The effect of Frozen Storage on the Microbiological and Biochemical Status of Camel (Camelus dromedarius) Meat

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ABSTRACT
This study was performed to evaluate the effect of frozen storage on microbial load, and biochemical characteristics of camel meat. Fifteen samples were collected from longissimusdorsi muscles (three replicates) of different camels slaughtered at Al Salam slaughter house in Khartoum state west Omdurman. Measurement of pH, water holding capacity (WHC), total volatile nitrogen (TVN), peroxide value, acid value, and microbial analysis was done on meat samples. Meat samples were frozen for 1, 2, 4, 6 and 8 weeks at -18 °C and undergone the microbial and biochemical analysis. On day 0, the Mean bacterial loads and Coliform counts of meat were $7 \times 10^3$ and $5 \times 10^3$ cfu/g respectively. On 8th week the mean bacterial loads and Coliform counts stored at -18°C were $3 \times 10^2$ and $2.5 \times 10^2$ cfu/g respectively. Types of the bacteria isolated were Staphylococcus aureus, E.coli, Citrobacter, Salmonella spp, Enterobacteraerogenes, klebsielaspp, Pseudomonas aeruginosaand Proteus spp. with percentage rate 12%, 11%, 15%, 1%, 16%, 12%, 5% and 15% respectively. The study indicated that the freezing storage increased the water holding capacity, total volatile nitrogen (TVN), acid value and pH significantly (p<0.05). The peroxide value was not affected by freezing storage time. In summary the study showed that the storage time at -18°C affects on the microbial and biochemical status of camel meat. 

Keywords: Camel, Freezing, Meat, Microbiological and Biochemical status

INTRODUCTION
The Dromedary one-humped camel (Camelus dromedarius) is representing two thirds of the world’s camel population, particularly in North East Africa, i.e. Somalia, Sudan, Ethiopia and Kenya (FAO, 2011). The role of the camel as a meat producer is becoming more important due to the versatile role it plays rather than as a symbol of social prestige, which was the role it used to play but which has since greatly diminished (Dawood and Alkanhal, 1995). The dromedary camel is a good source of meat especially in areas where the climate adversely affects the performance of other meat animals (Kadim et al., 2008). Camel can provide a substantial amount of high quality meat. The demand for camel meat appears to be increasing due to health reasons, as they produce carcasses with less fat as well as having less cholesterol and relatively high polyunsaturated fatty acids than other meat animals (Jouki, 2012) The quality of meat produced by younger animals was in comparable with beef in taste and texture (Babiker and Yousif, 1990).
flora from different sources. Also, several methods have been proposed for decreasing the microbial flora to a standard allowance for increasing the shelf-life and decontamination of microbial pathogens including cooking, freezing, fermenting, salting, smoking, drying, and pickling (Al Sheddy et al., 2004 and Kalalou, 2004). The aims of preservation methods are: (a) to inhibit the microbial spoilage and (b) to minimize the oxidation and enzymatic spoilage. Freezing is an excellent method of keeping the original characteristics of fresh meat. Freezing rate (slow and fast) affects the quality of frozen meat significantly. Fast freezing produces better quality meat than slow freezing. During slow freezing formation of large ice crystals damages the cell and results in protein denaturation. Concentration of enzymes and presence of other compounds govern the process of protein denaturation (Dave and Ghaly, 2011). Freeze storage cannot prevent oxidative spoilage and microbial/ enzymatic spoilage (Jay et al., 2005). Meat can be frozen for months in domestic refrigerators or in commercial freezers, some changes that affect certain properties of fresh meat can occur while frozen, such as, water holding capacity, pH, color, firmness and texture (Cristina et al., 2005). The aim of this study was to evaluate the changes in various biochemical properties and microbial loads of camel meat during the freezing storage.

MATERIALS AND METHODS

Samples collection: a sample 15 pieces (three replicates) of longissimusdorsimuscles was obtained randomly from different camel carcasses which slaughtered at Al Salam slaughter house in Khartoum estate west Omdurman. The samples were collected in sterile polyethylene bags and transported in a clean cool box contains ice cubes immediately to the laboratory for analysis. The Samples were stored at refrigerator - 18°C and undergone the biochemical and microbial analysis.

**Total volatile nitrogen (TVN):** TVN was determined according to the method described by Mwansyemela (1973). Apportion of 10 g of the minced meat was mixed with 2g magnesium oxide in 300 Ml of distilled water in a distilling flask of macro Kjeldahl for distillation. The distillate was collected in a receiving flask that contained 25 mL of 2% boric acid and a few drops of 0.1% methyl red and bromocresol green indicators. The distillate solution was finally titrated with 0.1 N sulfuric acid and the amount of TVN expressed as mg N/100 g meat.

**Biochemical analysis:**

**pH values:** pH were determined by taking 10 g of sample and mixed with 20 ml distilled water for one minute then measured by digital pH meter.

**Peroxide value (AV):** peroxide value indicated the lipid oxidation and rancidity. The PV value was determined according to AOAC method (1984).

**Water holding capacity (WHC):** water holding capacity was measured using the method of Hung and Zayan (1992).

**Microbial analysis:** isolation and identification was done according to Cowan and Steel's (1974). Aseptically 1gm of camel meat was diluted in 10 ml buffered peptone water. Serial decimal dilutions were made and following analysis were carried out on duplicate agar plates: (a) total viable count on nutrient agar incubation at 37°C for 24hrs, (b) total coliform count on MacConkeyagar, E.coli spp., Enterobacter aerogenes, Proteus spp, Pseudomonas aeruginosa and Citrobacter spp. on EMB agar, Salmonella sppandKlebsiela on XLD.
agar, and *Staphylococcus aureus* on manitol salt agar.

**Statistical analysis:** all samples were duplicated and the collected data were evaluated statistically by SPSS software (1979). One way ANOVA was used for comparing the means at (p<0.05).

**RESULTS AND DISCUSSION**

The effects of storage time on the pH of camel meat were determined (Table 1) the pH value increased significantly (p<0.05) during frozen storage period and this, is in agreement with those reported by Apata, (2014), and Kandeepan and Biswas, (2007). The increase of pH after 40 days of storage is assumed to be an accumulation of some basic substances due to denaturation of protein during frozen storage, also microorganisms and endogenous enzymes degrade meat proteins, produce ammonia and organic sulphides and amines, which increase pH (Azad and Akter, 2005).

The significant increase in pH with prolonged freezer storage may be attributed to the fact that meat undergoes autolysis resulting in decrease in extract release volume (ERV) and WHC with increase in pH (Strange *et al.*, 1977). Table (1) showed that WHC was decreased significantly (p<0.05) between 1st to 8th week and this results are in agreement with the findings of Banani *et al.*, (2006) who reported decrease in water holding capacity of frozen goat meat from 18.06 to 3.24% over 15 days of freezing preservation. The decrease of WHC in prolonged storage of meats may be due to the rate in post mortem pH falls, ice crystal formation, high ionic strength, protein denaturation, drip loss and above all, the bulk of meat stored and the capacity of the refrigeration facility (Lawrie, 1998), and also may due to increased denaturation of protein and partly due to enhanced movement of water into extracellular spaces (Kandeepan and Biswas, 2007). The extent of the damage caused by freezing depends on the size of the ice crystals, and slow freezing rates produce the largest crystals, which cause higher protein denaturation, this produces the loss of water holding capacity (Devine *et al.*, 1995).

**Table 1: Mean values (± STD) of biochemical parameters of camel meat during storage time (-18°C)**

<table>
<thead>
<tr>
<th>Storage time</th>
<th>pH</th>
<th>WHC%</th>
<th>PV%</th>
<th>TVN (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day0</td>
<td>5.57±0.08d**</td>
<td>47.25±1.43***</td>
<td>0.61±0.06**</td>
<td>9.87±1.25***</td>
</tr>
<tr>
<td>Week 1</td>
<td>5.58±0.27d</td>
<td>47.36b±1.98a</td>
<td>0.63±0.06c</td>
<td>11.36±1.47b</td>
</tr>
<tr>
<td>Week 2</td>
<td>5.61±0.10d</td>
<td>48.79±2.09c</td>
<td>0.66±0.07d</td>
<td>13.69±1.73d</td>
</tr>
<tr>
<td>Week 4</td>
<td>5.85±0.05b</td>
<td>44.93±2.31c</td>
<td>0.69±0.08e</td>
<td>15.40±1.10f</td>
</tr>
<tr>
<td>Week 6</td>
<td>5.96±0.09b</td>
<td>37.95d±0.91d</td>
<td>0.75±0.09a</td>
<td>17.27±1.05b</td>
</tr>
<tr>
<td>Week 8</td>
<td>6.18±0.11a</td>
<td>37.78d±0.91d</td>
<td>0.79±0.09a</td>
<td>19.21±1.27a</td>
</tr>
</tbody>
</table>

*= There is a significant difference (P≤0.05) **= There is a high significant difference (P≤0.001)

a,b,c,d,e,f: values in the same column with different superscript are significantly different (p<0.05).

Total volatile nitrogen (TVN) and Peroxide value (PV) were increased significantly (p<0.05) during frozen storage period (Table 1), this result is in agreement with Azad and Akter, (2005) who reported that the TVN and PV of frozen beef samples were gradually increased with advancement of storage period, also Simitzis and Deligeorgis, (2010) reported that Polysaturated fatty acids are more susceptible to lipid oxidation. Lipid oxidation is one of the main factors limiting the quality and acceptability of meat and meat products. This process leads to discoloration, drip losses, off odour and off flavour development, and the production of potentially toxic compounds. Hydroperoxides, the primary initial products of lipid
oxidation, are essentially odorless, but will decompose to a variety of volatile and non-volatile secondary products (Hamid et al., 2009). Hydroperoxides are produced due to the lipid oxidation of highly unsaturated fatty acid fractions of membrane phospholipids, which are susceptible to further oxidation/decomposition. This suggested that the camel meat would be susceptible to the development of rancidity due to its high content of polyunsaturated fatty acids (Kadim et al., 2006). As fresh meat is a rich medium for the growth of microorganisms, it will ultimately spoil as a consequence of such growth unless frozen to temperatures too low for microbial growth to occur. Proteolytic enzymes (internal or microbial source) decompose the structural meat proteins and produce nitrogenous compounds. Therefore, total volatile bases nitrogen (TVN) measurement can help spoilage diagnosis of meat. Freezing rate (slow and fast) affect the quality of frozen meat significantly. Fast freezing produce better quality meat than slow freezing. During slow freezing formation of large ice crystals damages the cell and results in protein denaturation. Concentration of enzymes and presence of other compounds govern the process of protein denaturation (Rahman, 1999). The results indicated that the total viable count and coliform count were decreased significantly (p<0.05) during the frozen storage (Table 2) and (Figure 1), this result is in agreement with the findings of Jouki et al., (2012) who reported that Microbial analysis indicated that freezing storage of Turkey meat samples had a significant effect (p<0.05) on the reduction of microbial loads.

**Table 2: Growth of microbial count (mean± STD) on camel meat during storage time (-18°C)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total viable count (cfu/g)</th>
<th>Coliform count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day0</td>
<td>$7.0 \times 10^{3} \pm 0.13 \times 10^{3}$ ***</td>
<td>$5.0 \times 10^{3} \pm 0.2 \times 10^{3}$ ***</td>
</tr>
<tr>
<td>Week 1</td>
<td>$4.7 \times 10^{3} \pm 0.60 \times 10^{3}$ b</td>
<td>$2.5 \times 10^{3} \pm 0.45 \times 10^{3}$ b</td>
</tr>
<tr>
<td>Week 2</td>
<td>$3.6 \times 10^{3} \pm 1.40 \times 10^{3}$ c</td>
<td>$1.6 \times 10^{3} \pm 0.30 \times 10^{3}$ c</td>
</tr>
<tr>
<td>Week 4</td>
<td>$3.3 \times 10^{3} \pm 1.31 \times 10^{3}$ c</td>
<td>$0.4 \times 10^{3} \pm 0.03 \times 10^{4}$ d</td>
</tr>
<tr>
<td>Week 6</td>
<td>$0.3 \times 10^{3} \pm 0.02 \times 10^{3}$ d</td>
<td>$0.3 \times 10^{3} \pm 0.06 \times 10^{3}$ d</td>
</tr>
<tr>
<td>Week 8</td>
<td>$0.2 \times 10^{3} \pm 0.02 \times 10^{3}$ d</td>
<td>$0.2 \times 10^{3} \pm 0.027 \times 10^{3}$ d</td>
</tr>
</tbody>
</table>

* **= There is a high significant difference (P≤0.001)
  a,b,c,d,e,f: values in the same column with different superscript are significantly different (p<0.05).*

**Figure 1: Change in total viable count and Coliform count during storage time**
The damage in cell membranes and DNA denaturation are the probably causes for the death of bacterial cells during freezing and thawing. It is also hypothesized that there is a damage of some proteins because of the increase of salt concentration into the cells (Panoff et al., 1998). Microbial contamination can reduce the quality of fresh meat, shorten its shelf life and cause economic losses and health hazards. The use of chilling, freezing as well as organic acids and their salts have been investigated for the preservation of beef, lamb and other types of meat (Al Sheddy, 1994).

Survival rates after freezing will depend on the precise conditions of freezing, the nature of the food material and the composition of its microflora, but have been variously recorded between 5 and 70 %. Bacterial spores are virtually unaffected by freezing, most vegetative Gram-positive bacteria are relatively resistant and Gram-negatives show the greatest sensitivity (Rahman, 1999).

Storage time can be extending through hygienic slaughtering and clean handling of the carcass (FAO, 1990).

Figure (2) shows the Enterobacteria isolated as contaminated bacteria from carcasses of camel and all are pathogenic or food born bacteria, the isolated bacteria were Staphylococcus aureus, E. coli, Citrobacter, Salmonella, Enterobacteriaerogenes, klebsiela, Pseudomonas aeruginosa and Proteus spp. with percentage rate 12%, 11%, 15%, 1%, 16%, 12%, 5% and 15% respectively. (Dave and Ghaly, 2011) reported seventeen genera of Predominant spoilage bacteria found in frozen meat include all bacteria isolated in this research. Meat and meat products provide excellent growth media for a variety of microflora (bacteria, yeasts and molds) some of which are pathogens, the intestinal tract and the skin of the animal are the main sources of these microorganisms (Dave and Ghaly, 2011).

CONCLUSION

Result of this study concluded that the frozen storage time at -18°C had reduced the quality of camel meat and a significant effect on biochemical and microbial status.

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