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Hydroperoxide formation in different lean meats

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ABSTRACT

Peroxide is one of the compounds that are indicated to be toxic in the human digestion system. Lean fresh meat samples were collected from beef, lamb, pork and chicken to investigate their hydroperoxide formation potential. Total peroxides of fresh comminuted raw meat were determined by analysing protein-bound peroxides and hydroperoxide compounds in water–methanol and chloroform extracted phases. The amount of total peroxides was ranked as: beef > pork > lamb > chicken. Hydroperoxide formation was examined at different pH values and at different incubation times, using beef and chicken samples. All peroxides were transient, with a maximum value after 2–4 h of incubation at 37 °C. When pH fell from 7 to 1.5, the different peroxides fell by 10–20%. Non-polar peroxide formation could largely (70%) be described by variation in fatty acid composition and hemin content of the meat, while protein-bound peroxide variation was less explained by these variables. Liposome addition increased (40%) the amount of protein-bound peroxides.

1. Introduction

Meat consumption from some land-based animals has come under attack due to unclear status regarding many diseases. Colon cancer is among these diseases, and it is one of the major causes of death in western countries (Sesink, Termont, Kleibeuker, & Van der Meer, 1999). It has been recognised that many genetic factors are involved as determinants of colorectal cancer (Fearon & Jones, 1992), but environmental factors have appeared to contribute to the incidences of colon cancer (MacLennan, 1997). The World Cancer Research Fund panel has judged that the evidence of red meat and processed meat being a cause of colon cancer is convincing (WCRF, 2007), and a western style diet with a high red meat consumption is suggested as a risk factor for colon cancer (Sesink et al., 1999). Increased consumption of meat can be due to improved efficiency in agriculture, which has then created sufficient amounts of relatively cheap meat products. Animal breeding has so far given most priority to rapid animal growth and cost-effective feeds. But meat should also have a good oxidative and microbial shelf life. Sufficient oxidative stabilization is paramount for meat flavour. A present understatement is that oxidised food can be consumed as long as the microbiology and sensory quality are acceptable to consumers. Compounds that could increase the genetic instability of colon cells and the appearance of cancer have received much attention (Ferguson, 2010). Lipid or lipid-derived peroxides are a major source of dietary pro-oxidants speculated to be of toxicological importance (Halliwell & Chirico, 1993).

An *in vitro* study on intake of fat and derived peroxides has identified this as one of many important factors in colon cancer (Angeli et al. 2011). Lipid peroxides are set with an acceptable upper level of 5–10 mmol/kg in oil or fat (Sattar & Deman, 1976). Peroxide limits are normally not defined for products other than oil/fats. However, it is more common to eat larger amounts of lean meat than of pure oil/fats in a meal. Heated turkey meat has been reported to have 1 mmol of lipid hydroperoxide/kg wet weight (Kuffa, Priesbe, Krueger, Reed, & Richards, 2009). This suggests a high peroxide value in the endogenous lipids (~100 mmol/kg lipid). In addition, proteins may also carry peroxides equal to 3–22 mmol/kg of protein (Salminen and Heinonen, 2008). Proteins damaged by free radicals in the presence of oxygen can yield relatively long-lived protein peroxides (Davies, Fu, & Dean, 1995; Gebicki & Gebicki, 1993), which have been shown to readily degrade to free radicals upon reaction with iron (II) complex. It is therefore necessary to include them in an assay for hydroperoxide measurements, in particularly in lean meat where the lipid content is low relative to the protein content.

With sufficient amounts of efficient antioxidants, meat should be a homoeostatic system which remains reduced or without oxidised compounds and reactive components. The aim of this study was: (1) to set up a new model system for measuring total hydroperoxide values of lean meat and the reactivity of lean meat towards liposomes, (2) to discover if the lipid peroxides were always dominant over the protein-bound peroxides, (3) to investigate whether the peroxides were stable when incubated

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over time and at different pH values, (4) to establish the hydroperoxide formation ability in some Norwegian regular diet meats.

2. Material and methods

2.1. Meat samples

Chicken muscles (*Musculus pectoralis major*) were collected on the day of slaughter from a hot boning line, vacuum-packed and frozen at −80 °C. The chicken-SO group was chicken fed with a wheat-based diet containing 4% soybean oil and 0.003% selenium-enriched yeast (Ultra Bio-logics., Inc., O.S.Y. 2000× containing 2.15 g Se/kg), whereas the chicken-LO group was fed with a wheat-based diet with 2.4% linseed oil, 1.6% rapeseed oil, and 0.04% selenium yeast. Beef muscles (*Musculus semimembranosus*) were obtained on the day of slaughter from a hot boning line, vacuum-packed and frozen at −40 °C until they could be brought to −80 °C (after 5 days). Pork muscles (*Musculus gluteus medius*) were collected 1 day after slaughter from the cold boning line, vacuum-packed, and frozen at −80 °C. The pig group was homogeneous, as all pigs were of the crossbreed Noroc that was produced to give higher intramuscular fat content than the regular Norwegian Landrace/Yorkshire crossbreed. All the pig samples were from the same farm. Lamb muscles (*Musculus psoas major*) were obtained 1 day after slaughter from a cold boning line, vacuum-packed, frozen at −40 °C until they could be brought to −80 °C (after 5 days). Each group contained 10 animals. These beef (*M. semimembranosus)*, pork (*M. gluteus medius*) and lamb (*M. psoas major*) muscles were randomly chosen from different Norwegian feeding farms from a local meat supplier (Nortura SA, Lillehammer, Norway).

2.2. Chemicals

L-α-Phosphatidylcholine 95% (egg, chicken) powder was purchased from Avanti Polar Lipids, Inc., (Alabaster, USA). Water was purified by a purification system (Millipore, Sydney, Australia). Chloroform (AR grad), sulphuric acid, methanol, acetone, iron (II) sulphate, hexane and Ringer's solution tablets were from Merck (Darmstadt, Germany). Guanidine hydrochloride, hydrochloric acid (37%), streptomycin and C13:0 internal standard were supplied by Sigma–Aldrich Chemical (Sydney, Australia). Butylated hydroxytoluene, xylenol orange sodium salt and triphenylphosphine (99% in purify) were purchased from Alfa Aesar (Lancashire, UK). Sorbitol and hemin were bought from Sigma–Aldrich (St. Louis, USA). Sodium dithionite and KOH were purchased from VWR Inc., (Oslo, Norway). All the other chemicals were of analytical grade as supplied.

2.3. Generation of liposomes

L-α-Phosphatidylcholine 95% (egg, chicken) powder (1 g) was first dissolved and mixed in 50 ml of chloroform to assure a homogeneous mixture of lipids. The organic solvent was evaporated to 1 ml by using a rotary evaporator (R215, Buchi Rotavapor, Switzerland). The solution was dried thoroughly by nitrogen gas to a lipid residue at room temperature. Hydration of the dry lipid cake was accomplished by adding 50 ml of Ringer's solution in a 60 °C water bath for 60 min. Liposomes were produced by using an extrusion technique, which yielded a polydisperse suspension of multilamellar liposomes. The mini-extruder was assembled by inserting two internal membrane filters and one polycarbonate membrane filter (0.1 μm pore size, Avanti polar lipids, Inc. Alabama, USA), and then the system was heated to 60 °C before use. One gas-tight syringe (Hamilton, Bonaduz, Switzerland) was loaded with 1 ml of solution and applied to one end of the mini-extruder while the other end of the mini-extruder was supported with an empty gas-tight syringe so that the fluid could be circulated through filters from both sides. This resulted in large, unilamellar liposome vesicles defined by the pore size of the membrane.

The lipid solution was completely transferred between the original and alternative syringes by gently pushing the plunger (1 min each time) 10 times (20 passes through the membranes). A successfully prepared liposome solution had no sediment after storage at 4 °C overnight. Liposome solutions were stored at −80 °C after preparation for later use.

2.4. Hydroperoxide value (PV) measurements by using the ferric–xylenol orange (FOX) method

Meat cuts were trimmed of all visible fat, frozen in liquid nitrogen and homogenised by blender (800 W Home blender, Invite) to meat powder. Hydroperoxide measurements were made on meat, with or without added liposomes. Triplicates of meat samples (0.1 g) were incubated in 1 ml of Ringer's solution and quadruplicate meat samples were incubated in 200 μl of liposomes (4 mg/ml) and 800 μl of Ringer's solution. To all systems, 10 μl of 20 g/l streptomycin was added and the systems were incubated for 2 h in a 37 °C water bath.

The measurements without added liposomes served to identify endogenous ability to produce peroxides, while the other measurement served to verify the potential of the meat samples to induce peroxides in liposomes (as an *in vitro* model for cell membranes). The samples were mixed with 1 ml of chloroform and methanol (2:1, volume-ratio), vortexed and centrifuged at 24,462*g* for 10 min at 4 °C. After centrifugation the system separated into three phases which were 1.33 ml of polar upper phase (25% methanol + 75% Ringer's solution, pH 7), an interphase (the meat protein aggregate) and 0.67 ml of non-polar lower phase (chloroform) containing soluble lipids. Each of the three phases was removed for separate hydroperoxide measurements. Upper phase (700 μl) was removed and the following chemicals were added immediately in this order: 5 μl of 4 mM BHT, 4 μl of 2 M H_2SO_4, 40 μl of H_2SO_4 at pH 1.8, 30 μl of 5 mM XO + 5 M sorbitol mixture at pH 1.8 and 40 μl of 1.67 mM $FeSO_4$ at pH 1.8. A blank containing the upper phase reduced with 10 μl of 1 M sodium dithionite and subjected to an identical protocol was used as a negative control. The protein aggregate at the interphase was washed three times with 2:1 chloroform:methanol before 1.7 ml of 6 M GuHCl were added to resolubilise the protein for optimal hydroperoxide exposure. The protein aggregate did not always solubilise to a transparent solution, but it swelled to an open system that allowed for low molecular weight diffusion (*i.e.* diffusion of the chemicals added). After 30 min of solubilisation, all chemicals were added immediately in this order: 12 μl of 4 mM BHT, 97 μl of H_2SO_4 at pH 1.8, 73 μl of 5 mM XO + 5 M sorbitol mixture at pH 1.8 and 73 μl of 1.67 mM $FeSO_4$ at pH 1.8. A blank containing suspended protein phase reduced with 10 μl of 1 M sodium dithionite and subjected to identical protocol was used as a negative control. Lower phase (50 μl chloroform) was removed and chemicals were added immediately in this order: 200 μl of chloroform, 460 μl of methanol, 5 μl of 4 mM BHT, 12 μl of 2 M H_2SO_4, 26 μl of 10 mM XO at pH 1.8 and 54 μl of 1.67 mM $FeSO_4$ at pH 1.8. A blank containing the lower phase reduced with 10 μl of 1 M triphenylphosphine and subjected to identical protocol was used as a negative control. All the samples were incubated for 60 min in enclosed Eppendorf tubes at room temperature to ensure colour development. The upper phases and the suspended protein interphases were centrifuged at 24,462*g* for a further 10 min at 4 °C to secure transparency before the measurements by the spectrophotometer, while the lower phases were measured spectrophotometrically at 590 nm immediately after the incubation. The initially obtained hydroperoxide