Sodium Chloride or Heme Protein Induced Lipid Oxidation in Raw, Minced Chicken Meat and Beef

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Abstract

Gheisari H.R., Møller J.K.S., Adamsen Ch.E., Skibsted L.H. (2010): **Sodium chloride or heme protein induced lipid oxidation in raw, minced chicken meat and beef**. Czech J. Food Sci., **28**: 364–375.

The objective of this study was to evaluate the effect of the salt (NaCl) level (0%, 1% and 6%) or the addition of metmyoglobin (MetMb) in the amount twice that in the natural muscle content on the oxidative stability of minced chicken meat or beef. The minced meat samples with the added NaCl or added MetMb were stored for 3 weeks during which the analyses of TBARS, peroxide value, and volatiles coming from lipid oxidation were assessed together with the quantification of vitamin E and fatty acid profiles. Heme pigment and indices of lipid oxidation were higher for beef than for chicken, except the volatile octanal regardless of the pretreatment. Peroxide value (POV) and TBARS increased significantly over storage in both minced chicken meat and beef. The minced meat added 6% salt group had the highest contents of TBARS and POV in both species. Vitamin E values decreased significantly over storage time in chicken meat and beef. 6% salt group had the lowest vitamin E content and salt had an increasing effect on hexanal content. At the end of the storage time, 6% salt group had the highest total content of saturated and the lowest one of polyunsaturated fatty acids. Added MetMb group showed no significant differences in lipid oxidation indices in comparison with those of the control group. In conclusion, higher lipid oxidation rate and total saturated and lower polyunsaturated fatty acids were observed in the salt groups. In contrast, adding MetMb had no increasing effect on lipid oxidation in chicken meat and beef.

Keywords: lipid oxidation; myoglobin; heme iron; salt; rancidity

Lipid oxidation is a major deteriorative process in meats during storage. It is responsible for a wide variety of undesirable reactions such as the loss of fresh meat colour and flavour, while protein oxidation products result in concomitant loss of protein functionality and nutritional value (Gray *et al*. 1996).

The major factors influencing lipid oxidation in raw meat include their fatty acid composition, endogenous prooxidative or antioxidative constituents, and non-meat additives (antioxidative or prooxidative). Salt (NaCl) is one such additive, and its prooxidative effect has been well recognised at concentrations (0.5–2.5%), which is normally used in meat products (Rhee 1999; Rhee & Ziprin 2001). However, some reports showed that salt at a high concentration (above 2.5%) can inhibit lipid oxidation. This may be due to the ability of NaCl to displace iron from the binding sites (macromolecules and membranous fatty acids) (Srinivasan & Xiong 1996), however, this suggested mechanism has not yet been affirmed experimentally.

Myoglobin (Mb) is the main pigment in skeletal muscles and its responsible for the muscle colour (Mancini & Hunt 2005). This protein has an important role during animal life providing the oxygen necessary for muscle metabolism. Iron exists in a protein bound form in Mb. Transition metals, notably iron, are believed to play a role in initiating lipid oxidation (Carlsen *et al*. 2005) and up until the early 1970s, catalysis of lipid oxidation in muscle had been attributed to Mb and other heme compounds, which are present at high concentrations in muscle. In contrast to earlier findings, it was found that non-heme iron rather than heme iron was the active catalyst of lipid oxidation in cooked meats (Baron & Andersen 2002). Igene *et al*. (1979) found that iron was released from heme pigments during cooking and proposed that the resultant increase in non-heme iron was responsible for the rapid oxidation of cooked meats. Tichivangana and Morrissey (1985) also reported that inorganic iron had a greater prooxidant effect than Mb in water-washed muscle residue. Mb had a significantly greater prooxidant effect in heated muscle residue when compared to the raw residue, in agreement with the findings of Igene *et al*. (1979). In the experiments with water-washed muscle residue, Johns *et al*. (1989) found that, at levels approaching those present in muscle, heme iron was a powerful catalyst of lipid oxidation while inorganic iron appeared to have little prooxidant activity. It is clear, therefore, that some uncertainty remains regarding the relative roles of heme and non-heme iron in the development of lipid oxidation in meats.

The objective of the present study was to compare the salt or heme protein induced lipid oxidation in raw minced chicken or beef meat. Salt addition was investigated at three levels of salt, while heme protein added as Mb was assessed at one level being twice that of the natural content in the included meat types. Lipid oxidation was evaluated by the measurement of primary and secondary lipid oxidation products as well as fatty acid composition and vitamin E content during three weeks of chill storage.

MATERIAL AND METHODS

Chemical. The following chemicals were used for the analyses: 2-thiobarbituric acid, trichloroacetic acid (TCA), propylgallate (PG), ethylenediaminetetraacetic acid, disodium salt (EDTA), chloroform, methanol, calcium chloride, potassium chloride, potassium hydroxide, ethanolic pyrrogalol, hexane, Butylated Hydroxy Toluen (BHT), ethanol, hydrochloric acid, acetone, 2-heptanon, ammonium thiocyanate, sodium chloride (≥ 0.99.0% purity), pentane, sodium (stored under petroleum), dry methanol, *n*-hexane, pyrogallol, Tetraethylpyrazine (TEP), barium chloride dihydrate, iron sulphate. All chemicals used were of analytical grade and were obtained from Sigma Chemical Co.(TOWN, STATE).

Animals and preparation of the meat samples. Two crossbreed (SDM × Danish Holstein) cows were slaughtered at the age of 4 years and 6 years. Muscles (2 kg of each cow) were obtained from the region of filet, loin end flank (psoas major muscles) one day *post-mortem* and were mixed with each other, ground through a plate with 0.25 cm holes. Chicken samples (8 pack of 0.5 kg) were obtained from filet bought from retail suppliers and ground through a plate with 0.25 cm holes. Each meat sample was divided into sixteen 500 g batches and four of them were randomly subsected to one of the following treatments: no additive (control) and NaCl (1% and 6%) and horse MetMb (0.2% for beef and 0.025% for chicken meat) additions. Twenty gram portions of each treatment were placed in small plastic bags which were closed and covered with a black plastic, and stored at 4°C for 0, 1, 3, 7, 14, and 21 days. The samples of day 0 were vacuum packaged. Upon the removal of the samples after each storage time, these were frozen at –20°C until analysed.

Dry matter analysis. 5 g of the sample was dried in an oven at 104°C for 6 h and weighed after allowing to cool for 15 minutes.

pH. The sample (5 g) was homogenised with 5 ml of Milli-Q water using an Ultra Turrax T-25 (Janke & Kunkel IKA-Labortechnik, Staufen, Germany). pH was measured using a Metrohm pH-meter (Herisan, Switzerland) with direct insertion of the probe electrode after calibration.

Water activity. Water activity (a_w) was measured by AQUA LAB (ADAB Analytical Devices AB, Stockholm, Sweden). The sample was filled into a small plastic container and analysed. The a_w-measurements were controlled by running a standard solution with known a_w before and after each measurement round.

Thiobarbituric acid reactive substances (TBARS). Lipid oxidation was measured as 2-thiobarbituric acid reactive substances (TBARS) by the extraction method described by Vyncke (1975) with a few modifications: the meat sample

(1.50 g) was homogenised for 45 s at 13 500 rpm using an Ultra Turrax T-25 (Janke & Kunkel IKA-Labortechnik, Staufen, Germany) with 6 ml of 7.5% trichloroacetic acid (TCA) solution including 0.1% propylgallate (PG) and 0.1% ethylenediaminetetraacetic acid disodium salt (EDTA). The homogenate was filtered through filter paper, 589.3. The extract (2 ml) was mixed with 0.020M 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, Clayton South, Victoria, Australia), and the absorbance difference, A_{532}–A_{600}, was calculated applying A_{600} as correction for the sample turbidity. TBARS, expressed as micromoles of malondialdehyde per kilogram of meat, was calculated using TEP/malonic aldehyde as standard.

Extraction of lipids. Meat (10 g) was homogenised with an Ultra Turrax with 100 ml chloroform/methanol (2:1 v/v) for 1 min at 13 500 rpm. After homogenisation, 25 ml 1.0mM $CaCl_2$ solution was added and the sample was further homogenised using an Ultra Turrax and centrifuged (HARRIER, 15/80, London, UK) at 1000 rpm for 20 minutes. The chloroform phase was removed and the extraction procedure was repeated. The chloroform phase containing the extracted lipids was dried by vacuum evaporation Büchi RE 11 (Büchi Laboratoriums-Technik AG, Flawill, Switzerland). Finally 2 × 2 ml chloroform/methanol and 2.0 ml $CaCl_2$ solution were added to the dried sample and the sample was mixed (Vortex-mixer VF2, Janke & Kunkel IKA-Labortechnik, Staufen, Germany) and centrifuged for 20 min at 2500 rpm. The lipid phase was removed, dried by vacuum evaporation, and weighed. The percentage of intramuscular fat was calculated from the weight of total lipid obtained after solvent extraction and the weight of the meat.

Fatty acid composition of the total fat. The extracted lipid (10 mg) was transformed into its constituent methyl esters by AOAC method. Methyl esters (1 µl) were injected into a HP 5890 series II gas chromatograph (Hewlett-Packard Company, Paolo Alto, USA) with a flame-ionisation detector and with a 25 m × 0.20 mm × 0.33 µm HP-FFAP column No. 19091F-102 (Hewlett-Packard) under the following oven temperature program and conditions: 50°C for 1 min; 50–180°C at 15°C/min; 180–220°C at 5°C/min; at 220°C for 10 min; injector temperature was 250°C, detector temperature 300°C. Helium was used as the carrier gas with a split ratio of 1:10 and the flow was 1 ml/min. The relative contents of the fatty acids C4:0, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:4, C22:1, and C24:0 were calculated from the chromatograms and from an external standard containing methyl esters of the fatty acids.

α-Tocopherol. α-Tocopherol was extracted using the procedure of Buttris and Diplock (1984) with some modifications: 2 ml homogenate (4 g meat sample homogenised with 20 ml 1.15% KCl-solution using an Ultra Turrax blender for 30 s at 13 500 rpm) was added to 0.20 ml saturated potassium hydroxide and 2 ml 0.50% ethanolic pyrrogalol. The sample was Vortex-mixed for 10 s and saponification was carried out at 70°C for 30 minutes. After cooling in ice water and the addition of 1.0 ml water and 4.0 ml hexane with 0.001% BHT, the sample was vigorously shaken, mixed on a Vortex mixer followed by centrifugation (HARRIER, 15/80, London, UK) at 2500 rpm for 5 minutes. The upper hexane layer was collected and the residue was re-extracted with further 3 ml of hexane containing 0.001% BHT. The combined fractions were evaporated under nitrogen and α-tocopherol was redissolved in 0.50 ml ethanol with 0.001% BHT. Quantification of tocopherols was performed according to Jensen *et al*. (1997).

Determination of total heme pigment. Total heme pigment content was measured according to Hornsey (1956) using 5 g of the meat sample to which were added 0.5 ml concentrated HCl, 0.75 ml Milli-Q water, and hereafter 20 ml acetone (30°C). The sample was homogenised immediately after adding the acetone on an Ultra Turrax for 45 s to avoid a fatty membrane formation. The sample glass was stored for 2 h on ice. It was stirred regularly. Then the sample was filtered into a 20 ml volumetric flask. The filtering paper used was S&S 589/3 which had been moistened before with 80% acetone. The absorbance was measured (scan 450–900 nm), and the total heme pigment content was calculated according to Hornsey (1956).

Hexanal and other volatile analysis. Minced meat sample (2 g) was transferred into a 20 ml sample vial and 100 µl of 2-heptanon (0.20683 g/100 ml) was added to insert tube as an internal standard and analysed with Hewlett Packard GC system.

Determination of peroxide value (POV). The IDF Standard method was used to determine the peroxide values of all samples (Shantha & Decker 1994). The extracted lipid of the respective sample (≤ 0.01–0.3 g) was mixed in a disposable glass tube

Table 1. Compositional analysis and chemical characteristic of raw, minced chicken or beef meat

Treament/ parameter	Control		1% Salt		6% Salt		Added metmyoglobin	
	chicken	beef	chicken	beef	chicken	beef	chicken	beef
pH	5.92 ± 0.03	5.99 ± 0.12	5.88 ± 0.06	5.81 ± 0.01	5.82 ± 0.01	5.75 ± 0.04	5.97 ± 0.03	5.96 ± 0.05
a_w	98.65 ± 0.35^a	99.15 ± 0.21^a	98.30 ± 0.28^a	98.65 ± 0.21^a	96.10 ± 0.57^b	94.65 ± 0.64^b	99.30 ± 0.28^a	99.35 ± 0.21^a
Dry matter	25.62 ± 0.48a	26.81 ± 0.44^a	26.84 ± 0.11^b	27.22 ± 0.90^a	29.62 ± 0.06^c	30.43 ± 0.43^b	26.33 ± 0.09^b	27.67 ± 0.48^a
Heme pigment	40.13 ± 0.95^a	292.38 ± 28.40^a	40.85 ± 0.36^a	299.88 ± 23.50^a	41.47 ± 0.40^a	312.80 ± 13.22^a	79.32 ± 1.24^b	605.46 ± 22.44^b

Comparison of pH value, water activity (a_w), dry matter (%) and heme pigment (ppm) for four different groups of minced meat samples: control, addition of 1% or 6% salt, or addition of 2-fold heme pigment (adjusted by addition of metmyoglobin. Means in the same row with different superscripts are different ($P < 0.05$)

with 9.8 ml chloroform-methanol (7 + 3, v/v) on a vortex mixer for 2–4 seconds. Ammonium thiocyanate solution (50 µl) was added, and the sample was mixed on a vortex mixer for 2–4 seconds. Then, 50 µl iron(II) solution was added, and the sample was mixed on a vortex mixer for 2–4 seconds. 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 by using a spectrophotometer (CARY 3 UV-visible, Varian Australia Ltd).

Statistical analysis. The data of different characters in two animal species during different storage times were summarised. Their means were compared to one another by using the *t*-test, one way ANOVA, repeated measure ANOVA, and Duncan's multiple range tests (SAS Software, version 9.1). The significance was defined at $P < 0.05$.

RESULTS

Compositional analyses of the meat samples were performed in order to characterise the two types of meats included. Total lipid contents for beef and chicken meat used in this study were 5.56 (SD = 1.15) and 2.86 (SD = 0.12), respectively. Heme pigment, dry matter, a_w and pH of different groups of chicken meat and beef are shown in Table 1. The highest dry matter content and the lowest a_w were found in 6% salt group. Figure 1 shows the overall fatty acid (FA) composition of 4 groups of two kinds of minced meat samples, with FA being monitored during the 3 weeks of chill storage like every other analysis, but no significant variation could be detected and thus, the reported results are average values from all these measurements ($n = 40$). At the end of the storage time, the 6% salt

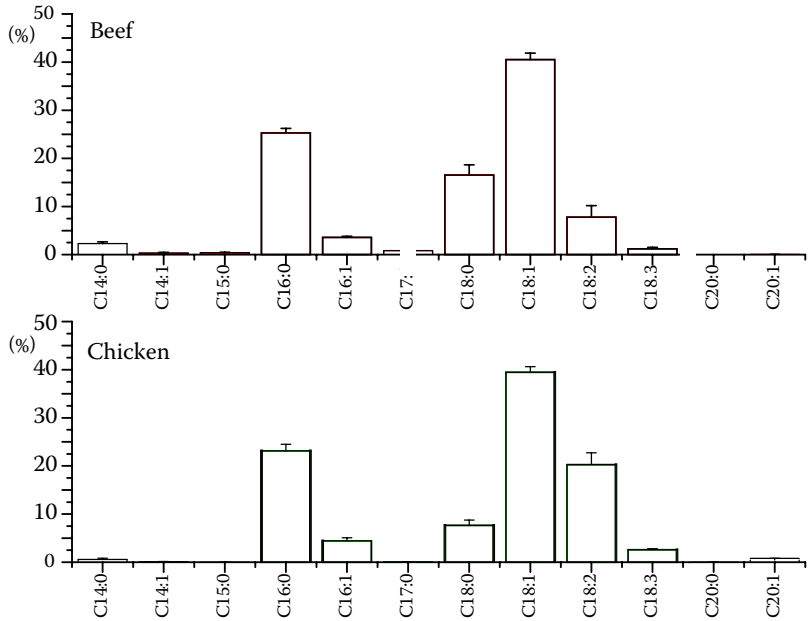

Figure 1. The overall fatty acid (FA) composition of 4 groups of raw minced chicken and beef meat samples

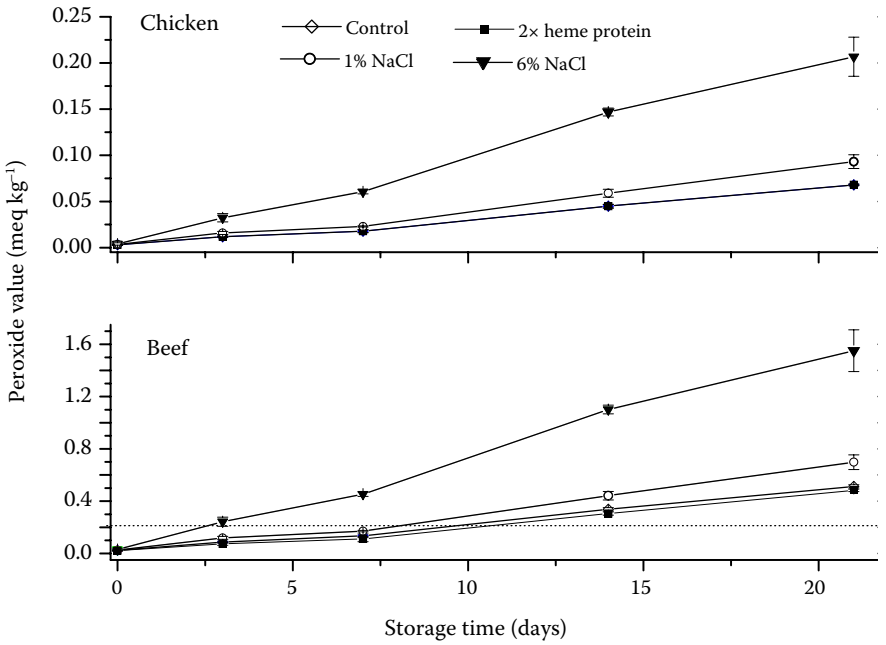

Figure 2. Comparison of peroxide values (meq kg^{-1}) between different groups of raw minced chicken and beef meat during storage time

group had the highest total saturated and lowest polyunsaturated fatty acids contents.

Heme pigment and all the fat oxidation indices studied were higher for beef than for chicken except octanal, regardless of the pre-treatment. POV and TBA values increased significantly over storage days in both minced chicken meat and beef (Figures 2 and 3). The minced meat added 6% salt had the highest TBA content in both species and after 3-week storage added MetMb group showed the lowest TBA content in beef meat. Except the latter result about the added MetMb group in beef meat, other POV value results changed same as did TBA values (Figure 2).

To characterise further the progress of the lipid autoxidation induced by either NaCl or heme protein additions various volatile carbonyl compounds were measured by static headspace GC. The hexanal content was higher in the minced meats added 6% salt as compared to the other groups after the storage time of 1 and 2 weeks. Beef meat samples added 1% salt had higher hexanal values than the samples with added MetMb after week 1, while beef meat added 1% salt had a higher hexanal content

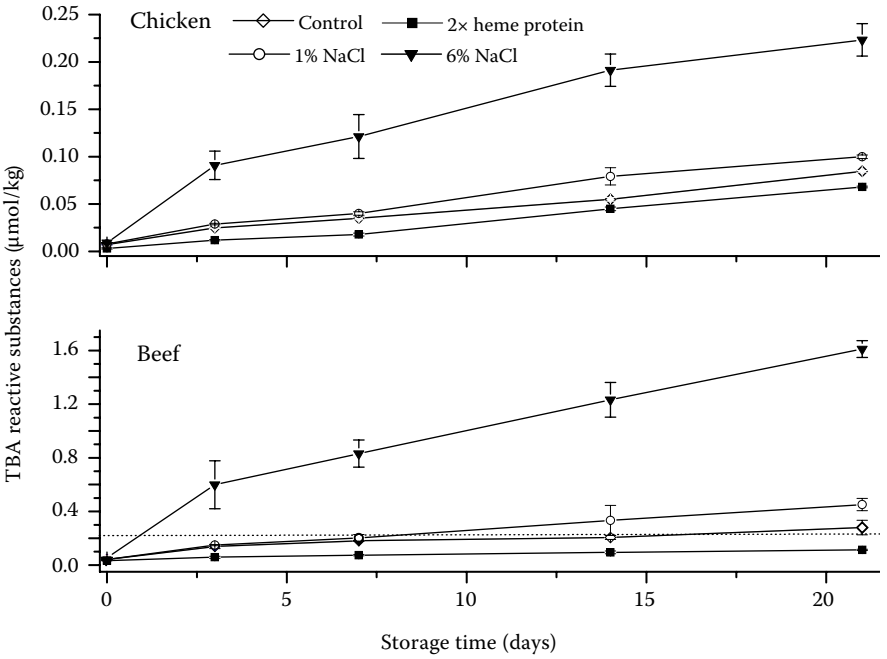

Figure 3. Comparison of TBA values (µmol/kg) between different groups of raw minced chicken and beef meat during storage time

Table 2. Comparison of hexanal values (mg/kg) between different groups of raw minced chicken and beet meat during storage time

	Day 0	Day 3	Week 1	Week 2	Week 3
Chicken meat					
Control	0.08 ± 0.01^{a}	0.24 ± 0.01^{b}	$0.47 \pm 0.03^{c,A}$	$0.65 \pm 0.01^{d,A}$	0.86 ± 0.02^{e}
1% salt	0.11 ± 0.01^{a}	0.32 ± 0.23^{ab}	$0.54 \pm 0.03^{abc,A}$	$0.75 \pm 0.03^{bc,A}$	0.95 ± 0.34^{c}
6% salt	0.14 ± 0.01	0.56 ± 0.36	0.92 ± 0.08^{B}	1.01 ± 0.05^{B}	1.12 ± 0.82
Added metmyoglobine	0.13 ± 0.01^{a}	0.23 ± 0.01 b	$0.44 \pm 0.03^{c,A}$	$0.64 \pm 0.01^{d,A}$	0.84 ± 0.02^{e}
Beef meat					
Control	0.36 ± 0.10^{a}	$0.61 \pm 0.05^{a,A}$	$1.42 \pm 0.01^{b,AB}$	$1.73 \pm 0.03^{b,A}$	$3.84 \pm 0.36^{c,A}$
1% salt	0.37 ± 0.08^{a}	$0.82 \pm 0.11^{b,A}$	$1.70 \pm 0.02^{c,B}$	$2.21 \pm 0.03^{d,B}$	$6.07 \pm 0.23^{e,B}$
6% salt	0.39 ± 0.04^{a}	$1.51 \pm 0.04^{b,B}$	$4.84 \pm 0.20^{c,C}$	$7.71 \pm 0.06^{d,C}$	$9.13 \pm 0.81^{e,C}$
Added metmyoglobine	0.30 ± 0.01^{a}	$0.58 \pm 0.13^{a,A}$	$1.29 \pm 0.09^{b,A}$	$1.57 \pm 0.07^{b,D}$	$3.48 \pm 0.50^{c,A}$

Means in the same row with different small letter superscripts are different ($P < 0.05$); means in the same column with different capital letter superscripts are different ($P < 0.05$)

than the control samples after week 2. The samples of beef meat added MetMb had the lowest hexanal value in week 2 (Tables 2). Propanal values of 6% salt group were the highest. The added MetMb group of beef meat had lower propanal values than were those from 1% salt on day 3, week 2 and week 3, and from the control in week 2 (Tables 3). Octanal values were much higher for chicken than for beef on day 0, all groups except 6% salt on day 3 and all groups except 1% and 6% salt in week 1. Time had no effect on octanal values of the control and 1% salt groups of chicken meat and 1% and 6% salt groups of beef meat. In chicken meat, 6% salt group had the highest octanal values in week 3. In beef meat, 6% salt group had higher octanal values than 1% salt on day 3 (Tables 4).

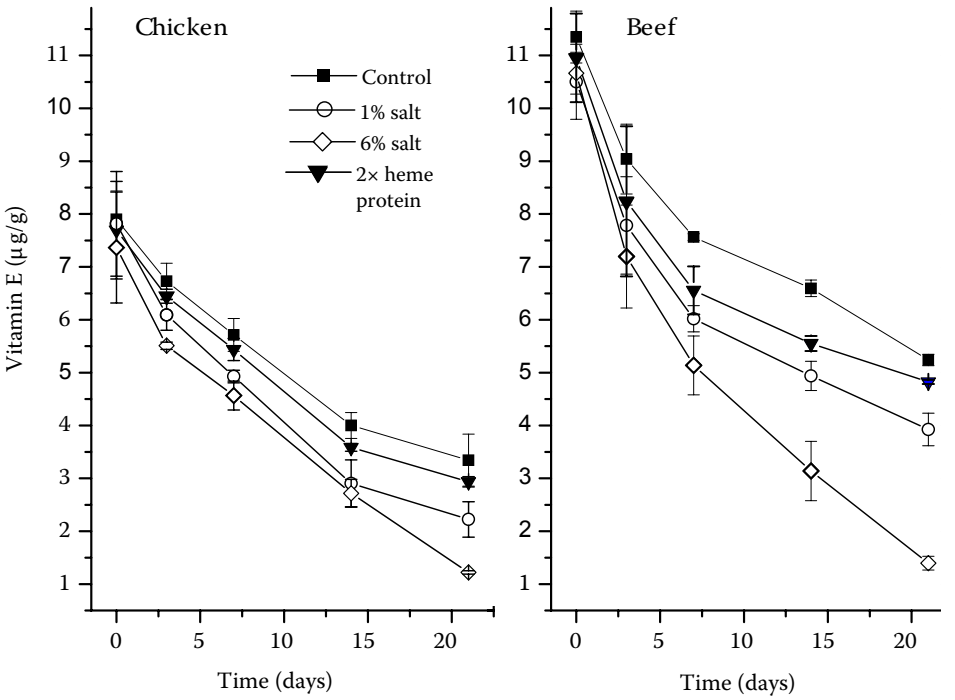

Figure 4. Content of vitamin E (µg/g) for different sample groups of raw minced chicken and beef meat added salt or myoglobin during 3 weeks of chill storage.

Table 3. Comparison of propanal values (mg/kg) between different groups of raw minced chicken meat during storage time

	Day 0	Day 3	Week 1	Week 2	Week 3
Chicken meat					
Control	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a,A}	0.15 ± 0.02^{b,A}	0.17 ± 0.07^{b,A}
1% salt	0.04 ± 0.02	0.07 ± 0.05	0.14 ± 0.03^{A}	0.19 ± 0.13^{A}	0.20 ± 0.14^{A}
6% salt	0.04 ± 0.01^{a}	0.11 ± 0.07^{a}	0.46 ± 0.03^{b,B}	0.53 ± 0.06^{bc,B}	0.59 ± 0.01^{c,B}
Added metmyoglobine	0.02 ± 0.01^{a}	0.04 ± 0.02^{a}	0.05 ± 0.04^{a,A}	0.16 ± 0.02^{b,A}	0.20 ± 0.03^{b,A}
Beef meat					
Control	0.32 ± 0.01^{a}	0.35 ± 0.25^{ab,AB}	0.51 ± 0.06^{ab,A}	0.68 ± 0.25^{ab,A}	0.87 ± 0.26^{b,AB}
1% salt	0.32 ± 0.24^{a}	0.56 ± 0.15^{ab,B}	0.66 ± 0.53^{ab,A}	0.80 ± 0.12^{ab,A}	1.18 ± 0.16^{b,B}
6% salt	0.35 ± 0.23^{a}	1.35 ± 0.09^{b,C}	1.81 ± 0.12^{bc,B}	2.07 ± 0.08^{c,B}	2.90 ± 0.38^{d,C}
Added metmyoglobine	0.06 ± 0.05^{a}	0.07 ± 0.06^{ab,A}	0.11 ± 0.04^{ab,A}	0.24 ± 0.08^{bc,C}	0.31 ± 0.07^{c,A}

Means in the same row with different small letter superscripts are different ($P < 0.05$); means in the same column with different capital letter superscripts are different ($P < 0.05$)

Vitamin E values decreased significantly over the storage time in chicken meat and beef as shown in Figure 4, and the relative decline in vitamin E content was most pronounced for the samples treated with 6% salt which, at the end of 3-week storage, had the lowest vitamin E content. Accordingly, the samples treated with 1% salt exhibited reductions in vitamin E content that were intermediate between the control and the samples added 6% salt. Interestingly, the samples added MetMb demonstrated a loss of vitamin E that was slightly more evident than in the control samples, which could indicate that the additional heme content caused a prooxidative effect which, however, was fully counteracted by the naturally present vitamin E (that was in function as antioxidant) in the meat, and thus the prooxidative effect of the additional heme proteins did not show up in the measures for primary or secondary oxidation products, e.g. POV or TBARS, respectively.

Table 4. Comparison of octanal values (mg/kg) between different groups of raw minced chicken and beef meat during storage time

	Day 0	Day 3	Week 1	Week 2	Week 3
Chicken meat					
Control	10.53 ± 0.16	1.11 ± 0.50	2.09 ± 1.40	2.31 ± 0.64	2.39 ± 0.35^{A}
Salt 1%	0.91 ± 0.34	1.33 ± 0.42	1.71 ± 0.72	2.34 ± 0.04	3.15 ± 1.92^{A}
Salt 6%	0.98 ± 0.08^{a}	1.88 ± 0.92^{a}	2.18 ± 0.14^{a}	4.09 ± 0.69^{b}	7.83 ± 0.49^{c,B}
Added metmyoglobine	0.88 ± 0.36^{a}	1.63 ± 0.43^{ab}	2.09 ± 0.83^{ab}	2.30 ± 0.03^{ab}	3.08 ± 1.19^{b,A}
Beef meat					
Control	0.25 ± 0.01^{a}	0.37 ± 0.14^{a,AB}	0.58 ± 0.02^{ab}	1.16 ± 0.51^{b}	2.22 ± 0.24^{C}
1% salt	0.30 ± 0.03	1.13 ± 0.01^{B}	2.13 ± 0.11	3.59 ± 3.43	5.21 ± 4.24
6% salt	0.32 ± 0.02	1.81 ± 0.50^{A}	3.86 ± 2.22	5.74 ± 2.01	6.34 ± 4.43
Added metmyoglobine	0.29 ± 0.05^{a}	0.39 ± 0.12^{a,AB}	0.66 ± 0.26^{a}	1.47 ± 0.51^{a}	3.46 ± 1.21^{b}

Means in the same row with different small letter superscripts are different ($P < 0.05$); means in the same column with different capital letter superscripts are different ($P < 0.05$)

DISCUSSION

In this study, lipid oxidation indices were higher in all cases for beef than for chicken, regardless of the treatment. The higher total fat, polyunsaturated fatty acids, and natural heme pigment contents in beef meat could be responsible for these observations. The samples added 6% salt showed a higher lipid oxidation than the other groups in both animal species.

The heme proteins hemoglobin (Hb) and Mb are normally considered as effective promoters of the lipid oxidation (Johns *et al*. 1989). Several authors have postulated that oxymyoglobin (OxyMb) and lipid peroxidation in muscle tissue are interrelated (Yin & Faustman 1993; Lin & Hultin 1997). It was found that OxyMb is a weak catalyser of membrane lipid peroxidation. In the presence of an iron redox cycle system, OxyMb affected muscle membrane undergoes lipid peroxidation by a mechanism that depends on OxyMb concentration. OxyMb at a concentration of 20μM slightly decreased membranal lipid peroxidation. However, at a higher concentration of 80–120 μM, lipid peroxidation was inhibited almost completely (Harel & Kanner 1989). Monahan *et al*. (2005) showed that lipid oxidation-induced oxygen depletion, as opposed to primary or secondary lipid oxidation products, is a likely cause of OxyMb oxidation in the muscle systems.

The formation of MetMb from OxyMb is positively correlated with lipid oxidation and appears to be dependent on the antioxidant status (Yin *et al*. 1993). MetMb reacts with H_2O_2 or lipid hydroperoxides to generate ferryl heme protein radicals, which can abstract hydrogen from polyunsaturated fatty acids and hence initiate lipid oxidation (Reeder *et al*. 2004). Alternatively, the displaced hemin or released iron can stimulate lipid oxidation (Ahn *et al*. 1993). Iron initiates lipid oxidation by generating free radicals capable of abstracting a hydrogen atom from unsaturated fatty acids (Gutteridge & Halliwell 1990). The formation of MbFe(III) is highly correlated with the extent of lipid oxidation in muscle foods and it is now generally accepted that MbFe(III) is an effective pro-oxidant at acidic pH and in the presence of hydroperoxides (Hogg *et al*. 1994). Mb has been postulated to possess specific electrostatic and hydrophobic binding sites for fatty acids (Yackzan & Wingo 1982). The specific sites for fatty acid binding have not been identified, however, the presence of at least two hydrophobic cavities in addition to the heme cavity has been suggested in MbFe(III) (Baron *et al*. 2000). For many years, it had been assumed that MbFe(III) was the only heme protein species responsible for the initiation of lipid oxidation in muscle-based foods. This has been the basis of the general assumption that the maintenance of the bright cherry-red colour of meat can delay the oxidative deterioration of muscle-based foods (Baron & Andersen 2002). However, whether or not the oxidation state of iron is of any significance in the initiation process of lipid oxidation by heme proteins has been questioned (Korycka-Dahl & Richardson 1980). Subsequently, $MbFe(II)O_2$ has been shown to initiate lipid oxidation as effectively as MbFe(III) even though the mechanism by which $MbFe(II)O_2$ initiates lipid oxidation still is unclear (Chan *et al*. 1997). Yin and Faustman (1994) report a high correlation between $MbFe(II)O_2$ oxidation and lipid oxidation both in microsomes and liposomes and believe that $MbFe(II)O_2$ oxidation and lipid oxidation are coupled. Most studies show that both $MbFe(II)O_2$ and MbFe(III) are pro-oxidants, and the difference observed between their abilities to initiate lipid oxidation might be trivial as stated by Hirano and Olcott (1971). Watanabe *et al*. (1998) observed faster TBARS increase in deer meat than in beef. One of the probable cause might be the difference in Mb content between the two species. Mb content in deer is 0.07–0.73% (unpublished data), which is higher than that in beef. In our study, no effect was observed of added MetMb, which contains the ferric iron form but apparently it had no influence on the oxidative measures carried out. The possible reason why the additional Mb cannot promote lipid oxidation could be that the physical location, when added in this way to the minced meat, is not similar to the naturally present muscle Mb as the muscle pigment. This may in effect result in an altered interaction with lipid substrates and thus provide no extra oxidation load on the meat samples, which holds for both meat types and therefore seems to be a general observation that is valid irrespectively of the muscle type investigated.

Sodium chloride (NaCl), which is an important additive to the meat, has multiple functions in the meat products. It tenderises meat by increasing ionic strength, enhances flavour, and increases water-holding capacity. At high concentrations, it also inhibits microbial growth, functioning as a preservative (Rhee & Ziprin 2001). However,

NaCl has some undesirable side effects at moderate concentrations (0.5–2.5%) normally used in the meat products. It promotes lipid oxidation in raw and cooked meat and accelerates MetMb formation and raw meat discoloration (Rhee 1999).

Increasing the concentration of NaCl enhances lipid peroxidation in raw minced muscle, especially after the freezing-thawing process. This effect seems to derive from the fusion of the intracellular compounds and the destruction of the cell structure by NaCl (Shomer *et al*. 1987). In addition, NaCl acts to displace iron ions from the binding sites. Muscle tissue contains a small amount of "free" iron ions; the addition of iron ions (10–20pM) to minced muscle increased slightly the rate of lipid peroxidation. However, the same treatment, in the presence of NaC1, enhanced lipid peroxidation approximately 3–5-times more than the control. One suggestion is the interaction of a large part of the added iron ions with the proteins. This interaction seems to prevent iron ions from affecting membranous lipids and acting as catalysers of lipid peroxidation. NaCl seems to disturb the interaction between iron ions and the proteins and, therefore, leaves more free iron ions to interact with the lipid fraction which enhances lipid peroxidation (Kanner *et al*. 1988).

One of the most possible theories involves the disruption by NaCl of the structural integrity of the membranes, bringing the catalysts into a closer contact with lipid reactants. For raw meat (particularly red meat), the mechanism of lipid oxidation catalysis by NaCl is likely to involve changes in meat pigments as well; salt may initially promote the formation of hypervalent ferrylmyoglobin (or activated MetMb), an initiator of lipid oxidation (Rhee 1999; Rhee & Ziprin 2001).

Little has been known about how antioxidant enzymes may be affected by different types of salt and their ionic strengths. Lee *et al*. (1997) reported no inactivation of catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase by NaCl in ground pork muscle during freeze 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. According to our result, an increasing percentage of NaCl in raw minced meat can produce a higher degree of lipid oxidation. We could find an antioxidative effect of higher NaCl concentrations (> 6%) in dry-cured meat products. However, our results did not show similar effects, and the physico-chemical environment that differs quite substantially for our minced meat samples and a dry-cured meat product could be one reason for this. In fact, it may be the high salt addition combined with the excessive dehydration occurring in the dry-cured meat products that accounts for such variation (Andres *et al*. 2004).

TBARS value is often used as an index of lipid oxidation in meat products during storage. It was found that the TBARS number, at which rancid odour is first perceived, is between 0.5 and 1.0. This threshold has served as a guide for interpreting TBARS test results (Kanner 1994). Over 21-day storage in our study, all groups except 6% salt in beef meat had TBARS values below 0.5 (μmol/kg meat). Salt addition and storage of samples had positive effects on TBARS values in chicken meat and beef.

Sallam and Samejima (2004) studied the effects of sodium lactate (NaL) and sodium chloride (NaCl), either alone (30 g/kg) or in combination (20 + 20 g/kg), on the lipid oxidation of raw ground beef during vacuum-packaged storage at 2°C. Ground beef was sampled at 3 days intervals during 21-day storage. The initial TBARS value of ground beef ranged from 0.177 (mg malonaldehyde/kg) in NaL-treated meat to 0.196 in NaCl-treated samples. In all ground beef samples, storage time had a significant effect on TBA values, which tended to increase with storage. On storage day 21, TBARS values of both NaCl-treated (0.309) and control (0.318) samples were significantly lower than those of the samples treated with NaCl (0.463).

Rhee and Ziprin (2001) ground beef or chicken breast muscle samples (either pretreated with 60 ppm chlortetracycline/0.2% potassium sorbate to control microbial growth or not pretreated) were mixed with 0 ± 5% NaCl and aerobically refrigerated for 0 ± 6 days. They showed that in beef as well as in chicken meat, NaCl increased TBARS content of the stored samples (3 days or 6 days) in refrigerator, whether pretreated or not. Beef (pre-treatment and no pre-treatment) TBARS content increased continually with the storage time. TBARS content in the no pre-treatment chicken did not change significantly over the storage days, regardless of NaCl level. For the pre-treatment chicken, day-6 TBARS values at 1 ± 5% NaCl were higher than the corresponding day-0 values. At 0% added NaCl, however, the TBARS content of the pre-treatment chicken changed lit-

tle throughout 6-day storage. TBARS values after 3 days or 6 days of storage were much higher ($P < 0.05$) for beef than for chicken, regardless of the pre-treatment.

It was hypothesised that the difference in susceptibility to lipid oxidation between raw beef and chicken breast might be related to their heme pigment levels. Specifically, it was hypothesised that, since beef has a higher Mb content than chicken breast meat, more MetMb and H_2O_2 would be formed in beef through oxidation of OxyMb, eventually resulting in more H_2O_2-activated MetMb (ferrylmyoglobin radicals), thus accelerating lipid oxidation. The higher TBARS values for beef samples as compared to chicken samples may also be partly due to the lower pH values of beef. Note that the fat in chicken breast meat is usually much more polyunsaturated than the fat from beef muscles or separable lean (Rhee *et al*. 1996).

Hernandez *et al*. (2002) indicated that ionic strength increase can affect the stability of antioxidant enzymes, with GSH-Px being affected more than catalase. Their results show the TBARS content to increase with increasing ionic strength (or salt concentration) and storage time and are consistent with those of previous studies.

Our results showed that with increasing added salt level vitamin E content in chicken meat and beef decreases. Vitamin E is especially important in slowing down *post mortem* oxidative changes due to its antioxidative properties. Vitamin E protects highly oxidisable polyunsaturated fatty acids from peroxidation by reactive oxygen species produced by adjacent membrane-bound enzymes (McCay & King 1980). Free radicals are neutralised 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 workers have shown that the rate and extent of lipid oxidation are dependent on the vitamin E concentration in the tissues. Kanner *et al*. (1990) reported that the differences in TBARS values between 'red' and 'white' muscles were largely dependent on the nature of the predominant form of Fe catalysts, e.g. Mb and 'free' iron. Iron catalysis is of particular interest in relation to thermal lipid oxidation since iron compounds in aerobic organisms are involved in oxygen transport, storage, and activation in the form of heme pigments. Heme pigments, including Mb and hemoglobin, may thus become catalytic under the conditions of oxygen depletion, pH decrease, or abruptly fluctuating oxygen supply in tissues, as under ischemic conditions (Chan *et al*. 1997).

Ruminant fat has a lower ratio of saturated fatty acid (SFA) to polyunsaturated fatty acid (SFA: PUFA) in comparison to non-ruminant fat, which is due to hydrogenation of dietary unsaturated fatty acids in the rumen (French *et al*. 2000). The rumen hydrogenates a high proportion of unsaturated dietary fatty acids so that muscle fatty acids in cattle and sheep are more saturated and less unsaturated than those in pigs. In particular, C18:2, which is the major plant fatty acid, is much lower in ruminant tissues. This difference occurs because C18:3 fatty acids are relatively high in ruminant animals, being the major fatty acids in grass. Although a high proportion of them is converted to C18:0 in the rumen, significant quantities pass through the rumen to be absorbed in the small intestine. Almeida *et al*. (2006) reported that the proportion of monounsaturated fatty acids (MUFA) was similar in the beef cuts and chicken meat. The SFA and PUFA proportions were similar in both beef cuts, but higher ($P < 0.001$) and lower ($P < 0.0001$) than in dark chicken meat, respectively.

In conclusion, our results indicate that beef a higher lipid oxidation rate in comparison with chicken meat. Salt is a prooxidant for meat, and lipid oxidation will increase progressively with increasing amounts of salt and storage time. Such potential would be greater for red meat (beef) than for white meat (chicken). Adding MetMb in this dosage and way (hand mixing) had no effect on lipid oxidation.

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