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Microbiological shelf life of fresh, chilled reindeer meat (*M. longissimus dorsi*)

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Abstract: In this pilot study loin muscles (*M. longissimus dorsi*) from six reindeer calves (aged 4 months) were used to determine shelf life of fresh, chilled reindeer meat stored at +4 °C, measured as microbiological quality (aerobic microorganisms and *Escherichia coli*). The loins were collected at boning 3 days post slaughter and divided in five pieces that were randomly assigned to five different storage times; sampling directly after packaging and after chilled storage for 2, 3, 4 and 5 weeks at +4 °C. Samples were vacuum packaged and transported chilled to Hjortens Laboratory in Östersund, Sweden (accredited by SWEDAC according to SS-EN ISO/IEC 17025:2005 for food analysis) where the storage, microbiological sampling and analysis took place according to the protocols of Nordic Committee on Food Analysis (NMKL). The total amount of aerobic microorganisms at the first sampling directly after packaging (three days post slaughter) was 3.4 ± 0.3 $\log_{10}$ CFU/g. After two and three weeks of vacuum packaged chilled storage at +4°C the microbiological quality of the samples was on the border-line to poor (6.8 ± 0.3 $\log_{10}$ CFU/g). At four and five weeks of chilled storage the levels of aerobic microorganisms were significantly highest ($P \leq 0.05$) and the limit for acceptable quality of 7 $\log_{10}$ CFU/g aerobic bacteria had been passed (7.3 ± 0.3 $\log_{10}$ CFU/g and 7.8 ± 0.3 $\log_{10}$ CFU/g, respectively). Very few of the reindeer meat samples were contaminated with *Escherichia coli* bacteria. The results from the present pilot study suggest that storage time for vacuum packaged fresh, chilled reindeer meat should not exceed 3 weeks at a temperature of +4 °C.

Key words: reindeer meat; microbiology; hygienic quality; shelf life; aerobic plate count; *Escherichia coli.*

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Introduction

Reindeer meat is a high quality product with several attributes attractive to consumers – it is tender, has low fat content, a favourable fat composition and high levels of minerals (Hoffman & Wiklund, 2006). In addition, production systems like reindeer husbandry where the animals graze during most of the year are usually considered more animal-friendly and ethical compared with the standard commercial production of beef, pork or poultry (Wiklund & Smulders, 2011).

Reindeer meat is traditionally sold as a frozen product in Sweden however, the demand for fresh chilled meat is slowly increasing. There is very limited knowledge available about the properties of chilled reindeer meat in relation to handling, packaging and storage. In contrast, reindeer meat quality has been investigated from a variety of aspects like pre-slaughter handling, stress, feeding strategies, carcass composition, chemical composition of the meat and carcass handling techniques (Wiklund *et al.*, 2007). Two of the most important meat quality attributes – tenderness and flavour - valued by

consumers as crucial when judging the eating quality of meat have been demonstrated to be unique for reindeer meat compared with other meat types. Reindeer meat is significantly more tender compared with beef (Barnier *et al.*, 1999) and the typical flavour of reindeer meat is a direct reflection of the natural pastures in the forest and on the mountain tundra consumed by the reindeer (Wiklund *et al.*, 2003). It is therefore not unlikely that other aspects of reindeer meat quality like the properties of fresh chilled meat in relation to packaging and storage will differ from those of other meats.

Shelf life of fresh meat is often determined by microbiological quality, *e.g.* the total amount and types of microorganisms present on the meat. A critical limit often used to judge microbiological/hygienic quality of meat is 7 $\log_{10}$ CFU (Colony Forming Units)/g of aerobic microorganisms. Values of 7 $\log_{10}$ CFU/g and above is indicating that the meat is not fit for human consumption (Wiklund *et al.*, 2001; 2010a). Factors of importance for the shelf life of chilled meat are pH value, slaughter hygiene and chilling conditions/temperatures. In countries that export large quantities of chilled meat (South America, Australia and New Zealand) meat pH determines if the meat is to be exported as frozen or chilled. New Zealand is the world-leading producer of deer meat (venison) and exports the best cuts from the superior carcasses as premium chilled products. These high value products are chilled to a temperature of -1.5 °C with a guaranteed shelf life of 12 weeks (Wiklund *et al.*, 2010b). Similar to reindeer meat Red deer (*Cervus elaphus*) venison has also been demonstrated to be different from beef and lamb handled and processed according to the same protocol. The meat industry has therefore been encouraged to review their packaging and storage methods to optimize the quality of each individual meat type (Farouk *et al.*, 2009).

The purpose of this pilot study was to determine shelf life of fresh, chilled reindeer meat stored at +4 °C, measured as microbiological quality (aerobic microorganisms and *Escherichia coli*).

Material and methods

Animals

Six reindeer calves (age 4 months) were included in the study. The reindeer were slaughtered according to standard procedure at Lossen reindeer slaughter plant in Härjedalen, Sweden. Three days post slaughter the chilled reindeer carcasses were transported from the slaughter plant to a processing facility (Blindh Ren AB, Funäsdalen, Sweden) where the sampling was carried out. At boning, pH values were measured in the left side loin (*M. longissimus*) at the last rib. These measurements were taken to guarantee that the six calf carcasses selected for further sampling all would have an ultimate pH value below 5.8 in the loin. The left side loins from the six carcasses were removed and divided in five pieces that were randomly assigned to five different storage times; sampling directly after packaging and after chilled storage for 2, 3, 4 and 5 weeks at +4 °C. All samples were vacuum packaged using a standard vacuum machine (Röscher VM-51/2/CL, Röscher Vakuumtechnik GmbH, Bersenbrück, Germany) and vacuum bags designed for meat products (PA/PE 2-layer material, thickness 140 µm, Finnvacum OY, Helsinki, Finland) and labelled clearly. The samples were then transported chilled to Hjortens Laboratory in Östersund, Sweden (accredited by SWEDAC according to SS-EN ISO/IEC 17025:2005 for food analysis) where the storage, microbiological sampling and analysis took place.

Meat pH

Ultimate pH values in *M. longissimus* (LD, at the last rib) were measured at boning using a

portable pH meter (pH 3310, WTW GmbH, Weilheim, Germany) equipped with a polymer electrode (SenTix®Sp, WTW GmbH, Weilheim, Germany). The pH meter was calibrated at pH 7.0 and 4.0 with buffers (Hamilton Duracal Buffer, Hamilton Bonaduz, Switzerland) stored at room temperature (20 °C).

Microbiological quality

All samples were treated according to the standard sampling protocol of Nordic Committee on Food Analysis (NMKL 91:6, 2010). From each vacuum packaged meat sample, a subsample of 10 g was removed, put in 90 ml of peptone water and homogenized for 30 s before further preparation for analysis of aerobic microorganisms (NMKL 86:4, 2006) and *Escherichia coli* (NMKL 125:4, 2005).

Statistical analysis

The statistical analysis was carried out as a variance analysis (ANOVA) with the JMP® Statistical Software, version 9.0.0 (SAS Institute, 2010). The model for comparing microbiological quality (aerobic plate count) of the samples included the fixed effect of treatment group. Significance was defined as $P \leq 0.05$. The method used for analysis of aerobic microorganisms had a lower detection limit of 3 $\log_{10}$ CFU/g and an upper limit of 8.5 $\log_{10}$ CFU/g. Therefore the values of 2.5 $\log_{10}$ CFU/g and 9 $\log_{10}$ CFU/g were used when the results were outside the detection limits to facilitate statistical analysis. Values below detection limit were found in three samples directly after packaging (three days post slaughter) and values above the detection limit in one sample after 2 weeks of chilled storage at +4 °C.

Results and discussion

For the reindeer samples in the present study the total amount of aerobic microorganisms at the first sampling directly after packaging

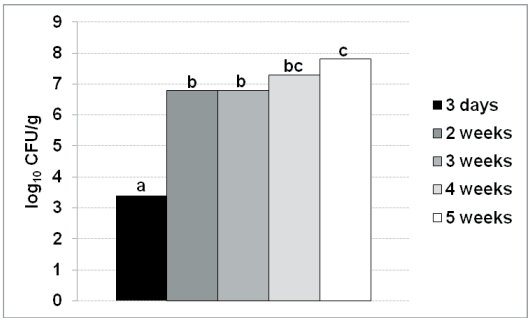

Fig. 1. Microbiological quality ($\log_{10}$ CFU/g aerobic microorganisms) in reindeer samples (*M. longissimus dorsi*, *n*=6 for each treatment) stored in vacuum bags at +4 °C for 3 days, 2, 3, 4 and 5 weeks post slaughter.Means with different letters are significantly different ($P \leq 0.05$).

(three days post slaughter) was 3.4 ± 0.3 $\log_{10}$ CFU/g (means and standard error; Fig. 1). This initial CFU value was significantly lower ($P \leq 0.05$) than any of the other measured values during the storage period. After two and three weeks of vacuum packaged chilled storage at +4 °C the microbiological quality of the samples was on the border-line to poor (6.8 ± 0.3 $\log_{10}$ CFU/g; Fig. 1). At four and five weeks of chilled storage the levels of aerobic microorganisms were significantly highest ($P \leq 0.05$) and the limit for acceptable quality of 7 $\log_{10}$ CFU/g aerobic bacteria had been passed (7.3 ± 0.3 $\log_{10}$ CFU/g and 7.8 ± 0.3 $\log_{10}$ CFU/g, respectively; Fig. 1). Very few of the samples were contaminated with *Escherichia coli* bacteria and no statistical analysis could be performed. In the samples collected three days post slaughter and after two weeks of chilled storage (*n*=6 for each storage treatment), *E. coli* was found in one out of six samples (values of 1.6 CFU $\log_{10}$/g and 3.1 CFU $\log_{10}$/g, respectively). After three and five weeks of chilled storage, *E. coli* was found in two out of six samples (values of 1.5 and 1.7 CFU $\log_{10}$/g and 1.0 and 2.1 CFU $\log_{10}$/g, respectively). In the samples stored at +4 °C for four weeks *E. coli* were found in three out of six samples (values

of 1.3, 2.2 and 3.7 CFU $\log_{10}$/g, respectively). The results from the present pilot study suggest that storage time for vacuum packaged fresh, chilled reindeer meat should not exceed 3 weeks at a temperature of +4 °C.

A recent venison study from New Zealand evaluated microbiological quality and shelf life of chilled red deer loins (*M. longissimus*) (Wiklund *et al.*, 2010a). The average initial value for aerobic microorganisms collected on the slaughter line, *i.e.* immediately after carcass grading and before entering the chilling room, was 0.65 ± 0.50 (mean and standard deviation) $\log_{10}$ CFU/cm^2. The meat samples were collected and vacuum packaged in a similar way to that of the reindeer samples in the present study, however the red deer samples were stored according to normal procedure for New Zealand premium chilled meat products at -1.5 °C. After 3 weeks of storage the CFU values for aerobic microorganisms measured 1.59 ± 0.36 $\log_{10}$/cm^2, and after 9 weeks of chilled storage at -1.5 °C CFU values reached 3.58 ± 2.24 $\log_{10}$/cm^2 (Wiklund *et al.*, 2010a). Comparing the present study on reindeer meat with the red deer venison study, it is clear that the microbiological quality was poorer and therefore the shelf life of the reindeer meat was substantially reduced. One factor of critical importance explaining the results from the present study is that the reindeer carcasses were transported from the slaughter facility to a processing facility before boning and packaging, which means extra manual handling – and higher risk of contamination - of every carcass. In New Zealand all deer are slaughtered and processed/boned at the same facility. The majority of Swedish reindeer carcasses are handled like those of the present study and it is therefore essential that the significance of hygiene during slaughter and handling of carcasses is stressed by the slaughter and processing companies. The increase in demand for fresh, chilled reindeer meat will require a completely different perspective on the relationship between microbiology and shelf life that what currently is the situation when most of the meat is still sold frozen.

Another factor determining microbiological spoilage of meat is the pH value. Meat pH is related to shelf life, tenderness, colour and water-holding properties, and is therefore a good indicator of meat quality. A pH value of 5.5 – 5.7 is within the normal range, while values over 5.8 result in reduced shelf life, especially for vacuum packaged meat (Gill & Newton, 1981). Meat with high pH, so called DFD (Dark, Firm, Dry) meat, is a persistent quality defect found in all meat species. Meat pH values are directly correlated to the levels of muscle energy (glycogen) at the time of slaughter (Gill & Newton, 1981). If the glycogen stores in the muscles are low, meat pH will be elevated. Low muscle glycogen stores might result from poor physical condition, intense physical activity or stress during pre-slaughter handling. A comprehensive survey of reindeer carcasses (n=3400) demonstrated that 29% of the measured pH values were 5.8 and higher, *i.e.* produced meat with an obvious risk of reduced shelf life (Wiklund *et al.*, 1995). Increased demand for and handling of vacuum packed fresh, chilled reindeer meat will immediately highlight any problems with pH values that are currently 'invisible' in the frozen products. As for other sectors in the meat industry, particularly the beef industry, the quality and shelf life of the fresh, chilled meat has to be guaranteed. Routine pH measurements of all reindeer carcasses intended for production of vacuum packaged fresh, chilled meat would contribute to optimizing meat quality and shelf life.

Except for storage time, temperature is the most important extrinsic factor influencing the storage life of fresh meat (O'Keeffe & Hood, 1981; Jeyamkondan *et al.*, 2000). Maximum storage life is achieved when meat is held at -1.5 °C, which is the lowest temperature that

can be maintained indefinitely without the muscle freezing (Gill & Jones, 1992). Hence, to obtain maximum shelf life of chilled beef, lamb, and venison from New Zealand, Australia and South America the meat should be equilibrated to -1.5 °C before load-out from the processing plant and kept at this temperature during storage and the extended transport period (Rosenvold & Wiklund, 2011). There was a substantial difference between the storage temperature used in the present pilot study (+4 °C) and the optimal storage temperature for fresh, chilled meat described above. However, the meat industry in Sweden – including the reindeer slaughter and processing companies – is not set up to operate according to *e.g.* New Zealand handling and transport standards which means keeping the whole handling chain of fresh, chilled meat to a temperature of -1.5 °C. Nevertheless, it would be essential for the reindeer meat companies that now are starting to export fresh, chilled reindeer meat to EU to standardize and possibly adjust/lower the storage temperatures throughout the handling chain. Improved chilling standards, increased awareness around the issues of hygiene and manual handling of carcasses and routine control of meat pH provide significant opportunities to extend the shelf life of fresh, chilled reindeer meat.

Conclusions

The reindeer meat companies that today sell vacuum packaged fresh, chilled reindeer meat normally guarantee a shelf life of 21 days (three weeks). That agrees well with the results from the present pilot study where it was demonstrated that the storage time for fresh reindeer meat should not exceed three weeks at +4 °C. Further studies focusing on aspects of fresh, chilled reindeer meat are recommended, particularly around chilling temperatures, slaughter and processing hygiene and meat pH control.

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Mikrobiologisk kvalitet hos färskt kyllagrat renkött (*M. longissimus dorsi*)

Abstrakt på svenska / sammanfattning: I denna pilotstudie användes ytterfilé (*M. longissimus dorsi*) från sex renkalvar för att studera hållbarheten på färskt, kylt renkött som lagrats vid +4 °C. Hållbarheten mättes som mikrobiologisk kvalitet (antal aeroba mikroorganismer och *Escherichia coli*). Ytterfiléerna samlades in vid styckning 3 dagar efter slakt, delades i fem bitar som slumpmässigt fördelades på fem olika lagringstider; provtagning direkt efter förpackning och efter 2, 3, 4 och 5 veckors kyllagring vid +4 °C. Köttproverna vakuumförpackades och transporterades kylda till Hjortens laboratorium i Östersund, Sverige (ackrediterat livsmedelslaboratorium enligt SWEDAC och SS-EN ISO/IEC 17025:2005), där lagring, provtagning och analys utfördes enligt metoder beskriva av Nordic Committee on Food Analysis (NMKL). Totalantalet aeroba mikroorganismer vid första provtagningen direkt efter vakuumpackning (tre dagar efter slakt) var 3.4 ± 0.3 $\log_{10}$ CFU/g. Efter två och tre veckors kyllagring vid +4 °C var den mikrobiologiska kvaliteten hos renköttsproverna på gränsen till dålig (6.8 ± 0.3 $\log_{10}$ CFU/g). Fyra och fem veckors kyllagring resulterade i de signifikant högsta värdena ($P \leq 0.05$) för aeroba mikroorganismer och att gränsen för acceptabel hygienisk kvalitet på 7 $\log_{10}$ CFU/g hade passerats (4 veckors lagring: 7.3 ± 0.3 $\log_{10}$ CFU/g och fem veckors lagring: 7.8 ± 0.3 $\log_{10}$ CFU/g). Några enstaka renköttsprover var kontaminerade med *Escherichia coli* bakterier. Baserat på resultaten från denna pilotstudie rekommenderas att lagringstiden för färskt vakuumförpackat renkött som hålls kylt vid +4 °C inte bör överstiga 3 veckor.