWOOD and ALKANHAL, 1995). The common opinion regarding camel meat as tough, coarse, watery and sweetish in taste, compared to meats from other animals, may be partly attributed to the fact that camel meat is usually a by-product of primitive traditional systems of production, where it is mainly obtained from old males and females that have become less effective in their primary roles of providing transportation, milk, or as breeding females (MORTON, 1984; WILSON, 1998). However, limited evidence suggests that the quality characteristics of camel meat are not greatly different from beef, if the animals are slaughtered at comparable ages (TANDON et al., 1988). However, camels are generally raised in less developed countries and research for improving their reproductive and productive characteristics has been limited (SKIDMORE, 2005).

We found that camel meat has good potential for producing cured meat and mixed methods of curing resulted in more tender and higher quality meat than other methods (GHEISARI and DANESH, 2012). The objective of the present study was to determine mixed curing effects on catalase and GSH-Px activity, TBA content, peroxide value and vitamin E level in camel meat during refrigerated storage.

Materials and methods

Animal and muscle samples. Camel muscles were obtained from local slaughterhouses. Five one-humped Iranian breed camels, all male adults (6 years old), were used for this study. Carcasses were allowed to chill for 24 hours at 3 ºC for completion of rigor mortis. Samples from the *Longissimus dorsi* muscles were removed and external fat and epimysial connective tissues separated.

Meat curing. Each muscle was divided into the two sections and one section was used for curing and the other considered as control. In the mixed method, at the first curing salts [NaCl (24 g), sucrose (3.6 g), sodium nitrite (0.12 g), spices (1.2 g), and ascorbic

acid (0.018 g) for 1 kg meat] was rubbed on the meat surface, and the meat was then suspended in the solution containing a 6% concentration of the curing salts. The samples were stored at 4 °C for 0, 4, 8 or 12 days. Upon removal of the samples after each storage time, they were vacuum packed and frozen at -70 °C until analyzed. All treatments were done in triplicate.

Gross composition, pH and nitrite residue. Moisture, protein (N × 6.25), fat and ash were determined according to the AOCS methods (2010).

20 g of ground meat was blended with 20 mL distilled water for 1 min using an Ultra Turrax T-25 (Janke & Kunkel IKA-Labortechnik, Staufen, Germany). A CG822 pH meter was used to determine the pH at 20 °C.

The nitrite levels in the cured meat samples were determined by colorimetric method according to AOAC (2010).

Assays of antioxidant enzymes. A 5-g muscle sample was homogenized in 25 mL phosphate buffer (0.05 M, pH = 7) and centrifuged at 4 °C for 2 min at 7000×g. The supernatant fraction was filtered through four layers of cheesecloth and used to determine catalase and GSH-Px activities. Catalase activity assay was performed as described by AEBI (1983) and MEI et al. (1994). The supernate (0.1 mL) was reacted at room temperature (~22 °C) with 2.9 mL of 11 mM H_2O_2 in phosphate buffer, and the reaction (H_2O_2 loss) was monitored by measuring the absorbance at 240 nm during the initial 30 s. One unit (U) of catalase was defined as the amount of extract needed to decompose 1 mmol of H_2O_2 per min.

GSH-Px activity was determined by measuring the oxidation of NADPH at 22 °C (DEVORE and GREENE, 1982; GUNZLER and FLOHE, 1985). The assay medium (3 mL) consisted of 1 mM reduced glutathione, 0.15 mM NADPH, 0.15 mM H_2O_2, 40 mM potassium phosphate buffer (pH = 7), 0.5 mM EDTA, 1 mM NaN_3, 1.5 units of glutathione reductase, and 300 μL of muscle extract. Absorbance at 340 nm was recorded over 3 min. An extinction coefficient of 6300 M^{-1} cm^{-1} was used for calculation of NADPH concentrations. One unit of GSH-Px was defined as the amount of extract required to oxidize 1 mmol of NADPH per min at 22 °C.

Determination of lipid oxidation. 1. TBARS determination. TBARS was measured by the extraction method described by VYNCKE (1975) 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% propylgallate (PG) and 0.1% ethylenediaminetetraacetic acid, disodium salt (EDTA) for 45 sec 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 (1975). The absorbance was measured

at 532 and 600 nm using a CARY 3 UV-visible spectrophotometer (Varian Australia Pty Ltd), and the absorbance difference, $A_{532\ nm} - A_{600\ nm}$, was calculated with $A_{600\ nm}$ correcting for sample turbidity. TBARS, expressed as micromole of malonaldehyde per kilogram of meat, was calculated using TEP/malonic aldehyde as standard.

2. *Extraction of lipids.* Meat (10 g) was homogenized by an Ultra Turrax (Ultra Turrax T-25, Janke & Kunkel IKA-Labortechnik, Staufen, Germany) with 100 mL chloroform/methanol (2:1 v/v) for 1 min at 13,500 rpm. After homogenization, 25 mL 1.0 mM $CaCl_2$ solution was added and the sample was further homogenized using an Ultra Turrax and centrifuged (Harrier,15/80, UK.) for 20 min at 1000 rpm. The chloroform phase was removed and the extraction procedure repeated. The chloroform phase containing the extracted lipids was dried by vacuum evaporation (Büchi RE 11, Büchi Laboratoriums-Technik AG, Flawill, Schweiz). Finally 2×2 mL chloroform/methanol and 2.0 mL $CaCl_2$ were added to the dried sample and the sample was mixed (Vortex-mixer VF2, Janke & Kunkel IKA-Labortechnik) and centrifuged for 20 min at 2500 rpm. The lipid phase was removed, dried by vacuum evaporation and weighed.

3. Determination of peroxide value. The IDF standard method was used to determine the peroxide values of all samples (SHANTHA and DECKER, 1994). The extracted lipid of sample(≤0.01-0.3 g) was mixed in a disposable glass tube with 9.8 mL choloform-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 in a vortex mixer for 2-4 s. After 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, by using a spectrophotometer(CARY 3 UV-visible, Varian Australia Ltd).

Determination of vitamin E. 1. *Instrumentation.* Chromatography was performed using Waters Associates chromatographic equipment (Milford, MA, USA) consisting of a 501 pump, a model 717 plus auto sampler, a model 470 scanning fluorescence detector, a pump control module, a SAT/IN module, and the program Millenium32 (version 3.05.01). A Lichrosphere Si 100 silica column (5 µm, 250mm × 4.6 mm) was used for all separations.

2. *Mobile phase.* Mobile phase consisted of 4% 1.4-dioxane, 0.04% acetic acid and 0.02 mg/L α-tocopherol in hexane. It was filtered through a PTFE 0.45 µm filter (Millipore C, Bedford, MA) and degassed before each HPLC session.

3. *Standard solutions.* Stock standard solutions of α-tocopherol (2000 mg/L), α-tocopherol acetate (2000 mg/L), γ-tocopherol (1000 mg/L), and each of the tocotrienols (2000 mg/L) were prepared in hexane. A combination standard containing α-tocopherol (10 mg/L), α-tocopherol acetate (75 mg/L), γ-tocopherol (5 mg/L), α-tocotrienol (2 mg/L), β-tocotrienol (2 mg/L), γ-tocotrienol (5 mg/L), and δ-tocotrienol (2 mg/L) was prepared

by diluting appropriate volumes of stock solutions with hexane containing 200 mg/L BHT, and used for quantification of the muscle samples. The internal standard solution added to each muscle sample was prepared by diluting α-tocopherol acetate stock solution with hexane to 200 mg/L. All the above solutions were frozen at −20 °C. The combination standard was frozen as small aliquots, and thawed daily as required.

4. *Sample preparation.* A 25 g of each meat sample was minced, and a representative sample of 1 g was placed in a 50 mL polyethylene tube. A 300 μL aliquot of 200 mg/L α-tocopherol acetate solution (internal standard), and 4 mL of absolute ethanol were added. The mixture was homogenized for 30 s in an UltraTurax T25 (Janke et Hunkel IKA-Labortechnik, Kuala Lumpur, Malaysia) homogenizer, with a 18G dispersing element. Five milliliters of distilled water were added to the tube and the content homogenized for 15 s. A 4 mL aliquot of hexane containing 200 mg/L BHT was pipetted and the sample homogenized for a further 15 s. The tubes were capped and centrifuged at 1500 rpm for 10 min. A 100 μL injection volume of the upper (hexane) layer was used for HPLC analysis.

5. *Chromatographic conditions.* Chromatography was performed at ambient temperature. The flow rate used was 1 mL/min. The column was equilibrated for 15 min with the mobile phase prior to injection of each sample or standard. All samples were detected using fluorescence at 295 nm excitation and 330 nm emission.

6. *Qualitative and quantitative analysis.* Qualitative analysis was performed by spiking the sample with the appropriate standard to observe the growth of the peak, and by comparing the order of elution with that reported in other studies.

Quantitative analysis was performed by injecting a range of volumes of the combination standard (in order to cover the expected concentrations of analytes in the extracts) with each batch of samples analyzed on the HPLC. The concentrations of the analytes in the samples were determined using a 4-level calibration curve constructed from the standard data. Quantification was performed by the internal standard method to compensate for the sample losses during extraction and chromatographic analysis.

Statistical analysis. Data of different traits in the control and cured meat groups after different storage times were summarized. Their means were compared to each other using the t-test, one way ANOVA, repeated measure ANOVA and Duncan's multiple range tests (SAS software, version 9.1). $P<0.05$ was considered as the level of significance.

Results

Moisture, protein, fat, ash percents and pH content of camel meat samples were 74.27 ± 2.73, 24.27 ± 0.50, 4.20 ± 0.60, 1.26 ± 0.25 and 6.49 ± 0.16, respectively. Changes in antioxidant enzyme activities during refrigerated storage of each meat group are shown in Tables 1 and 2. Catalase activity was quite stable during refrigerated storage. However,

in the control group GSH-Px activity decreased significantly over the storage days. Tables 3 and 4 show TBARS and peroxide values over storage days. These lipid oxidation indices increased significantly during refrigerated storage. Vitamin E values decreased significantly over storage time as shown in Table 5. Results showed no significant difference in catalase and GSH-Px activities and peroxide value between the control and cured groups. Cured meat samples have more vitamin E and less TBA content than the control group. The values of sodium nitrite in cured meat samples at day 4 and 12 were 118 ± 13.85 and 95 ± 8.37 ppm, respectively.

Table 1. Comparison of catalase activity (U/g) between control and cured groups at different storage times

Day	0	4	8	12
Control group	550 ± 16.6	601 ± 461	546.6 ± 293.3	527.7 ± 127.7
Curing group	570.6 ± 168.5	653.9 ± 167.3	502.4 ± 98.4	522.7 ± 191.9

Table 2. Comparison of glutathione peroxidase activity (U/g) between control and cured groups at different storage times

Day	0	4	8	12
Control group	2.09 ± 0.08 ^{a}	1.92 ± 0.10 ^{ab}	1.80 ± 0.07 ^{b}	1.58 ± 0.10 ^{c}
Curing group	1.97 ± 0.21	1.90 ± 0.27	1.81 ± 0.23	1.73 ± 0.21

Means in the same row which are not followed by a common letter differ significantly (P<0.05).

Table 3. Comparison of TBA value (μ mol/kg) between control and cured groups at different storage times

Day	0	4	8	12
Control group	0.03 ± 0.02 ^{a}	0.23 ± 0.05 ^{b}	0.49 ± 0.36 ^{bc}	0.71 ± 0.21 *^{c}
Curing group	0.03 ± 0.01 ^{a}	0.16 ± 0.07 ^{b}	0.22 ± 0.10 ^{b}	0.32 ± 0.13 ^{b}

Means in the same row which are not followed by a common letter differ significantly (P<0.05). * indicates significant difference (P<0.05) between the same column

Table 4. Comparison of peroxide value (meq o_2/kg) between control and cured groups at different storage times

Day	0	4	8	12
Control group	0.05 ± 0.01 ^{a}	0.15 ± 0.04 ^{b}	0.26 ± 0.08 ^{bc}	0.39 ± 0.11 ^{c}
Curing group	0.06 ± 0.02 ^{a}	0.17 ± 0.06 ^{b}	0.28 ± 0.10 ^{bc}	0.36 ± 0.13 ^{c}

Means in the same row which are not followed by a common letter differ significantly (P<0.05).

Table 5. Comparison of vitamin E (mg/kg) between control and cured groups at different storage times

Day	0	4	8	12
Control group	6.16 ± 2.11 [a]	4.70 ± 0.75 [a]	2.37 ± 0.85 *[b]	1.57 ± 0.12 *[b]
Curing group	6.67 ± 1.30 [a]	5.24 ± 1.43 [ab]	4.13 ± 0.65 [b]	2.34 ± 0.47 [c]

Means in the same row which are not followed by a common letter differ significantly (P<0.05). * indicates significant difference (P<0.05) between the same column

Discussion

Recently, more attention has been paid to the nutritional value of camel meat, with the aim of creating additional value for various camel meat products (ULMER et al., 2004). Thermal processing, curing and smoking are the three most common methods used for camel meat preservation and processing (KALALOU et al., 2004; ZEGEYE, 1999). Generally, consumers are prejudiced against fresh unprocessed camel meat. If camel meat could be converted into processed meat products, it might be more acceptable to domestic consumers.

One of the oldest methods for conserving meat is, without doubt, curing. Today, curing has become more a method of diversification and adaptation to the habits of the consumer more than a method of preservation. Traditional cured meat products, made from whole meat pieces of pork or beef, are abundantly elaborated and consumed in different countries throughout the world.

Little has been known about how antioxidant enzymes may be affected by different types of salt and their ionic strength. LEE et al. (1997) reported no inactivation of catalase, GSH-Px and superoxide dismutase by NaCl in ground pork muscle during frozen storage. However, they observed a decrease in the activity of these enzymes when NaCl was added to the muscle extract used in the enzyme assays; hence, they suggested that the enzymes could be inhibited in salted pork. SARRAGA et al. (2002) studied the effect of different NaCl percentages used in the manufacturing processes of dry-cured loins on the GSH-Px activity and the TBARS values. The interaction of salt concentration and meat quality was not statistically significant. NaCl percentages have a significant effect on both GSH-Px activity and TBARS (P<0.05). Samples with 2% of NaCl presented higher enzyme activity than samples manufactured with 3% of salt. GHEISARI and MOTAMEDI (2010) indicated that ionic strength elevation can decrease GSH-Px activity in chicken meat. In the present study curing had no effect on antioxidative enzyme activities.

A few previous studies have indicated the stability of catalase in refrigerated chicken, beef, pork and camel muscles (RENERRE et al., 1996; PRADHAN et al., 2000; GHEISARI and MOTAMEDI, 2010). The stability of catalase in refrigerated turkey varied with the type of

muscle (RENERRE et al., 1999). Our study confirmed the catalase stability in refrigerated raw and cured camel meat. The stability of catalase during frozen storage has also been reported in pork, beef and chicken muscles (LEE et al., 1997; PRADHAN et al., 2000).

Our results, showing a GSH-Px activity decrease in control group during refrigerated storage, differ from those in fish (WATANABEE et al., 1996), several beef muscles (RENERRE et al., 1996), and beef Psoas major and Longissimus dorsi and pork Longissimus dorsi muscles (DAUN et al., 2001), which indicated the stability of GSH-Px. However, our results were in agreement with those in turkey, chicken breast, beef and camel Longissimus dorsi muscles (RENERRE et al., 1999; GHEISARI and MOTAMEDI, 2010).

Cured meat products are based on the addition of salt, which has some undesirable side effects at moderate concentrations (0.5-2.5%) normally used in meat products. It promotes lipid oxidation in raw and cooked meat, and accelerates MetMb formation and discoloration in raw meat (RHEE, 1988). According to LEE et al. (1997) an increase in muscle salt concentration decreased antioxidant enzyme activities and increased TBA values. These authors proposed that the observed decrease in oxidative stability could be attributed to the reduced catalytic activity of the antioxidant enzymes. The observations by GHEISARI and MOTAMEDI (2010) differ in that higher levels of GSH-Px activity were accompanied by higher levels of TBA values, suggesting that the muscle-stabilizing action of the curing process leads to a decrease in the need for the antioxidant enzyme action.

Our results show TBARS and peroxide values that increase with storage time, which are consistent with those of previous studies (RHEE et al., 1983, 1996; GHEISARI and MOTAMEDI, 2010; GHEISARI et al., 2010). However, the current study has additionally shown the decreasing effect of curing on TBARS values. SARRAGA and GARCIA REGUEIRO (1998) showed that the main oxidation of membrane lipids, taking place during the curing process, would occur during the first steps of the manufacturing processes, when temperatures are between 3 and 5 °C; and, furthermore, samples containing in vitro 3 and 5% NaCl showed lower TBARS values than samples with 0 and 1% of salt.

KURT and ZORBA (2010) showed that peroxide values decrease significantly with increasing nitrite levels in a dry fermented Turkish sausage. The antioxidant effect of nitrite is likely due to the same mechanisms responsible for cured color development, involving reactions with heme proteins and metal ions, chelating of free radicals by nitric oxide, and the formation of nitriso- and nitrosyl compounds with antioxidant properties (SEBRANEK, 2009). MORRISSEY and TICHIVANGANA (1985), in experiments with pork, chicken and mackerel meat, reported that the antioxidative effect of nitrite was apparent even at 20 ppm and also that nitrite and nitrosylmyoglobin behaved synergistically toward

the inhibition of lipid oxidation. In studying the feasibility of replacing nitrite with sorbate in mortadella, AL-SHUIBI and AL-ABDULLAH (2002) also showed that the antioxidant effect of nitrite is present at low levels (40 ppm). Also the antioxidant activity of spices has been investigated and confirmed by many researchers (SEBRANEK and BACUS, 2007; HONIKEL, 2008; ZAHARAN and KASSEM, 2011). Therefore, the lower lipid oxidation in the cured meat samples in this study was in agreement with these facts.

Vitamin E is a major lipid-soluble and chain- breaking antioxidant that protects the integrity of membranes, by inhibiting lipid peroxidation in the body (SEN et al., 2004). Free radicals are neutralized by α-tocopherol before lipid oxidation propagates among highly unsaturated fatty acids in cellular and sub-cellular membranes. This delay in the production of lipid oxidation breakdown products (e.g. peroxides) may indirectly prolong the life of OxyMb (LYNCH et al., 1999).

Many studies have shown that the rate and extent of lipid oxidation are dependent on the vitamin E concentration in the tissues. GHEISARI et al. (2010) showed that increasing salt level led to decreasing vitamin E content, both in chicken meat and beef. Curing had a preservative effect in the present study. This is connected to the addition of antioxidant agents to cured meat and its lower lipid oxidation.

The sodium nitrite residues of cured camel meat samples were lower than the Iran standard value (143 ppm). Thus, the products are safe from the point of view of nitrite residue for consumers.

Taken together, camel is a useful potential source of meat, particularly in the arid tropics. If it is cured, it ise less susceptible to lipid oxidation. More research work in the areas of meat production, technology, marketing, and social awareness is needed to exploit the potential of camels as a source of meat and related products.

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SAŽETAK

Oksidacija masti glavni je čimbenik smanjenja kvalitete hrane, osobito u mesnim proizvodima. Cilj je ovog rada bio istražiti učinke salamurenja na aktivnost katalaze i glutation peroksidaze i pokazatelje oksidacije lipida u mesu deva za vrijeme pohrane u hladnjaku. Uzorci su bili uzeti od najdužeg leđnog mišića pet odraslih zaklanih mužjaka. Nakon salamurenja uzorci su bili pohranjeni pri 4 °C tijekom 0, 4, 8 i 12 dana. Nije ustanovljena značajna razlika u aktivnosti katalaze i peroksidaze između kontrolne skupine i skupine koja je bila salamurena. Ipak, za vrijeme pohrane došlo je do smanjene aktivnosti glutation peroksidaze, a to je smanjenje bilo veće u kontrolne skupine. Sadržaj vitamina E bio je veći u uzorcima salamurene nego kontrolne skupine. Sadržaj tiobarbiturne kiseline u salamurenim uzorcima bio je manji nego u kontrolnima. Zaključno se može reći da salamurenje devina mesa može smanjiti užeglost zbog oksidacije lipida i sačuvati više hranjivih tvari u mesu, primjerice vitamina E.

Ključne riječi: oksidacija lipida, vitamin E, antioksidacijski enzimi, salamurenje, meso deva