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Microbiological quality of minced meat sold in butcher shops of Oujda city, Morocco

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Abstract

This work revolves around the assessment of the microbiological quality of ground beef in the Moroccan market. Samples of minced meat were collected from retail outlets (artisanal butcher shops and modern butcher shops) in Oujda city (Morocco). The samples were analyzed for the enumeration of microbiological process hygiene criteria (Total Plate Count, *Escherichia coli*, coagulase positive staphylococci and sulphite-reducing clostridia) and food safety criteria (*Salmonella* sp. and *Listeria monocytogenes*). The results indicated a significant contamination of all the analyzed samples. The mean values obtained (expressed in log CFU/g) were 4.94, 2.7, 1.42 and 1.14 respectively for total plate counts (TPC), *E. coli* (ECC), coagulase positive staphylococci (CPS) and Sulphite-reducing clostridia (SRC). Further, the results show the absence of safety indicators in all the samples. According to the regulations applied in Morocco, 60%, 13%and 3% of the samples were ofpoor or unsatisfactory microbiological quality regarding *E. coli*, coagulase positive staphylococci and Sulphite-reducing anaerobes criteria (SRC) respectively. And 7%, 13% and 30% of the samples have acceptable microbial quality for ECC, CPS and SRC respectively. The highest bacterial counts in the samples used in this research were recorded in traditional Butcher shops ($P < 0.05$). These high levels of microbial contamination and occurrence of pathogenic bacteria reflect the poor hygienic quality of ground beef under these conditions.

Keywords: Ground beef, Microbiological quality, Butcher shops, Morocco.

Introduction

Foodborne diseases often occur after eating contaminated foods. Meat and meat products considered highly perishable and susceptible to contamination by pathogens, such as *Staphylococcus aureus*, *Salmonella* sp., *Listeria monocytogenes* and *Escherichia coli* O157, are frequently associated with this type of infection (Samadpour *et al.*, 2006). In Morocco, 2,655 cases, 43.7% of which are cases of collective poisoning, were recorded in 2017. Foodborne diseases are in second place with a prevalence of 15.7%. The most incriminated elements are meat and meat products with a prevalence of 25.8% (CAPM, 2018). In the US, the Center for Disease Control (CDC) counted during the year 2012, 831 epidemic outbreaks, 14,972 diseases and 794 hospitalizations of foodborne. Among animal products meat and meat products have been incriminated in 13% of cases(Roberts, 1980).

The hygienic quality of meat depends first on the contamination during the slaughter and cutting process, and secondly the development and growth of contaminating flora during cooling, storage and distribution (Jouve, 1990). The objective of this study is to evaluate minced meat's safety in order to assess the

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risk to public health, specifically via the assessment of its bacteriological quality by the enumeration of hygiene process indicators (Total Plate Count, *Escherichia coli*, *Staphylococci*, sulfite reducing bacteria) and the detection of safety indicators (*Salmonella* sp., *Listeria monocytogenes*).

Materials and methods

60 samples were collected from butchers of Oujda city over a period of three months. (March to June 2014, and April to June 2015). Two types of butchers were chosen for this study, with a frequency of 2 times per butcher.

The first group is represented by modern butchers and supermarkets. These butchers have wide display fridges ensuring the respect of the cold chain. This type of butchers also uses semi-automatic cutting utensils. The second group includes traditional butchers, who use manual cutting systems and display surfaces that do not allow for the separation between the different groups of products. Also, in this type of shops, the cold chain is not guaranteed. The samples, collected aseptically, were maintained at 6°C in a thermoelectric cooler for less than 24 hours, until analysis.

The Microbiological analyses concerned the enumeration of the Total Plate Count (TPC), *E. coli* (ECC),*Staphylococcus aureus (S. aureus)* and sulphite-reducing clostridia (SRC),carried out according to ISO 4833-1 (ISO, 2013), ISO16649-2(ISO, 2001), ISO 6888-2(ISO, 1999) and ISO 15213 (ISO, 2003) respectively. And the detection of *Salmonella* sp. and *Listeria monocytogenes*, carried out according to ISO 6579(ISO, 2002) and ISO 11290-1(ISO, 2004) respectively. Sample preparation and decimal dilutions were performed according to the ISO 6887-2 method (ISO, 2010).

Interpretation of the results was made according to the Order No 624-04 of 17 Safar 1425 related to the microbiological standards to be met by animal and animal-origin products(SGGM, 2004). For comparative purposes we also included the regulation applied in Luxembourg, Table 1 (Health, 2015).

Table 1. Acceptability threshold required in minced meat according to local standards in Morocco and in Luxemburg (Health, 2015; SGGM, 2004). **Legend:** TPC, total plate count; *E. coli, Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; SRC, Sulfite Reducing bacteria growing under anaerobic conditions.

Standards		**TPC** x10^5 CFU/ml	***E. coli*** x 10^2 CFU/ml	***S. aureus*** x 10^2 CFU/ml	**SRC** CFU/ml	***Salmonella* spp.** in 10 g	***Listeria monocytogenes*** in 10 g
Moroccan	m	5	1	1	10	Absence	Absence
	3m	15	3	3	30	Absence	Absence
	M	50	5x10^2	5x10^2	10^2	Absence	Absence
European	m	5	0.5	5	----	Absence	----
	3m	15	1.5	15	----	Absence	----
	M	50	5	50	----	Absence	----

The means were calculated for each microbe from duplicate plate counts. All bacterial counts were expressed in log10 colony-forming units per gram (log10 CFU/g). Mean log10 (x) value and SD were calculated on the assumption of a log-normal distribution. To compare log10 values of microbial counts, the data were analyzed using Student's "t" test for each type of micro-organism. The data from the different retail outlets were combined to compare the microbial loads according to

the type of outlet (artisanal butcher shops, modern butcher shops and supermarket. Significance was determined at the 5% level (Kim, T. K., 2015).

Results and Discussion

For raw meat products, potential safety and quality can be estimated with the use of microbiological process hygiene criteria such as the enumeration of TPC, ECC, SRC, CPS and food safety criteria like *Salmonella* sp. and *Listeria monocytogenes*.

In this study, the results of the microbiological analysis (TPC, ECC, SRC, CPS) of ground beef samples are shown in Table 2. These results revealed that ground beef samples collected during the period of the study present a significant contamination. The Enumeration of the TPC ranged between 3.2 x 10^3 and 3.6 x 10^6 CFU/g, with an average count of 8.6 x 10^4CFU/g (Table 2). These values remain below the allowed threshold of 3m = 15 x 10^5CFU/g prescribed in Morocco (Table 1). While 17% of samples are considered acceptable, 83% have a satisfactory status (Table 3).Total Plate Count ranging between 1.3 x 10^4 –2.5 x 10^8CFU/g of minced meat have been reported by various workers in different geographical areas(Aslam *et al.*, 2000; ERDEM *et al.*, 2014; Siriken, 2004). In our study, we observed that the TPC in ground beef were closer to those reported by (Emswiler *et al.*, 1976)and (Bouzid *et al.*, 2015) who reported values of 3.9 x 10^4CFU/g and 7.6 x 10^4CFU/g respectively. On the other hand our results are much lower than those reported by (Oumokhtar *et al.*, 2008)in minced meat sold in butcher shops of the city of Fez and (10^6CFU/g).

This variation in the levels of contamination between the studies, including our research, can be explained by the fact that this parameter (TPC) indicates a deficiency in terms of the application of good manufacturing practices (GMP), and can be associated with the microbiological risk of the finished product. An elevated enumeration of aerobic mesophilic bacteria is a general indicator of poor practice in an establishment (broken cold chain, improper cooling, preparation in advance, prolonged storage, inadequate hot holding temperature, hygiene and sanitation, etc.) and not just an indicator of alteration in the strict sense. A difference in the level of controlling such practices partly explains the variations observed in the level of contamination between the different studies (Jouve, 1990).

Enumeration of ECC ranged from <10 to8.5 x 10^3 CFU/g, with an average of 5.0 x 10^2CFU/g (Table 2). This average value slightly exceeds the minimum threshold of tolerance (3m = 3.0 x 10^2CFU/g) allowed in Moroccan regulation for the preparation of raw ground beef prepared in advance (Table 1). According to this regulation,33% of samples have a lower load than the minimum threshold (≤3m), and are therefore of satisfactory quality, 7% of samples are of acceptable microbiological quality. However, nearly 60% were of unacceptable status (Table 3). Counts of *E. coli* that exceed the limits established by regulations have been frequently reported throughout the world. Most of the enterobacteria present in meat come from fecal contamination. The prevalence of *E. coli* in our study was 60%. This is in the same order of that previously reported by Stagnitta *et al.* (2006)in Argentina which was about 58.3%. However, this prevalence is higher than that which was recorded by Siriken (2004) in Turkey (30%).The average count of 5x10^2CFU/g recorded in our study was less than2.2x10^4CFU/g, previously reported by Salihu *et al.* (2010). However, the results (4.75 log CFU/g) recorded in another Moroccan town (Fez) were higher than our results. This average value of high contamination is probably due to improper handling during slaughtering and

transformation or the materials that were used. A lack of precaution at this level leads probably to direct or crossed contamination. This, as well as the contribution to the contamination during the carcasses transport in butchers, the break of the cold chain or during the mincing process for the minced meat.

Table 2. Charge (CFU/g) of microorganisms in ground meat collected in Oujda city Morocco. **Legend:** TPC, total plate count; SRC, Sulfite Reducing bacteria growing under anaerobic conditions.

	TPC	***E. coli***	***S. aureus***	***SRC***
Minima	$3.2x10^3$	<10	<10	<10
Mean	$8.6x10^4$	$5.0x10^2$	$2.6x10^1$	$1.4x10^1$
Log (Mean)	(4.94)	(2.7)	(1.42)	(1.14)
Maxima	$3.6x10^6$	$8.5x10^3$	$4.2x10^3$	$2.0x10^2$
Variation coefficient	17%	39%	88%	67%

Table 3. results' compliance with Moroccan law (SGGM, 2004). **Legend:** TPC, total plate count; SRC, Sulfite Reducing bacteria growing under anaerobic conditions.

	TPC	***E. coli***	**S. *aureus***	**SRC**
% of **satisfactory** samples (**≤3m**)	83%	33%	73%	67%
% of **acceptable** samples (**>3m, ≤10m**)	17%	7%	13%	30%
% of **unsatisfactory** samples (**>10m**)	0%	60%	13%	3%

Coagulase positive staphylococci counts showed that these bacteria were found in more than 60 % of the samples. 13 % of these samples were above the maximum permissible threshold (10^3 x CFU/g). While 73 % of samples didn't exceed the minimum tolerance threshold (3m), 13 % of samples have shown values which lie between 3m and M=10m (the maximum tolerance threshold) (Table 3). The average load that we recorded is below the one obtained in Algeria (4.45 log CFU/g) by Bouzid *et al.* (2015)and in Nigeria (5-7 log CFU/g) by Salihu *et al.*(2010) but higher than the one reported in the USA (0.74 log CFU/g) by Emswiler and Kotula (1979)and the UK (0.5 log CFU/g) by Roberts (1980).In Morocco, Cohen *et al.* (2008)and Oumokhtar *et al.* (2008) found an average loads of 2.3 log CFU/g and 2.27 log CFU/g respectively. Many authors reported that coagulase positive staphylococci ranged from 10^2 - 10^4/g(Klein & Louwers, 1994; Sofos *et al.*, 1999). On the other hand, several studies have revealed variable prevalence of contamination in minced beef: 69.9 % in Nigeria (Salihu *et al.*, 2010); 21.4% in Turkey (Siriken, 2004) and between 16.7 % and 25 % in Morocco(Cohen *et al.*, 2008; Oumokhtar *et al.*, 2008). The contamination of minced beef by coagulase positive staphylococci in butcher shops can be linked to an improper handling or hygiene. The handling of carcasses in a narrow space, and the use of equipment that is difficult for cleaning on a regular basis lead to a more frequent contact with the manipulator which would increase contamination with *S. aureus*. Otherwise, the product's longer exposure to ambient temperature would contribute to the proliferation of these microorganisms (GBPH). Also, workers with poor hygiene can be asymptomatic carriers of *S. aureus* may be a major source of such contamination. In this sense, it has been suggested that in raw foods, *S. aureus* indicates contamination due to nasopharyngitis or human dermatosis (Al-Bahry *et al.*, 2014; Ho *et al.*, 2015; Kadariya *et al.*, 2014).

SRC bacteria were present in all the samples and, 67 % of them show values

lower than the minimum allowable threshold (≤30 CFU/g) and in a satisfactory level. The remaining 30% of samples have an acceptable status and 3% of the samples exceed the maximum permissible threshold value (Table 3). The presence of this microorganism is probably related to the crosses between contaminated and healthy sector. The mean value of SRC 1,14 log CFU/g found in our study (Table 2) is much higher than those reported elsewhere in U.S.A.(0.22 log CFU/g) by Emswiler & Kotula (1979), in United Kingdom (0.63 log CFU/g) and is almost similar to those obtained in Morocco 1.3 and 1.54log CFU/g(Cohen *et al.*, 2008; Oumokhtar *et al.*, 2008).Ground meat poses a major public health problem than raw meat (intact muscle) because mincing meat allows the surface bacteria to penetrate and with meat juice secretion throughout preparation operation, they find the favorable conditions to proliferate (Güngör & Gökoğlu, 2010). Additionally, we observed the absence of safety indicators (*Salmonella* spp and *Listeria monocytogenes*) in all the samples used in this research.

In our study, the comparison between traditional butchers (AB) and modern butchers (MB) can confirm this observation (Table 4). Statistically, the microbiological quality of the samples taken from the AB are more contaminated in terms of hygiene criteria than those taken in MB (Table 4).

Table 4. Microbiological profile of ground meat between artisanal and modern retail mean **CFU**/g (± SD).
Legend: AB, Artisanal butcher shops; MB, Modern butcher shops.

Item	AB (n = 15)	MB (n = 15)
Aerobic plate counts	$1.7\ 10^5 \pm 11$	$4.4\ 10^4 \pm 3$
Escherichia coli	$2.0\ 10^3 \pm 3{,}2^a$	$1.3\ 10^2 \pm 14^b$
Staphylococcus aureus	$3{,}4\ 10^1 \pm 16^a$	$2.0\ 10^1 \pm 22^b$
Sulfite Reducing bacteria	$2.1\ 10^1 \pm 6^a$	$9.3 \pm 5{,}4^b$

a,b Means in the same row with different superscript letters are different ($P < 0.05$).

The average values recorded of APC, ECC, CPS and SRC respectively for AB and MB are1.7 105 vs 4.4 104, 2.0 103 vs 1.3 102, 3,4 101 vs 2.0 101 and 2.1 101 vs 9.3 for APC, ECC, CPS and SRC respectively (Table 4). This difference can be explained by the use of manual techniques in AB, the improper design of the shops and low cooling capacity may contribute to an increase in contamination.

The presence of *E. coli* at a high level in meat products generally correlates with higher levels of food-borne pathogens originating from fecal origin (Kim & Yim, (2016); Martin *et al.*, 2016). Meat contamination continues during production operations and meat preparation in butcher shops. Cutting, grinding and especially chopping at room temperature contribute to the distribution of surface bacteria, and the release of muscle juice, which is very rich in nutrients, contributing to the amplification of the contamination of these products (Podpečan *et al.*, 2007; Salihu *et al.*, 2010).

Conclusion

This study focused on the microbiological quality of red meat marketed in Oujda, Oriental Morocco. In order to contribute to the assessment of the level of hygiene of this food and the danger it poses to public health, we concluded from this study, that:

- ✓ The high coliform concentration in the analyzed samples indicates a recent fecal contamination in the case of *E. coli* and this non-compliance is due to poor pre-slaughter and slaughter conditions, lack of respect for good

hygiene practices, and poor preparation and preservation conditions;

- ✓ The presence of *Staphylococci* is indicative of the contamination of the carcasses by workers who don't respect clothing hygiene and general hygiene rules;
- ✓ The *Clostridial* contamination observed in some of the analyzed samples indicates a break in the cold chain.

Several factors can lead to the modification of the meat's bacterial flora, they can generally be linked to non-compliance with good hygiene practices, so it is essential to act at this level by insisting on the respect of those rules by installing a HACCP system for risk management.

This work is of great importance in the field of biotechnology in its microbiological aspect, public health, nutrition; food hygiene... The results of this study can be used by health authorities to strengthen the measures to be taken in the field of public health, hygiene, epidemiological surveillance and the fight against food poisoning and foodborne diseases.

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