



ANTIOXIDANT EFFECT OF NIGELLA OIL ON HEATED CAMEL AND RABBIT MEAT

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Abstract

The exposure of meat to heating (cooking) or cold storage could alter its chemical composition. In this work, the oxidizing effect of heating on rabbit and camel meat, and the protective effect of nigella oil (NO) were evaluated and compared. Samples of rabbit and camel meat were treated with increasing doses of NO, and then were heated until reaching an internal temperature of 80 °C. Water loss, thiobarbituric acid reactive substances (TBARS) and carbonyls levels, as well as activities of catalase (CAT), glutathione peroxidase (GSHPx) and superoxide dismutase (SOD), were analyzed at different meat storage times. The results showed that heating of camel and rabbit meat induced a significant increase in the water loss, and TBARS and carbonyls levels, associated with a significant decrease in the activities of CAT, GSHPx and SOD. In both species, TBARS and carbonyls in the meat samples treated with NO before heating were significantly ($P < 0.05$) lower than those in the untreated samples, and decreased more and more when the NO dose increased. In the samples treated with NO, all enzymatic activities were significantly ($P < 0.05$) higher than those observed in the untreated samples, and increased more and more with increasing NO dose. The findings reveal a potential antioxidant power of NO preserving the organoleptic composition of heated camel and rabbit meat.

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Introduction

Meats are an excellent source of nutritional and bioactive compounds for the consumer [1]. While health benefits are the main factor influencing consumer demand for all meat products available on the market, dromedary [2,3] and rabbit [4] meat have excellent nutritional and dietary features, characterized by high protein and essential amino acid content, low fat and cholesterol content, and high B vitamin content. It is clear that meat processing by cooking and cold storage sometimes leads to a deterioration of its nutritional quality. Therefore, scientists are increasingly interested in the evaluation of nutritional losses caused by meat product processing methods. It is mainly lipids and proteins that are affected by both cold storage [5] and heat treatment [6,7], which could compromise health properties due to newly formed oxidation compounds. In combination with oxygen, free radicals generated during oxidative stress (OS) generally trigger oxidative chain reactions, which mainly target lipids, pigments, proteins, and vitamins of meat and meat products, altering their organoleptic composition and quality parameters such as color, flavor, tenderness, and water-holding capacity [8]. In addition, the antioxidant stability of meat is impaired due to the reduced activity of endogenous enzymes such as cata-

lase (CAT), glutathione peroxidase (GSHPx) and superoxide dismutase (SOD), which play an important role in the elimination of reactive oxygen species, causing lipid and protein degradation. Most of the processes affecting the activity of these enzymes and organoleptic composition occur through heat and/or low-temperature storage of camel [9,10] and rabbit meat [4]. Therefore, researchers are moving towards the use of natural antioxidants to prevent meat spoilage due to OS during heat treatment and cold storage [11,12]. These antioxidants are able to minimize lipid oxidation [13] and meat rancidity, thus preserving its organoleptic and nutritional quality, increasing its acceptability, and reducing the development of unpleasant odors in meat products [14,15]. Natural antioxidants of plant origin, such as fruits, vegetables, herbs, spices and seeds, scavenge free radicals and inhibit oxidative reactions, including lipid and protein oxidation in meat and meat products, which allows the preservation of their quality, extension of their shelf life, improvement of their stability and maintenance of their organoleptic characteristics [16,17]. As an aromatic plant with multiple biological activities, one could cite the example of nigella (*Nigella sativa* L.) (NS) or black cumin, which is an annual herbaceous plant belonging to the *Ranunculaceae* family. The seeds of this plant have

extraordinary and promising curative properties allowing a wide spectrum of medicinal applications, such as antioxidant, immunostimulant and antitumor [14,18]. NS seeds contain phytochemicals such as thymoquinone, α -pinene, carvacrol, p-cymene, β -pinene, α -thujene and longifolene. These compounds have biological properties that make them exploitable by researchers for their application in food and drug manufacturing [18]. The objective of our investigation was to evaluate and compare the oxidizing effect of heating on meat of rabbits and camels, and the protective effect of nigella seeds oil against this thermal stress, by analysis of water loss upon heating, thiobarbituric acid reactive substances (TBARS) and carbonyl contents, and enzymatic activities of CAT, GSHPx and SOD.

Objects and methods

Study site and animals

The study was carried out on five healthy male camels (*Camelus dromedarius*) from the municipal slaughterhouse of Casablanca, Morocco (2 to 3 years, 170 to 260 kg) and five male *New Zealand* rabbit (1.5 to 2 kg) carcasses purchased from a market located in the same city. Casablanca is located in the west of Morocco (North Africa, latitude 33° 34' 42" N, longitude 7° 36' 24" W). The animals lived in similar conditions, were exposed to the same conditions before slaughter and were fed, respectively, barley concentrate and dry hay straw. They were deprived of water and food, transported and carefully unloaded upon arrival. They were calmly handled and then slaughtered using the halal method without any stunning. The slaughter and then all handling of the carcasses were carried out according to common traditional manual practice. No ethical approval was obtained because this study only involved non-invasive procedures in the form of meat samples collection.

Muscle sampling and treatment

Skinning, evisceration and then cutting of the camel and rabbit carcasses were carried out four hours after slaughter. A portion of the long dorsal muscle (*longissimus* or *Longissimus dorsi*) on the right side of the carcass of each animal was collected in a sterile plastic bag, using a sharp knife. The muscles were transported for 10 to 15 minutes in a cooler at a temperature of $4 \pm 1^\circ\text{C}$, from the slaughterhouse to the Physiopathology and Molecular Genetics laboratory in Ben M'Sik Faculty of Sciences, Casablanca (Morocco), and all external fat and connective tissue were removed. The meat portion of each animal was ground two times at a temperature of 4°C through a meat grinder plate with 6 mm holes. Each minced portion of about 200 g was divided into five patties (P) (numbered P₁ to P₅) of about 30 ± 5 g in weight and 6 ± 1 cm in diameter. The patties were treated as follows (Table 1):

- P₁ (control 1) and P₂ (control 2) were untreated with NO.
- P₃, P₄ and P₅ were treated with 0.5 mL NO.100 g⁻¹; 1 mL NO.100 g⁻¹ and 2 mL NO.100 g⁻¹ of meat, respectively.

These patties were separately mixed with NO by hand protected by a latex glove.

All the patties were immediately cooled and stored at a temperature of 4°C until 72 hours after slaughter. At the end of this time, P₂, P₃, P₄ and P₅ were heated to reach a temperature of 80°C and then were immediately cooled on ice. All patties were stored separately in plastic bags in a freezer at a temperature of -80°C until the analysis of water loss, TBARS, carbonyls, CAT, GSHPx and SOD (Table 1).

Meat samples heating

In the absence of ingredients, the meat patties were packed separately in polyethylene bags and then completely immersed in a water bath preheated to 100°C for 10 min, until reaching a temperature of 80°C . The temperatures were monitored using a Digital Kitchen Cooking Thermometer (model Alla France, Reference 91000AF003) with the food-grade stainless steel piercing probe and a measurable temperature range from -50 to $+300^\circ\text{C}$ (accuracy: $\pm 1^\circ\text{C}$).

Table 1. Experimental Design

Patties	NO (mL.100 g ⁻¹)	Internal temperature	Parameters analyzed
P ₁ (control 1)	0 (Untreated)	Unheated	Water loss, TBARS, carbonyls, CAT, GSHPx and SOD
P ₂ (control 2)	0 (Untreated)	80 °C	
P ₃	0.5		
P ₄	1		
P ₅	2		

TBARS: thiobarbituric acid reactive substances, CAT: catalase, GSHPx: glutathione peroxidase, SO: superoxide dismutase.

Determination of water loss during heating

The water loss during heating (heating loss: HL) was calculated as the percentage of the weight of the heated samples compared to the weight of the unheated ones, using the method of Honikel [19]. After heating, each sample was removed and dried with a paper towel. HL was calculated as the difference in the sample weight before and after heating, and expressed as a percentage of the initial sample weight:

$$\text{HL (\%)} = [(\text{initial sample weight} - \text{final weight}) / \text{initial weight}] \times 100. \quad (1)$$

Determination of values of thiobarbituric acid reactive substances

Lipid oxidation of raw and heated minced meat was estimated by the thiobarbituric acid reactive substances (TBARS) assay using the method described by Botsoglou et al. [20] with slight modifications. Briefly, 3 g of meat samples were homogenized at $37,000 \times g$ for 1 min, with 20 mL of ultrapure water. After adding 5 mL of 25% trichloroacetic acid, the homogenate was centrifuged (SIGMA centrifuge, model 2-16 K, Germany) at a temperature of 4°C for 15 min at $10,000 \times g$, and then the supernatant was filtered. In a test tube, 3.5 mL of the extract solution was added to 1.5 mL of 0.6% aqueous 2-thiobarbituric acid, and

the solution was kept in a water bath at a temperature of 70 °C for 30 min, then cooled in tap water for 10 min. The absorbance was measured at 532 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB) to calculate the TBARS value, and the results were expressed as nmoles of malondialdehyde/mg of proteins.

Estimation of carbonyl content

Protein oxidation was assessed by the absorbance of protein carbonyl groups at 370 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB), using the molar extinction coefficient of the hydrazone ($22000 \text{ L.M}^{-1}\text{cm}^{-1}$) formed after derivatization of the carbonyl groups with 2,4-dinitrophenyl hydrazine (DNPH) [21]. The carbonyl content was expressed as nmole of incorporated DNPH/mg protein. Protein concentration was calculated by measuring the absorbance at 280 nm (UV-visible spectrophotometer, model UVILINE9400, Ref: SOC-UVILINE9400, France) and comparing it with that obtained in the case of bovine serum proteins used as standards.

Analysis of catalase activity

An enzymatic fraction was prepared from 2 g of sample homogenized in 6 ml of ice-cold phosphate buffer (100 mM, pH 7.4) using a homogenizer IKA model T18 Digital Ultra-Turrax (IKA-Werke GmbH & Co. KG, Germany) at 13,000 rpm for 1 minute. The homogenate obtained was centrifuged at 2,000 rpm for 30 minutes (SIGMA centrifuge, model 2–16 K, Germany) at a temperature of 4 °C. CAT activity was analyzed in the final supernatant. This activity was continuously monitored by the decomposition of hydrogen peroxide (H_2O_2) into H_2O and O_2 , using the method of Sinha [22]. Fifty microliters of meat extract was placed in a UV cuvette with 2.9 ml of H_2O_2 solution (11 mM H_2O_2 in 50 mM phosphate buffer) and the absorbance kinetics at 240 nm were monitored at 1-s intervals for 3 minutes (UV-visible spectrophotometer, model UVILINE9400, Ref: SOC-UVILINE9400, France). The same phosphate buffer was used to prepare a blank. CAT activity was calculated using the molar extinction coefficient of H_2O_2 ($39.5 \text{ L.M}^{-1}\text{cm}^{-1}$) in U/g, with U corresponding to the amount of enzyme required to decompose 1 μmol of H_2O_2 per minute, and was expressed in μmol of H_2O_2 /min/mg of protein.

Analysis of superoxide dismutase activity

Total SOD activity was quantified according to the method of Paoletti et al. [23]. The procedure is based on the oxidation of NADH in the presence of superoxide anions generated by EDTA, MnCl_2 and β -mercaptoethanol. As SOD transforms superoxide anions into hydrogen peroxide and the oxidation of NADH is itself linked to the availability of these anions in the medium, then, as soon as SOD is added to the reaction mixture it inhibits the oxidation of the nucleotide. Therefore, at high concentrations of the enzyme, the absorbance at 340 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB) remains

stable, however, in the control (not added to SOD) it decreases. Twenty microliters of meat extract was placed in a UV tank with 167 μl of TDB (100 mM triethanolamine buffer, 100 mM diethanolamine, pH 7.4), 5 μl of 100 mM EDTA/50 mM MnCl_2 pH 7 and 8 μl of 7.5 mM NADH. The decrease in absorbance at 340 nm was monitored in kinetic mode during each 42-second cycle for 21 minutes. One unit (1 U) of SOD activity was defined as the amount of enzyme required to inhibit the NADPH oxidation rate of the control by 50%. Enzyme activity was calculated in $\mu\text{mol}/\text{min}/\text{mg}$ protein.

Analysis of glutathione peroxidase activity

GSHPx activity was determined according to the method described by Chen et al. [24]. Meat extract was obtained from 5g samples homogenized in ice-cold phosphate buffer (50 mM, pH 7) using a homogenizer IKA model T18 Digital Ultra-Turrax (IKA-Werke GmbH & Co. KG, Germany) at 13,000 rpm for 1 minute. Sample tubes were always kept in an ice-water bath during homogenization. After centrifugation at 2,800 g for 20 minutes at a temperature of 4 °C, supernatants were collected in microtubes and centrifuged again (10,000 g, 10 minutes, a temperature of 4 °C) and stored at –80 °C until analysis. Twenty microliters of meat extract was placed in a UV cuvette with 80 μl of 50 mM phosphate buffer, 500 μl of the assay medium (100 mM potassium phosphate buffer, pH 7, 1 mM EDTA, 2 mM NaN_3), 100 μl of glutathione reductase (2.4 U/ml), 100 μl of 10 mM L-glutathione, and 100 μl of NADPH (1.5 mM NADPH in 0.1% NaHCO_3). After 1 min, 100 μl of 1.5 mM H_2O_2 was added and the kinetics of absorbance at 340 nm (spectrophotometer JENWAY6320D visible range, JENWAY, GB) were monitored at 20-s intervals for 2 min and compared to a blank consisting of the phosphate buffer. GSHPx activity was calculated using the molar extinction coefficient of NADPH ($6,220 \text{ L.M}^{-1}\text{cm}^{-1}$) and expressed as units (U)/g protein, with U corresponding to the amount of enzyme required to oxidize 1 μmol NADPH/min.

Statistical analysis

For each of the analyzed parameters of each animal, measurements were performed in duplicate on five animals and SD values were therefore calculated on ten values. The results are presented as mean \pm standard deviation (SD). The effect of two factors, namely nigella oil as a natural antioxidant and storage duration (0, 1, 3 and 6 days), was analyzed by ANOVA (analysis of variance) followed by Tukey's post-hoc test on GraphPad Prism 8. A *P* value < 0.05 was considered statistically significant.

Results

Water loss

Camel and rabbit meat samples pretreated with NO at a dose of $2 \text{ mL.}100 \text{ g}^{-1}$ and then heated to reach an internal temperature of 80 °C showed a significant decrease ($P < 0.05$) in water loss upon heating (%), compared to their controls (untreated with NO) (26.23 ± 2.62 vs. 33.26 ± 3.32

and 22.53 ± 2.44 vs. 29.67 ± 3.21 , respectively) (Figure 1). Water losses of untreated and NO-treated samples for all doses showed no significant variation between camel and rabbit meat (Figure 1).

Thiobarbituric acid reactive substances and carbonyls

After heating untreated samples of the camel and rabbit meat until reaching an internal temperature of 80°C , the contents of TBARS (nmol malondialdehyde/mg protein) and carbonyls [nmol (2,4-dinitrophenyl)-hydrazine/mg protein] as indicators of peroxidation of lipids and proteins, respectively, increased significantly ($P < 0.05$) compared to unheated ones (9.12 ± 0.65 vs 3.87 ± 0.12 and 7.62 ± 0.54 vs 3.67 ± 0.22 , respectively, in the camel meat, and 6.78 ± 0.54 vs 2.35 ± 0.11 and 5.65 ± 0.46 vs 2.85 ± 0.18 , respectively, in the rabbit meat) (Figure 2). In both species, heated meat samples showed significantly ($P < 0.05$) lower levels of TBARS and carbonyls when pretreated with NO compared to untreated ones. Thus, at $1 \text{ mL NO} \cdot 100\text{g}^{-1}$, these levels were 6.81 ± 0.55 vs 9.12 ± 0.65 and 5.55 ± 0.51 vs 7.62 ± 0.54 , respectively, in the camel meat, and 4.85 ± 0.35 vs 6.78 ± 0.54 and 4.48 ± 0.37 vs 5.65 ± 0.46 , respectively, in the rabbit meat (Figure 2). In addition, these contents were significantly ($P < 0.05$) lower in heated samples pretreated with $2 \text{ mL NO} \cdot 100\text{g}^{-1}$ than those measured in heated samples pretreated with $1 \text{ mL NO} \cdot 100\text{g}^{-1}$ (Figure 2). Furthermore, TBARS and carbonyl contents in the untreated and unheated samples, and the NO-treated and heated ones were significantly ($P < 0.05$) higher in the camel meat than those observed in the rabbit meat (Figure 2).

Activity of enzymatic antioxidants

In the camel and rabbit meat, heating of untreated meat samples to reach 80°C decreased significantly ($P < 0.05$) the antioxidant enzymatic activities of CAT ($\mu\text{moles of H}_2\text{O}_2/\text{min}/\text{mg}$ of proteins), GSHPx (GSHPx) (U/g of proteins) and SOD ($\mu\text{moles}/\text{min}/\text{mg}$ of proteins), compared to unheated samples (3.52 ± 0.53 vs 7.48 ± 0.65 , 1.81 ± 0.22 vs 2.81 ± 0.32 and 3.54 ± 0.17 vs 5.24 ± 0.23 , respectively, in the camel meat, and 4.06 ± 0.57 vs 9.11 ± 0.71 , 1.52 ± 0.13 vs 2.12 ± 0.31 and 3.51 ± 0.18 vs 6.26 ± 0.21 , respectively, in the rabbit meat) (Figure 3). Heated samples of the camel and rabbit meat, showed significantly ($P < 0.05$) higher activities of CAT, GSHPx and SOD when pretreated with NO compared to untreated ones. Thus, at $1 \text{ mL NO} \cdot 100\text{g}^{-1}$, these activities were 5.65 ± 0.46 vs 3.52 ± 0.53 , 2.58 ± 0.16 vs 1.81 ± 0.22 and 4.78 ± 0.18 vs 3.54 ± 0.17 , respectively, in the camel meat, and 5.76 ± 0.45 vs 4.06 ± 0.57 , 2.52 ± 0.14 vs 1.52 ± 0.13 and 4.76 ± 0.21 vs 3.51 ± 0.18 , respectively, in the rabbit meat (Figure 3).

The results showed that the enzyme activities of CAT, GSHPx and SOD were significantly ($P < 0.05$) higher in the heated samples pretreated with $2 \text{ mL NO} \cdot 100\text{g}^{-1}$ than those measured in the heated samples pretreated with $1 \text{ mL NO} \cdot 100\text{g}^{-1}$ (Figure 3). In addition, these activities in the untreated and unheated samples, and the NO-treated and heated ones, were significantly ($P < 0.05$) lower in the camel meat than those observed in the rabbit meat (Figure 3).

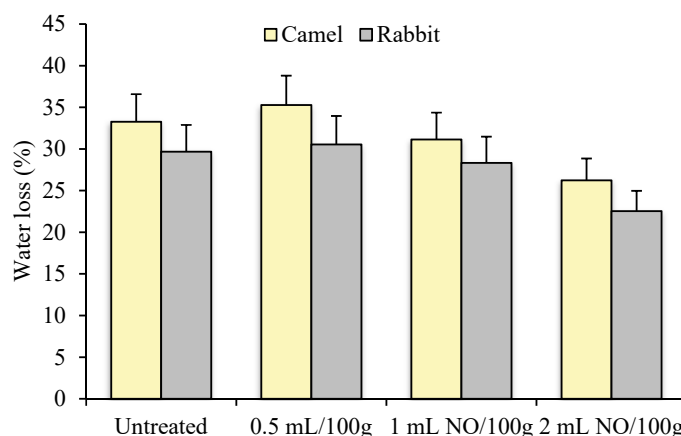


Figure 1. Values of water loss (%) in camel and rabbit meat pretreated with nigella oil (NO) at different doses, then heated until reaching an internal temperature of 80°C . (Means \pm SD, measurements were carried out in duplicate on five animals and SD values were therefore calculated on ten values)

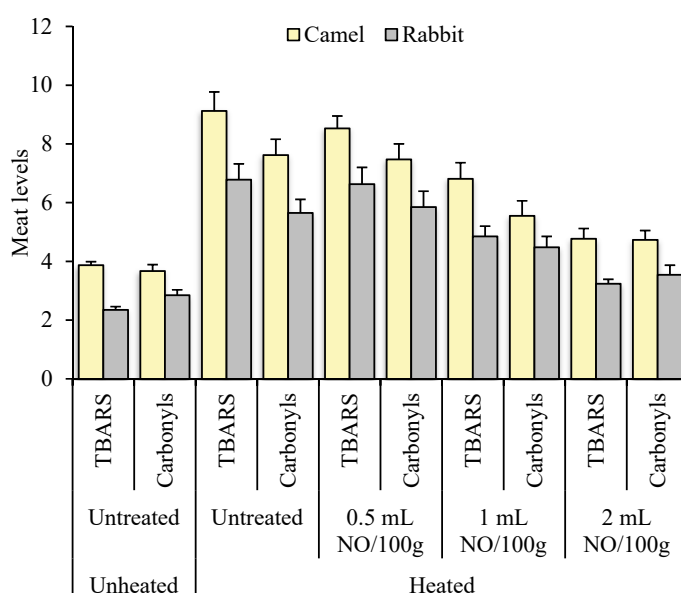


Figure 2. Values of thiobarbituric acid reactive substances (TBARS) (nmol of malondialdehyde/mg of proteins) and carbonyls [nmol (2,4-dinitrophenyl)-hydrazine/mg protein] contents in camel and rabbit meat pretreated with nigella oil (NO) at different doses, then heated until reaching an internal temperature of 80°C . (Means \pm SD, measurements were carried out in duplicate on five animals and SD values were therefore calculated on ten values)

Discussion

In the present study, heating of camel and rabbit meat induced a significant increase in the amounts of water loss, MDA and carbonyls, associated with a significant decrease in the activities of CAT, GSHPx and SOD. However, the effect of heating on oxidative stability was more pronounced in the camel meat. In both species, the treatment of meat with NO before heating, reduced significantly these levels and increased significantly these enzymatic activities in a dose dependent manner.

Water losses of camel and rabbit meat observed during heating up to 80°C are consistent with those reported by Purslow et al. [25] for beef. These losses appear to be mainly due to thermal denaturation of some of the myosin and actin molecules at $70\text{--}80^{\circ}\text{C}$, and may be accompanied by

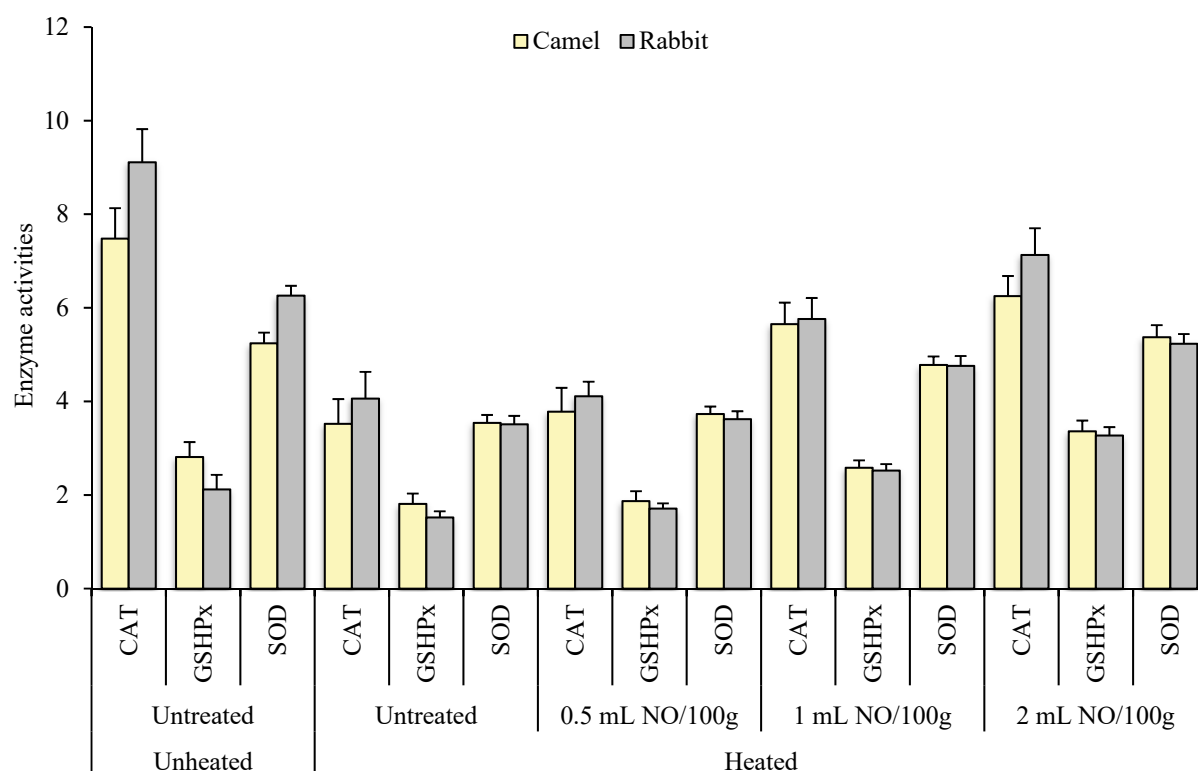


Figure 3. Activity of catalase (CAT) ($\mu\text{moles of H}_2\text{O}_2/\text{min}/\text{mg}$ of proteins), glutathione peroxidase (GSHPx) (U/g of proteins) and superoxide dismutase (SOD) ($\mu\text{moles}/\text{min}/\text{mg}$ of proteins) in camel and rabbit meat pretreated with nigella oil (NO) at different doses, then heated until reaching an internal temperature of 80°C . (Means \pm SD, measurements were performed in duplicate on five animals and SD values were therefore calculated on ten values) (1 GSHPx unit is the amount of enzyme to oxidize $1 \mu\text{mol}$ NADPH/min)

leakage of lipids, peptides and other nutrients, resulting in a decrease in meat quality [25]. Furthermore, water losses are largely dependent on the temperature and duration of heat exposure, and are dominated by longitudinal shrinkage for long sarcomeres and transverse shrinkage for short sarcomeres [25]. Haghighi et al. [26] studied the impact of protein denaturation and muscle tissue contraction during heating resulting in rapid loss of juice and moisture from meat, and the authors found that values of these parameters were closely related to temperature and not to cooking time.

In the present work, exposure of meat to heat resulted in OS revealed by high TBARS and carbonyls contents similar to what was reported for beef [27], camel [6,28,29], rabbit [30] and other species [31]. These effects could be promoted by the production of free radicals and pro-oxidant compounds, and the destruction of endogenous enzymatic antioxidants present in the muscle, thus accelerating the exposure of the lipid [28,30] and protein [27] fractions to oxidative attack and disruption of cell membranes. In their study, Shen et al. [27] examined the effect of increasing the cooking temperature up to 72°C on the quality and carbonylation of beef proteins. These authors observed that cooking losses and protein carbonyls significantly increased with temperature [27]. The significant increase in TBARS and carbonyl contents in camel and rabbit meat during heating noted in the present work has also been reported by other studies in camel [6,28,29] and rabbit [30]. In fact, Tabite et al. [6] found that camel meat cooked at 200°C to reach an internal temperature of

72°C for 30 minutes, cooled to ambient temperature, then stored for 10 days at 4°C , showed significant lipid-protein oxidation compared to raw meat. Additionally, Bahwan et al. [28] have evaluated the effects of heat treatment of camel meat samples until reaching an internal temperature of 70°C to 75°C , and have measured high levels of TBARS (4.5 mg/kg). These oxidations are a consequence of reactive oxygen species and generation of other oxidizing agents, which induced rapid peroxide formation and subsequently affected the structure, function and biological activity of lipids and proteins. However, these undesired modifications in physicochemical characteristics of meat by thermal stress, vary across heating methods (boiling, grilling, microwave, and frying), so, the microwave heating showed the highest levels of meat oxidation in camel [28,29] and rabbit [30].

The low oxidative stability of dromedary meat compared to rabbit meat observed in this study could be explained by the high levels of polyunsaturated fatty acids, myoglobin and other heme pro-oxidants in camel meat [3,9,32,33]. In fact, free ionic iron released from heme pigments and ferritin are considered major catalysts of lipid oxidation in cooked meat [34,35]. Furthermore, chicken or fish minced meat patties cooked to reach an internal temperature of 75°C showed lipid oxidation closely related to their content of unsaturated fatty acids and different iron species [36]. Oxidation of oxymyoglobin to metmyoglobin generates intermediates that can accelerate the oxidation of oxymyoglobin and/or unsaturated fatty acids. This process occurs specifically after the formation of a superoxide

anion, which further generates hydrogen peroxide. The latter can react with metmyoglobin to form an activated metmyoglobin complex, ferryl myoglobin, and thus increase lipid oxidation [37,38].

In camel and rabbit meat samples having been treated with NO, then heated, the TBARS and carbonyl contents were lower, and the activities of CAT, SOD and GSHPx were higher compared to untreated and heated samples. The effect of NO was dose-dependent suggesting that this oil might promote a significant protection of the camel and rabbit meat against thermal stress. In lamb, dietary supplementation with *Nigella sativa* seeds (1%) for 3 months increased resistance to lipid peroxidation in meat [39], the *longissimus dorsi* muscle and the semitendinosus muscle [40]. In addition, black cumin extract showed excellent antioxidant potential on cooked beef patties when stored in refrigerator for 15 days, by increasing the stability of lipid quality which was revealed by significant low peroxide value and malondialdehyde contents [41]. Furthermore, Asghar et al. [42] found that the addition of *N. sativa* seeds to the diet (1 to 4%) increased the shelf life of Japanese quail meat, by reducing lipid peroxidation. In one study, Morshdy et al. [43] evaluated the antioxidant effects of NO at different concentrations (0.1%, 0.25% and 0.5%) on the physicochemical characteristics of rabbit meat stored in the refrigerator for 12 days, and the authors found that NO was able to reduce lipid peroxidation. In addition, dietary supplementation with *N. sativa* seeds in the presence of heat stress caused a reduction in TBARS levels and an increase in CAT, SOD and GSHPx activities in serum and liver in rabbits [44] and broiler chicken [45] suggesting a reduction in OS. Other studies reported that NO treatment induced an improvement in antioxidant potential by progressively decreasing TBARS values in cooked beef patties [41] and minced pork meat [46] during refrigerated storage for several days. Additionally, the work of Muzolf-Panek et al. [47] revealed that *N. sativa* seeds were able to protect ground chicken meat against SO during cold storage for 12 days. Zwolan et al. [48] showed that the ethanolic extract of *N. sativa* seeds, added to chicken meatballs at a rate of 1.2 g/100 g, reduced lipid oxidation during refrigerated storage of 14 days.

The most important free radicals generated by heat stress are oxygen derivatives, especially superoxide an-

ion and hydroxyl radical [49]. In the present study, the improvement in the stability of lipids and proteins in camel and rabbit meat during heating in the presence of NO, could be explained by a richness of this oil in different bioactive and antioxidant compounds. According to Alberts et al. [18], black seed contains many phytochemical antioxidants such as thymoquinone (TQ), α -pinene, carvacrol, p-cymene, β -pinene, α -thujene, longifolene, tocopherols, ascorbic acid, flavonoids, thymol, tannins, magnesium and zinc, which can directly scavenge free radicals, inhibit the production of pro-oxidant nitric oxide, and modulate the activity of endogenous antioxidant systems. Indeed, TQ stimulates the activity of antioxidant enzymes such as CAT, GSHPx, and SOD, increases the expression of GSH levels, and inhibits the formation of TBARS [18]. According to another study, it was reported that polyphenol compounds showed a protective effect against OS induced by cooking and cold storage of meat in camel [10,12], sheep and goat [16].

Although studies have reported that ante-mortem dietary supplementation with black seed has improved the antioxidant potential of meat from many livestock species [39,40,42,45], a similar effect as observed in the present in vitro study following the incorporation of NO into minced meat remains to be confirmed in dromedary.

Conclusion

The study highlights, on the one hand, an impact of heat-induced oxidative stress on dromedary and rabbit meat revealed by an increase in water losses and TBARS and carbonyl contents, associated with a decrease in the enzymatic activities of CAT, GSHPx and SOD. On the other hand, the same study shows the protective role of NO against this OS by a reduction in the oxidation of lipids and proteins and by an increase in the activities of antioxidant enzymes according to a dose-dependent effect. The results of the study make it possible to promote nigella as an aromatic plant capable of playing an important antioxidant role to preserve the organoleptic composition of dromedary meat during heating. They also make it possible to consider the use of the seeds of this plant as a food supplement before the slaughter of an animal in order to minimize post-mortem OS in meat.

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