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MICROBIOLOGICAL CHARACTERISATION OF ONE HUMPED CAMEL MEAT IN MOROCCO

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ABSTRACT

In this study, samples of camel meat (*Camelus dromedarius*) were collected from 2 slaughterhouses. All the samples were taken from carcasses after set up of the rigor mortis, the same day they are slaughtered. Samples were analysed for their microbiological characteristics, which included Standard Plate Count (SPC), *Enterobacteriaceae*, enterococci, staphylococci, *Salmonella* spp, sulphite-reducing *Clostridium*. Results showed that the microbial profiles were relatively low for all the micro-organisms studied. The average SPC was 7.17×10^4 cfu/g, coliform numbers ranged from less than 10 to 5×10^4 cfu/g. Enterococci reached an average of 3.84×10^3. Staphylococci were the most abundant micro-organisms in the product and ranged from 110 cfu/g to 2.4×10^4 cfu/g. Salmonella was not detected in any sample. 5.88% of the staphylococci isolates revealed DNAse positive and phosphatase positive. Fifty three per cent of the coliforms were identified as *Klebsiela* spp/*Enterobacter* spp, 31% as *E.coli* and 3% as *Citrobacter* spp.

Key words: Camel, meat, microbiology, quality

The one humped camel (*Camelus dromedarius*) is a very interesting species for meat and milk production (Yousif and Babiker, 1989; Kadim *et al,* 2008). In Morocco, as well as in many other African and Asian countries, the food customs include camel meat as a very popular eatable.

Furthermore, the demand for camel meat appears to be increasing due to health reasons. Camel meat contains less fat, lower cholesterol and relatively high polyunsaturated fatty acids compared to beef (Rawdah, *et al,* 1994; Dawood and Alkanhal, 1995; Kadim *et al,* 2008, Gheisari *et al,* 2009). These characteristics help reducing cardiovascular diseases risk related to high saturated fat consumption (Giese, 1992).

The hygienic quality of red meat continues to attract attention globally. Potential spoilage bacteria and pathogens are generally associated with red meat include *Bacillus cereus, Staphylococcus aureus*, *Pseudomonas* spp, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* spp (Eisel *et al,* 1997; Nel *et al,* 2004; Jones *et al,* 2008). Elevated numbers of these organisms have been said to be related to food-related illness. In contrast there is little information on the hygienic quality of camel meat, even they are widely consumed in African and Asian countries and they may be components in range of processed meat products.

The aim of this research focused on the microbiological quality of camel meat purchased from retail stores in Morocco.

Materials and Methods

Samples collection

Fresh meat samples of camel meat were collected from 2 slaughterhouses in Morocco (Temara and Settat). Carcasses of one year old dromedary camels are sold the same day they are slaughtered. A 500 g of meat was put in a plastic bag and transported using refrigerated box (4°C) to the laboratory for the microbiological analyses which were done immediately.

Microbiological determinations

Ten gram of each sample was blended in 90 ml of saline water (8.5gm/l) with a warring blender to prepare the initial dilution (10^{-1}). From this dilution serial dilutions up to 10^{-6}, were prepared in tubes containing 9 ml of saline water.

SPC: Standard Plate Count

Appropriate serial dilutions (10^{-1} to 10^{-6}) of the samples in saline water (8.5% NaCl) were pour plated on standard plate count agar (PCA) (Biokar, France). The plates were incubated at 30°C for 48h.

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Enterobacteriaceae ***counts***

These were enumerated on deoxycholate agar (Merck, Germany). The plates were incubated at 37°C for total coliforms and at 44°C for faecal coliforms for 24h. The appeared colonies on the medium were streaked on the same medium for more purification. Isolated colonies were cultured on trypticase soy agar slants and incubated for 24h. Cultures were stored at 4°C until identification according to the IMViC test.

Staphylococci

Dilutions up to 10^{-6} were plated on mannitol salt agar (Merk, Germany). The plates were incubated at 37°C for 24h. The small yellow colonies on the medium were counted and checked for their catalase and Gram reactions. Catalase positive Gram negative colonies were spread cultured on trypticase soy agar slants for further determinations of DNAse and phosphatase.

Phosphatase: The strains were grown on BHI (Brain heart Infusion, Biokar, France). The 0.5 ml of culture was added to 0.5 ml of nitrophenylphosphate in small tubes and incubated at 37°C for 5 to 18h. A positive reaction is revealed by a yellow colour of the reagent.

DNAse: The deoxyribonucleic acid agar (Merck, Germany) was prepared, autoclaved and poured in plates which were allowed to solidify. The strains were surface spot inoculated (5 spots/ plate) on the medium and the plates were incubated at 37°C for 24h. Reactions were revealed by pouring a chlorhydric acid solution (1N) on the plate surface. Clear zones around the cultures indicate a positive reaction.

Enterococci

The MPN (most probable number) using 3 tubes per dilution was determined on Azide Dextrose Broth (Difco Laboratory, USA). Incubation was done at 37°C for 24h. Tubes that had shown growth were propagated on Ethyl Violet Azide broth (Difco Laboratory, USA) and incubated at 37°C for 24h. Positive tubes were revealed by growth and formation of a violet precipitation in the bottom of the tubes. The number of positive tubes is reported to the table for the most probable number of the enterococci in the sample.

Salmonella

Twenty five gram of the sample were added to 100 ml of sterile buffered peptone water (BPW) (Merck, Germany) and incubated for 18h at 37°C. Two tubes of tetrathionate broth and 2 tubes of selenite cystein broth (Merck, Germany) were inoculated with 1 ml from the BPW and incubated for 24h at 37°C. Positive tubes of both media were streaked on Hektoen agar (Merck, Germany). The method described by Poelma *et al* (1984) was used for the identification of the suspected colonies blue green white with or without dark center.

Spore forming bacteria

The initial dilution was heat activated at 80°C for 10 min and immediately cooled in iced water. Anaerobic sulfite reducing *Clostridium* were grown on SPS medium (Merck, Germany) in tubes which were then inoculated with 2, 1 and 0.5 ml of the heat activated dilution and incubated at 30°C for 24h. Dark colonies were counted.

Results and Discussion

In healthy animals, combination of the immune system and the physical barrier adequately protect organs and muscles against microbial invasion. Therefore, muscle tissues from freshly slaughtered carcasses should be relatively free of bacterial contamination. The surface of the skin and gastrointestinal tract are, however, heavily colonised with bacteria and provide a source of cross contamination during processing. For example, faeces and soil can bring micro-organisms such as *Micrococcus, Staphylococcus* and *Pseudomonas* spp. As faeces and soil can come into direct contact with animal surfaces, removal of hides during processing can contaminate tissues via the skinning or handling. Fresh meat is an ideal source of nutrients (rich in nitrogenous compounds, minerals, water etc) and therefore, bacterial spoilage and food borne pathogenic bacteria of meat are greatly influenced by the sanitary conditions of the carcass and processing systems (Davies and Board, 1998; Nel *et al,* 2004; Castellano *et al,* 2008).

Several studies have been conducted on the microbiological quality of red meat, poultry and their products (Chawla and Chander, 2004; Phillips *et al,* 2006; Gill, 2007). Bacteria originating from the animal during slaughter, contaminate the carcass, and subsequently be distributed via cut or raw meat intended for further processing (Borch and Arinder, 2002).

Standard plate count

Aerobic plate counts are widely used to determine the general degree of microbial contamination (Aberle *et al,* 2001). The standard plate

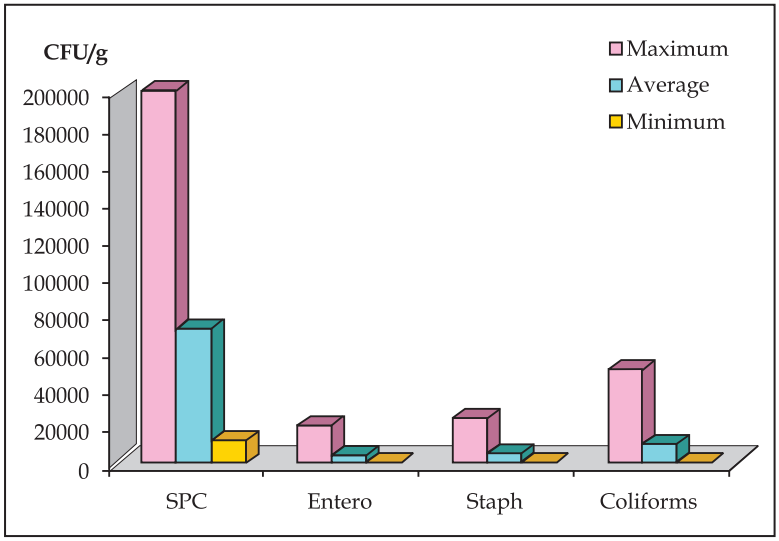

Fig 1. Minimum, maximum and average of standard plate count (SPC), total Coliforms counts, *staphylococci* counts and Enterococci counts in fresh camel meat samples.

counts ranged between $1.2x10^4$ and $2x10^5$ cfu/g. with an average of $7.17x10^4$ cfu/g (Fig 1). This value was significantly lower, either than the maximal limit ($5x10^6$ cfu/g) or than the minimal limit ($5x10^5$ cfu/g) set by the National Health Ministry for red retail meat (Kingdom of Morocco, 2004). This is a low microbial load regarding the conditions where carcasses are slaughtered. The low counts would not indicate suitable conditions of slaughtering but it could suggest that this kind of meat is fresh and also let suppose that it would resist food invading micro-organisms.

Enterobacteriaceae

The family *Enterobacteriaceae* includes *E. coli*, *Shigella* spp, *Edwardsiella* spp, *Salmonella* spp, *Citrobacter* spp, *Klebsiella* spp, *Enterobacter* spp, *Serratia* spp, *Proteus* spp, *Morganella* spp, *Providencia* spp and *Yersinia* spp and therefore, present a holistic view of the presence of these organisms on the product. The results for *Enterobacteriaceae* obtained during this study are also indicative of possible faecal contamination, ranged from 100 to $5x10^4$ cfu/g, with an average of $9.5x10^3$ cfu/g. This range is lower than the maximum national limit stipulated by the the Kingdom of Morocco (10^4 cfu/g). Coliforms colonies from each sample were isolated and characterised by the IMViC test. This showed that 31% of the isolates were identified as *E. coli*, 53% of the checked colonies were identified as *Klebsiella* or *Enterobacter* while *Citrobacter* represented 3% (Table 1). The maximum limit proposed by the Kingdom of Morocco (2004) is 500 cfu/g (*E. coli*). In a study by Eisel *et al* (1997), the average counts for *E. coli* on retail cuts were between 1 and 2 cfu/g (product) and 1–2 cfu 100 cm^{-2} (surface), much lower compared to this study. *E. coli* may be used as an indicator micro-organism because it provides an estimate of faecal contamination and poor sanitation during processing (Eisel *et al*, 1997). It should be kept in mind that high levels of this organism could be indicative of exposure to faecal pollution originating from improper slaughtering techniques, contaminated surfaces and/or handling of the meat by infected food handlers. Therefore, it is of the utmost importance that *E. coli* levels be kept as low as possible during slaughtering, through sanitary practices during initial carcass handling such as the evisceration process where intestinal material (source of *E. coli*) may come into contact with the carcass.

Salmonella spp

In this study *Salmonella* spp was not detected in any sample. The National limit stipulated by the Kingdom of Morocco is 0 cfu/25 g. The likely sources of this micro-organism probably due to incorrect slaughtering practices, which implies that intestinal material known to contain *Salmonella* spp contaminated the meat. Workers who practise poor personal hygiene and are carriers of *Salmonella* spp; thus may also contaminate the meat with the organism. According to Berends *et al* (1997), elevated levels of *Salmonella* spp that may be associated with animals slaughtered during a particular day may lead to elevated levels of the organism on meat derived from such animals. Berends *et al* (1997) furthermore, reported that once a production line is contaminated with *Salmonella* spp, the micro-organisms will establish itself on the machinery, equipment and hands of workers and cause cross-contamination.

In a study done by Bacon *et al* (2002), the prevalence of presumptive *Salmonella* spp was reported to be 1.3% in 8 slaughtering plants. In a

Table 1. Proportions of coliforms species isolated from camel meat samples.

	E. coli	*Citrobacter* spp	*Klebsiela* spp/ *Enterobacter* spp	**Non identified**
Number	10	1	17	4
%	31.25	3.12	53.12	12.5

similar study by Madden *et al* (2001), similar levels were found with 1.5% of the samples being positive for presumptive *Salmonella* spp.

Other studies in UK concerning the prevalence of Salmonella in red meats also found the rate of Salmonella contamination to be higher in other meats (Hare, rabbit, venison, goat, mutton, rabbit) (2.1%), followed by pork (1.9%), lamb (1.7%) and beef (1.1%), respectively (Little *et al,* 2008).

These results are notably much higher than the results obtained in the present study with camel meat despite the poor hygienic condition during slaughtering, cutting and sale of these meat samples. The absence of *Salmonella* spp in all samples, may also be explained by the evidence that these micro-organism could not be isolated from low contaminated materials or it may exist in low numbers that their research is not usually successful.

Staphylococcus aureus

Staphylococci counts reached an average of $5.26x10^3$ cfu/g in camel meat. This level indicates that the product is highly contaminated; it exceeds the maximal level authorised by the Moroccan regulations $5x10^2$ cfu/g (Kingdom of Morocco, 2004). Colonies from each sample were isolated and characterised for the DNAse and phosphatase formation. This showed that 2 isolates out of the 34 colonies studied showed positive reactions for both tests (table 2). The occurrence of *Staphylococcus aureus* on raw meat would be expected, because it is a principal component of the skin of humans and animals (Genigeorgis, 1989; Costa *et al,* 2004; Kloos and Bannerman, 2005).

The pathogenicity of *S. aureus* and its ability to cause diseases is attributed to a number of virulence factors such as the heat stable enterotoxins (Sandel and McKillip, 2004).

The mean count range of *S. aureus* in the different meats was examined by Al-Tarazi *et al* (2009) ranged from $5.3x10^2$ to $4.3x10^4$ cfu/g. They reported that 10^3 cfu/g is the highest permissible count of *S. aureus* commonly specified by the international agencies (Sally and Mark, 2003), then they consider this number of Staphylococcus as low degree of contamination. In more, Le Loir *et al* (2003) also noted that this low contamination is tolerated in most foodstuffs and they are not considered a risk for public health. This is expected because in fresh or chilled meat, *S. aureus* is not a good competitor with normal microflora (Jay *et al,* 2005). In addition, freezing meats significantly reduces the mean viable population of *S. aureus* (Bachhil, 1998). The minimum number of $5x10^6$ cfu/g *S. aureus* is required to produce a sufficient amount of enterotoxin to cause Staphylococcal food poisoning (Garbutt, 1997). High *S. aureus* counts and possible food poisoning usually results from food abuse particularly in cooked or ready to serve food. Therefore, presence of low number of *S. aureus* in fresh food does not necessarily guarantee that such meat is not hazardous for consumers (ICMSF, 1986).

Table 2. Proportions of toxigenic *staphylococci* strains isolated from camel meat samples.

Number	%	DNase	Phosphatase
24	70.58	-	-
6	17.64	+	-
2	5.88	-	+
2	5.88	+	+

Al Tarazi *et al* (2009) noted that camel meat possess the highest prevalence rate of *S. aureus* in samples examined and this might be related to the sticky fresh camel's meat, which enable the adherent micro-organisms to resist dislodging by the subsequent carcass washing (Gracey *et al,* 1999; Wilson, 1999).

In a study done by Vorster *et al* (1994) on ground beef, the mean *S. aureus* count was 2.5x10 cfu/g^{-1}. This count is much lower when compared with the results of this study, keeping in mind that the retail cuts is not yet processed, which may subsequently increase the levels of *S. aureus* in these meat samples to even higher levels. In another study by Desmarchelier *et al* (1999), the incidence of *S. aureus* ranged between 33%, 60% and 12.5% for carcasses after overnight chilling at three different abattoirs compared with the 5.88% incidence of *S. aureus* in this study. The high incidence of *S. aureus* could be the result of frequent contamination during slaughtering, dressing and eviscerating. Desmarchelier *et al* (1999) mentioned that such high incidences of *S. aureus* on beef samples is of particular concern because it may be a source of contamination to other foods and may represent a risk in processed foods (as mentioned earlier for ground beef). Therefore, it is essential to reduce the incidence of *S. aureus* on retail cuts. Furthermore, if this high incidence of *S. aureus* is added to the temperature and storage abuse, a possible outbreak can occur (Forsythe, 2000).

Clostridium was not detected in any sample. The anaerobic micro-organisms involved in food

alterations and or food poisoning are represented by sulfite-reducing *Clostridium* strains. In a study conducted by Nel *et al* (2004) in South Africa, meat samples were collected from a deboning room of a high throughput abattoir. The average count over the sampling period was $1.72x10^5$ cfu/g for *S. aureus* and for *E. coli* 3.4 10^5 cfu/g. Sixty per cent of the samples were positive for presumptive *Salmonella* spp while the aerobic plate and *Enterobacteriaceae* counts were $1.7x10^7$ and $4.6x10^6$ cfu/g, respectively.

Conclusion

The microbiology of meat is greatly dependent on the conditions under which animals are reared, slaughtered and processed. Thus the physiological status of the animal at slaughter, the spread of contamination during slaughtering and processing, the temperature, and other conditions of storage and distribution are the most important factors that determine the microbiological quality of meat. As the inherent antimicrobial defence mechanisms of the live animal are destroyed at slaughter, the resultant meat is liable to rapid microbial decay.

Although, some hazardous micro-organisms were present in the camel meat samples, proper cooking, manipulation, and processing of the camel meat could decrease the hazard. However, considering the poor slaughtering practices and an inappropriate hygienic conditions in retail stores, thus can say that the microbiological quality of camel meat in Morocco is acceptable. Therefore, there is a need to stress the importance of correct handling of fresh meat and meat products, both at a domestic and commercial level. Thus there is a need to establish standards of compliance for camel meat to give the consumer a reliable safe product.

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