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Research Article

Assessment of Factors Influencing the Hygienic Quality of Retail Beef Meat in Meknes City, Morocco

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ABSTRACT

In Morocco, beef meat consumption has undergone a remarkable evolution in the last years. The aim of this study is to evaluate the microbiological quality of beef meat marketed in the city of Meknes and to determine the factors influencing its contamination. Ninety representative samples were taken from different sites located in supermarkets, butcheries and souk (Weekly market) during winter and summer seasons of 2017. These samples were submitted to enumeration of Total Aerobic Bacteria (TAB), Total coliforms (TC), faecal coliforms (FC), *Staphylococcus aureus* (St), *Clostridium perfringens* (Cp), *Salmonella*, and *Listeria monocytogenes.* The average contamination was 6.33 for TAB; 5.50 for FC; 5.41 for TC; 2.21 for Cp and 1.68 for St in log CFU/g. *Listeria monocytgenes* was detected in 8.8% of samples, while 10% showed the presence of *Salmonella*. 66.67% of samples were unfit for consumption. The high compliance to standards was registered in summer (35.55%) and samples collected from Souk were the least contaminated during two seasons (62.5%). The level of contamination depends on sampling site, and seasonal variation. The comparison between sampling sites showed that the length of conservation has an important effect on the hygienic quality of beef meat.

Key words: Beef meat, Foodborne diseases, Hygiene, Seasonal variation, Morocco

INTRODUCTION

According to the World Health Organization (WHO), Foodborne diseases represent a major public health concern worldwide causing many illnesses (WHO, 2015). In USA, the pathogens transmitted generally by foodstuffs accounted each year for 9.4 million episodes of illnesses, 55,961 hospitalizations, and 1,351 deaths (Scallan *et al.*, 2011), moreover they were responsible for 24 029 infections and 98 deaths in 2016 (Marder *et al.*, 2017). In France, from 2008 to 2013 1.28–2.23 million foodborne illnesses are registered each year, with 16,500–20,800 hospitalizations and 250 deaths (Van *et al.*, 2017). In Morocco, since 1980, foodstuffs are often classified among the three most incriminate toxins. According to the to the Poison Control Center of Morocco (MPCC), 17,896 cases of Food borne diseases were registered by health facilities in Morocco during 1989-2008, representing the first poisoning cause in the country (Hadrya *et al.*, 2012), while 7729 diseases were declared by the center during 2013-2015. Red meat is responsible for a high proportion of these diseases worldwide (Fegan *et al.*, 2018). In 2016, the global production of red meat reached 320.6 million tons in which 68.3 million tons of beef meat. Beef carcass contains an important level of protein, vitamins, minerals and micronutrients which are very adequate to the development of pathogenic microorganisms (Bender, 1992). In Morocco, beef cattle is the first source of red meat and it reached 3.1 million head in 2014, of which 60% was destined for the meat sector. Moreover, Moroccan Production of beef meat was increasing from 2.8 to 3.3 million head during 2007-2016 (Ministry of Agriculture, 2017).

In order to determine the hygienic quality of red meats and more specifically those of cattle, and to highlight the factors influencing its contamination, also to fight against foodborne diseases caused by this foodstuffs, our study will contribute, for the first time, to the improvement of the quality of beef meat, marketed and consumed in Meknes city, Morocco.

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MATERIALS AND METHODS

Samples collection: Ninety samples of beef carcass were collected from many shopping sites in Meknes city, three different supermarkets, butcheries in seven areas, and Souk (Weekly market combines the population of the small villages around the city). The collection was carried out under aseptic conditions during two periods, 45 samples in winter (from 1 December to 28 February 2017) and 45 in summer (from 1 June to 31 August 2017). The sampling frequency is 15 samples per month (4 from supermarkets, 7 from butcheries and 4 from Souk). They were brought to the laboratory in a cooler maintained at 4°C, within a maximum of 24 hours.

Microbiological evaluation: Microbiological analysis concerned microorganisms whose isolation is required by the microbiological criteria and that affect the hygienic quality of meat. 25 g of beef meat was mixed with 225 mL of buffered peptone water (Oxoid, England), after than the mixture was grounding in a Masticator (Stomacher 400 Circulator, Seward) for 1 min at 260 rotation per minute (RTM). A series of decimal dilution were prepared to carry out the enumeration of total aerobic bacteria (TAB) using the incorporation technique on the medium Plate Count Agar (PCA, Biokar, France) before incubation at 30°C (NF V08-051, 1999). Total coliforms (TC) and faecal coliforms (FC) were performed by the incorporation technique into of Violet Red Bile Lactose Agar (VRBL, Biokar, France), before incubation at 30°C for total coliforms (ISO 4832, 2006) and at 44°C for faecal coliforms (NF V08-060, 2009). Clostridium perfringens (Cp) was counted using the Tryptose Sulfite Cycloserine Agar medium supplemented with D-cycloserine (TSC, Biokar, France) with an anaerobic incubation at 37±2°C for 24 to 48 hours (ISO 7937, 2004). The enumeration of Staphylococcus aureus (St) is carried out using the Baird Parker medium (BP, Biokar, France) with incubation at 36±2°C for 24 to 48 hours (NF V08-057, 2004). The research and isolation of Listeria was performed according to the ISO standard (ISO 11290-1, 2004), while Salmonella Spp. is researched and isolated using the AFNOR standard (NF U47-100, 2007).

Statistical analysis: Statistical analysis of data was performed by Microsoft Office Excel (2010) and IBM SPSS Statistics 22. The results of this study were interpreted according to rules of the Moroccan Minister of Agriculture and Health, while the effect of season and sampling sites on the hygienic quality of analyzed meat was performed using Student test with α=0.05.

RESULTS AND DISCUSSION

The results of microbiological analysis of beef meat and the detection of pathogenic bacteria showed that 66.67% (60/90) of analyzed samples do not meet the standards hygiene. The Table 1 summarizes the averages, the standard deviations, minimum and maximum values, of microorganisms counted in CFU/g, percentages of compliance and samples with a satisfactory quality.

Total aerobic bacteria (TAB)

The average contamination of beef meat by TAB is 6.33 log CFU/g, with a minimum value of 4.22 log CFU/g registered in a Souk, and a maximum value of 7.17 log CFU/g recorded in a butcher shop. The compliance study classifies 78/ 90 (86.66%) have an acceptable hygienic quality for TAB, while just 24/90 (26.66%) have a satisfactory hygienic quality. This result is lower than that of retail meat in the region of Fes-Boulemane in Morocco (7.91 log CFU/g) (Lazar, 2013) and in Benin (6.97 log CFU/g) (Salifou *et al.*, 2013), higher than that found in India (4.78 log CFU/g) (Selvan *et al.*, 2007) and in Australia (3.13 log CFU/g) (Vanderlinde *et al.*, 1998). Comparing to the contamination of beef meat in slaughterhouses by this germ, it is slightly greater than that found in Kenitra, Morocco (5.15 log CFU/g) (Dennai *et al.*, 2001), much higher than that obtained in the slaughterhouses in Australia (1.82 log CFU/g) (Summer *et al.*, 2003), lower than that found in Burkina Faso (8.33 log CFU/g) (Alboudo *et al.*, 2016). TAB reflects the degree of global bacterial contamination of meats, and a higher than defined number of colonies, may indicate a lack of hygiene during slaughter chain, transportation and storage temperatures of meat (Salifou *et al.*, 2013). This germ causes a putrefaction of meat when it exceeds 10^7 CFU/g (Ghafir and Daube, 2007).

Total coliforms (TC)

With regard to contamination by TC, the average is 3.92 log CFU/g, with a minimum value of 2.07 log CFU/g in a Souk and a maximum of 7.02 log CFU/g in a butcher shop. The average indicates a relatively high level of contamination, indicating a less in hygienic conditions of preparation, storing, or commercialization of the beef meat. This value is higher than that of commercial beef meat in Tanzania (4.13 log CFU/g) (Ntangapius, 2013), in India (2.07 log CFU/g) (Selvan *et al.*, 2007), and Comparing to contamination with this germ in the slaughterhouse, this average is much higher than that found in Kenitra, Morocco (3.85 log CFU/g) (Dennai *et al.*, 2001).

Faecal coliforms (FC)

The average contamination of beef meat by FC is 4.56 log CFU/g, it varies between a minimum of 1.60 log CFU/g and a maximum of 6.64 log CFU/g registered in a butcher shop. 40/90 (44.44%) presents an acceptable hygienic quality with 17/90 (18.88%) of satisfactory quality. Analyzed beef meat was highly contaminated with this germ (4.56 log CFU/g), this result is significantly higher than that of retail beef meat in Algeria (3.41 log CFU/g) (Bouzid *et al.*, 2015), and that of the slaughterhouse in Rabat, Morocco (3.32 log CFU/g) (Oumoukhtar *et al.*, 1998). The presence of FC beyond the standards in Supermarkets and butcheries respectively for 16/24 and 29/42 analyzed samples comparably to samples taken from Souk (5/24), indicates a decrease of meat freshness in these sites, a poor hygienic condition of slaughter (Dennai *et al.*, 2001).

Clostridium perfringens (Cp)

Beef meat samples were contaminated by Clostridium perfringens with an average of 2.01 log CFU/g, the minimum and maximum values vary between 1.30 log CFU/g and 3.23 log CFU/g recorded in a supermarket. It was detected in 22.22% of analyzed samples, that are acceptable for consumption with 89/90 (98.88%) of

satisfactory quality. This average is lower than that of retail beef meat in Algeria (2.29 log CFU/g) (Bouzid *et al.*, 2015) and in India (3.20 log CFU/g) (Selvan *et al.*, 2007). The contamination of meat by Clostridium perfringens is due to the contact of carcass with the intestine contents at the evisceration time (Cavalli, 2003), while its presence beyond the standards reflects the slow cooling of meat carcasses (it can grow rapidly at room temperature) and also the poor hygienic practices during processing (Salifou *et al.*, 2013).

Staphylococcus aureus (St)

The average contamination of beef meat by Staphylococcus aureus is 1.56 log CFU/g, it varies between a minimum of 1.30 log CFU/g and a maximum of 2.18 log CFU/g and it was detected in just 5.55% of samples. The compliance study classifies all samples are acceptable for consumption and have a satisfactory quality regarding the contamination by this germ. This percentage is much lower than that found in USA (50%) (Abdalrahmane *et al.*, 2015). This germ is the third most important cause of food-borne diseases in the world (Tong *et al.*, 2015), and its existence in meat is due to the direct contact between human and beef carcass (Salifou *et al.*, 2013).

Listeria monocytogenes

Eight strains of Listeria monocytogenes were detected in 8.8% of analyzed samples. This result showed a high level of contamination by this germ comparably to that found in Egypt (6.67%) (Ismaiel *et al.*, 2014), in Belgium (4.25%) (Uyttendalle *et al.*, 1999), in Madagascar (0%) (Ravaonindrina *et al.*, 2014), and lower than that found in China (34.8%) (Wu *et al.*, 2015). Listeria monocytogenes is among three pathogens that cause illnesses, hospitalizations, and deaths due to foodborne diseases in USA (Mead *et al.*, 1999). It is known for its ability to survive and grow at refrigeration temperatures (Farber and Peterkin, 2011), therefore, its presence beyond the standards reflects the long-term preservation of beef meat.

Salmonella

Concerning the level of contamination by Salmonella recorded in this study, it was detected in 9 (10%) analyzed samples. This prevalence is higher than that of retail beef meat in USA (6%) (White *et al.*, 2001), in India (0%) (Selvan *et al.*, 2007), in the slaughterhouses of Kenitra, Morocco (6.25%) (Dennai *et al.*, 2001), lower than that found in Senegal (87%) (Stevens *et al.*, 2006), and lower also than that of beef sausages in the same city of study (15%) (Ed-Dra *et al.*, 2017). Salmonella is the most pathogens germ isolated in this study and it is the first cause of death by foodborne illness each year in USA (Mead *et al.*, 1999).

Season effect on the hygienic quality of beef meat

About seasonal effect on the hygienic quality, The Table 2 presents the averages of contamination of samples by total aerobic bacteria (TAB), Total coliforms (TC), faecal coliforms (FC), Clostridium perfringens (Cp) and Staphylococci aureus (Sa) according to the seasons in log CFU/g (Figure 1A).

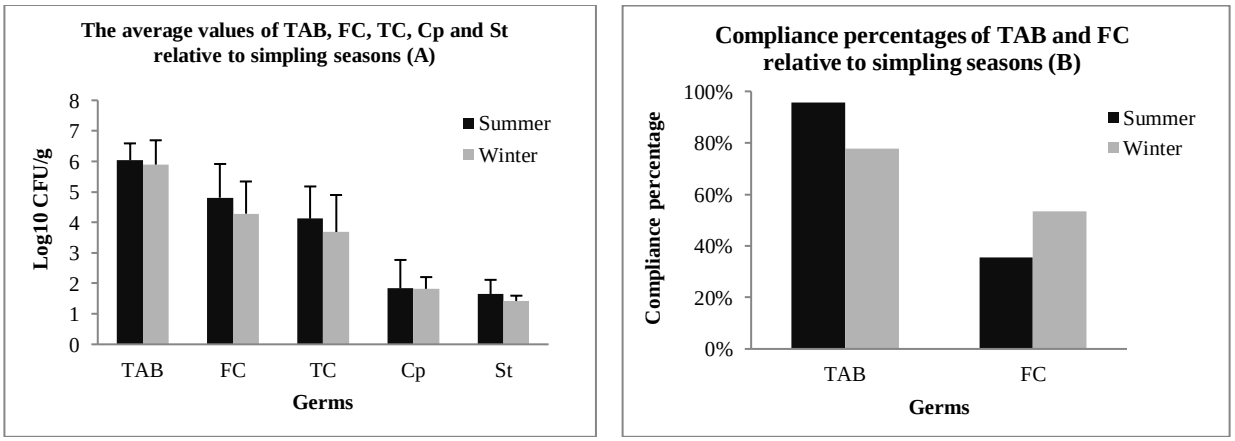

Fig. 1: A: The average values in log CFU/g of microflora counted (TAB, FC, TC, Cp and St) in beef meat according to sampling season. B: compliance percentage of microflora counted (TAB and FC) in beef meat according to sampling season.

Table 1: Averages, Standard deviation, minimum, maximum in log CFU/g, compliance percentages, and satisfactory quality percentages of microflora counted in beef meat sold in Meknes city (Morocco).

	TAB	FC	TC	Cp	St
The average	5.97	4.56	3.92	2.01	1.56
Standard Deviation	0.67	1.11	1.14	0.54	0.32
Minimum (m)	4.22	1.60	2.08	1.30	1.30
Maximum (M)	7.17	6.64	7.03	3.23	2.17
Criteria [m-M]	5.69-6.69	3.69-4.69	---	3-4	4-5
Compliance percentage	86,66%	44.44%	---	100%	100%
satisfactory quality percentage	26,66%	18.88%	---	98,88%	100%

TAB: Total Aerobic Bacteria, TC: Total coliforms, FC: Faecal coliforms, St: Staphylococci, Cp: Clostridium perfringens, E.coli: Escherichia coli, m: desired minimum threshold of contamination, M: maximum threshold of tolerable contamination, X=number of CFU/g, $X \leq m$: satisfactory quality, $m \leq X \leq M$: acceptable quality, $X \geq M$: unacceptable quality.

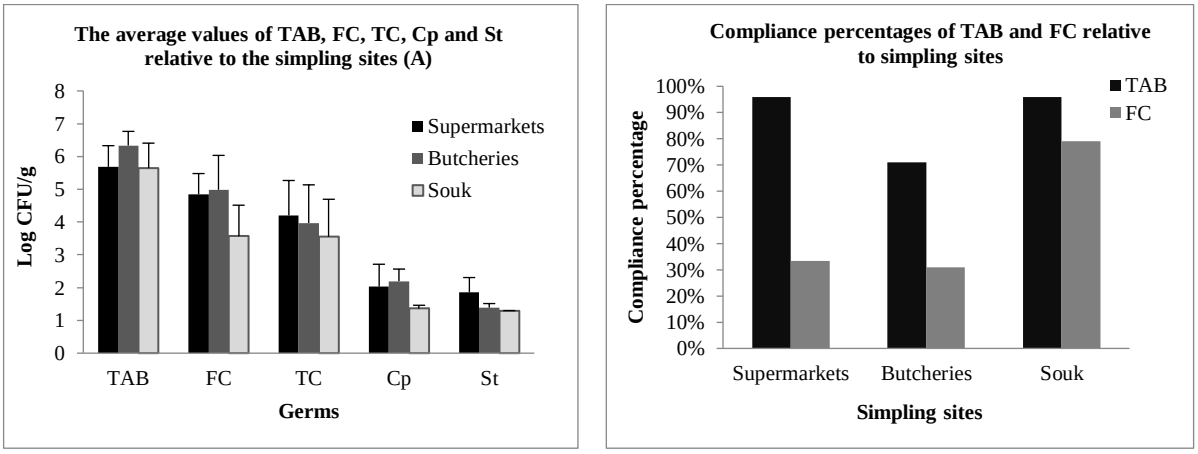

Fig. 2: A: The average values in log CFU/g of microflora counted (TAB, FC, Cp, and St) in beef meat according to sampling sites. **B:** compliance percentage of microflora counted (TAB and FC) in beef meat according to sampling site.

Table 2: Averages of microflora counted in beef meat sold in Meknes city (Morocco) relative to the sampling seasons in log CFU/g.

	TAB	FC	TC	Cp	St
Averages contamination in summer	6.04a	4.81a	4.12a	2.12a	1.65a
Averages contamination in winter	5.89a	4.28b	3.68a	1.82a	1.42a

a,a: no significant difference; a,b: Significant difference.

Table 3: Averages of microflora counted in beef meat sold in Meknes city (Morocco) relative to the sampling sites in log CFU/g.

	TAB	FC	TC	Cp	St
Averages contamination of supermarket meat	5.68a	4.85a	4.20a	2.03a,b	1.86a
Averages contamination of butcheries meat	6.34b	4.98a	3.97a	2.20a	1.39a
Averages contamination of Souk meat	5.65a	3.57b	3.55a	1.36b	1.30a

a,a: no significant difference; a,b: Significant difference.

The study shows that among 78/ 90 samples that have an acceptable hygienic quality for TAB, 43/45 (95.55%) are detected in winter and 35/45 (77.77%) in summer, and among 40/90 samples that meet the standards for FC, 24/45 (53.33%) are detected in winter and just 16/45 (35.55%) in summer (Figure 1B).

The level of contamination in winter by all researched germs was lower than which is found in summer, that indicates humidity level and the hot temperature in summer in the slaughterhouse has in important effect on the microflora present in meat (Gram *et al.*, 2002), knowing that the majority of foodborne bacteria prefer a temperature between 32 and 43°C to grow (Al-Jasass, 2013) and an activity of water varies between 0.980 and 0.995 (Addis, 2015).

The application of student test shows that there is no difference between samples taken in summer and those taken in winter concerning the contamination by TAB (P=0.327), TC (P=0.0872), Cp (P=0.964) and St (P=0.560), while there is a significant difference concerning the contamination by FC (P= 0.030).

Sampling site effect on the hygienic quality of beef meat

About the effect of sampling sites on the contamination of beef meat, the hygienic quality of meat depends on the conditions of slaughtering, handling and storing, temperature during transport and chilling, and hygienic conditions (Al-Jasass, 2013). The Table 3 summarize the average contamination by total aerobic bacteria (TAB), Total coliforms (TC), faecal coliforms (FC), Clostridium perfringens (Cp) and Staphylococcus aureus (St) in log CFU/g according to the sampling sites (Figure 2A).

Among 78/ 90 samples that meet the standards for TAB, 23/24 (95.83%) samples are detected in supermarkets, 32/42 (76.19%) detected in butcheries and 23/24 (95.83%) in Souk. While for FC, from 40/90 samples that have an acceptable hygienic quality, 8/24 (33.33%) in supermarkets, 13/42 (30.95%) in butcheries and 19/24 (79.16%) in Souk (Figure 2B).

The application of student test shows that there is a significant difference between the samples taken from butcheries and those from supermarkets ($P=1.21x10^{-5}$), and Souk ($P=2.23x10^{-5}$) regarding the contamination by TAB. However, there is no difference between the samples taken from supermarkets and souk (P=0.86).

Regarding faecal coliforms, there is no difference between the samples taken from butcheries and those from supermarkets (P=0.596), while there is a high significant difference between simples of Souk and those of supermarkets ($P=4.79x10^{-6}$), and those of butcheries ($P=1.44x10^{-6}$).

Concerning the contamination by Clostridium perfringens there is a significant difference between the samples taken from butcheries and those from Souk (P=0.003), while there is no significant difference between the samples taken from Supermarkets and those from butcheries (P=0.532), and Souk (P=0.136).

However, with regard to the contamination by total coliforms and Staphylococci aureus there no difference between sampling sites.

The high level of contamination of samples taken from butcheries by TAB in comparison with author sites

shows the poor hygienic conditions during slaughter operations of this beef meat. While the high level of contamination of samples taken from butcheries and supermarkets by FC indicates the decrease of meat freshness because of the long-term preservation, what is confirmed by the comparison with the samples taken from Souk that are sales in the same day of slaughtering, so they are significantly less contaminated by FC.

Conclusion

The microbial assessment of retail beef meat in Meknes city classifies 66.67% samples as unfit for consumption. Seasonal variation necessary related to the temperature factor has a very important impact on the level of meat's contamination. While regarding the effect of retail sites, despite the total disrespect of hygiene practices, the lowest level of contamination is that of retail beef meat in Souk during two seasons, reaching 66.66% of compliance in winter and 58.33%, in summer, which means that the Length of refrigerated storage in supermarkets and butcheries has an important effect on its hygienic quality. Salmonella sp. and Listeria monocytogenes as the most pathogenic germs were detected in analyzed samples, which indicates the real need to ensure the correct application of quality systems during slaughtering process and commercialization of beef meat.

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