Global Science Research Journals

ISSN: 2449-1799 Vol. 5 (2), pp. 198-204, February, 2017.

http://www.globalscienceresearchjournals.org/

Global Journal of Microbiology Research

Full Length Research Paper

Evaluation of microbiological quality of the Merguez as a meat product largely consumed in Algeria

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Received 15 December, 2016; Accepted 20 January, 2017

In order to assess the microbiological quality of Merguez and influence of temperature on the rate of contamination, from April 2 to May 12, 2016, a study was undertaken in two types of meat retailing in the region of M'Sila, Algeria. A total of 60 samples of Merguez were collected in ten sites from five markets and five independent butcher's shops, for purposes of microbiological analysis. The majority of the samples were contaminated by coagulase positive staphylococci and thermotolerant coliforms. The average counting in both types combined trade were 2.0±0.2 $\log_{10}$ cfu/g for thermotolerant coliforms and 2.2±0.2 $\log_{10}$ cfu/g for coagulase positive staphylococci. However, all samples were free of *Salmonella* spp. and sulphite-reducing *Clostridium*. Compared to the days of sampling, both bacterial indicators counted in covered markets were significantly different from one day to another ($p<0.05$). However, no significant difference ($p>0.05$) were reported between daily concentration for these two groups of bacteria counted in samples from independent butchers. These results show disrespect the rules of good hygienic practices during preparation, storage and sale of Merguez. The potential consequences of the consumption of these foods on the health of consumers should motivate disease control measures.

Key words: Algeria, thermotolerant coliforms, coagulase positive staphylococci, sulphite-reducing *Clostridium*, *Salmonella*, microbiological, covered markets, independent butchers, sausages, temperature.

INTRODUCTION

Merguez is a red colored, spicy lamb or beef-based North African fresh sausage. Merguez is regarded as food of choice because of their nutritive values. Its high contents in proteins and the nature of those made these products as essential food for a balanced feed ration. Its safety depends on the presence of factors such as relatively low water activity (aw) and low pH value. According to Shelef et al. (1997), acidity has a bacteriostatic effect on the evolution of germs. In parallel, water activity is a parameter that characterizes the water content of food

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stuffs and most bacteria develop well for Aw between 0.995 and 0.980. Pathogens are inhibited for values below 0.94 except for *Staphylococcus aureus* (Akollor, 1997).

Nevertheless, like all fresh products, it deteriorates quickly in particular under bad conditions of storage.

Thus putrefied Merguez was sometimes sold on the Algerian market. Moreover, because of its nutritional composition, it constitutes a rich medium very favorable to pathogens growth (Oumokhtar et al., 1998). It was often responsible of collective food borne diseases in Algeria (Mouffok, 2011).

A better knowledge of the factors involved in the bacterial contamination of these meat products is necessary to provide reliable data which allow the improvement of the strategies of prevention and to facilitate the implementation of correct conditions throughout the chain production of Merguez.

The main objective of this study was to evaluate the microbiological quality of the Merguez as a meat product largely consumed in Algeria, assessing consequently the hazards on public health. Moreover, high levels of contamination, during production and selling, obviously affect the hygienic quality of this sausage.

MATERIAL AND METHODS

Sampling

During 6 weeks, from April 2 to May 12, 2016, we collected, one day a week, 60 samples of Merguez, from ten different retailing sites, located in M'Sila. The day of sampling varied each week (Saturday to Thursday) in order to achieve representative results. Two types of trade were targeted: independent butchers and covered markets (five points of retailing from each trade type). The temperature of storage was also recorded for each sample. The samples were taken within four hours after its exposure for selling. The samples (250 g) were conditioned in sterile bags, clearly labeled and identified, maintained in a refrigerator containing frozen cooling blocks. These bags are immediately sent to the quality control laboratory (DOUMI-C-Q-A-D) from M'Sila area. These samples were analyzed immediately upon their arrival.

Microbiological analysis

At the laboratory, each sample initially separated into 5 units then was cut out separately in small pieces in sterile Petri dish, a Stomacher sachet is tared and 25 g of each unit were weighed exactly there. Then, 225 ml of a solution of tryptone salt (TSE) (Pasteur Institute, Algeria) were introduced into the sachet. The unit was crushed during 2 to 3 min in Stomacher. The supernatant obtained after crushing was recovered in a sterile bottle (it is the stock solution (SM) of concentration 10^{-1}). The latter was left at rest during 45 min, to allow the reactivation of the shocked or stressed microorganisms.

Various dilutions were performed using TSE (Pasteur Institute, Algeria) starting from the stock solution in accordance with the standards ISO 6887-1 (Norme ISO 6887-1, 1999).

The bacteria investigated and counted were the thermotolerant coliforms, the coagulase positive staphylococci, the sulfite-reducing *Clostridium* at 46°C and the *Salmonella* spp. (Arrêté interministériel, 1998).

The thermotolerant coliforms (TTC) were grown on Red Bile Agar supplemented with lactose (VRBL) (Pasteur Institute, Algeria), after incubation for 24 h at 44°C (Norme NF V08-060, 2009).

The coagulase positive staphilococci (CPS) were cultivated in Baird Parker agar (Pasteur Institute, Algeria) supplemented with Egg yolk and potassium tellurite. The bacterial number is evaluated after an incubation of 48 h at 37°C, their identity was confirmed by Gram stain, and the enzymatic based research of catalase and coagulase (Norme NF V08-057-1, 2004).

Sulfite-reducing *Clostridium* (SRC) at 46°C were counted after heating of the stock solution at 80°C for 10 min, then quickly cooled in order to destroy the vegetative forms of the clostridia and to activate their spores. After culture on the Tryptose-Sulfite medium with Cyclosérine (TSC) (Pasteur Institute, Algeria) at 46°C for 20±2 h, only the characteristic colonies were counted (Norme NF V08-061, 2009).

Salmonella spp (SLM) were detected according to the experimental protocol indicated by ISO 6579; it consists of: A pre-enrichment in buffered peptone water (BPW) (Pasteur Institute, Algeria). A double enrichment in 100 ml of Selenite Cystine broth (SCB) (Pasteur Institute, Algeria) and in 10 ml of Rappaport-Vassiliadis (RV) (Pasteur Institute, Algeria). An insulation on Hektoen agar (Pasteur Institute, Algeria) for the SCB and an isolation on brilliant green agar (BGA) (Pasteur Institute, Algeria) for the RV. Then, Galleries Api 20E kits (Bio Merieux) were applied (Norme ISO 6579, 2002).

All microbial counts were expressed as decimal logarithm of colony forming units per gram ($\log_{10}$ cfu/g). The results were compared with the criteria required by the Algerian inter-ministerial decree of January 24th 1998, related to the quality of foodstuffs. The maximum concentrations accepted for the counted bacteria are: 2 $\log_{10}$ cfu/g for TTC, 2 $\log_{10}$ cfu/g for CPS, 1.5 $\log_{10}$ cfu/g for SRC and the absence of SLM (Arrêté interministériel, 1998).

Statistical analyses

The average bacterial burdens were calculated per day, taking into account the trade type and for each bacterium. The day of sampling (from Saturday to Thursday) and the temperature of storage were considered as sources of variations. The bacterial count was evaluated five times and the mean was taken into account in the statistical calculations. A factorial analysis of the variance was applied to compare the results of the enumeration of the bacteria between the two types of trade. It is also used to compare means of bacterial enumeration according to sampling day.

The parametric Pearson's correlation coefficient (r) was calculated to assess potential association between the average concentrations of counted bacteria and the temperature of storage at the moment of sampling.

The test of Student was used for comparison between the average number of bacterial colonies with the threshold of acceptability for each type of bacterium.

All calculations were carried out using STATISTICA software version 2007, after transformation decimal logarithmic of the results expressed as cfu/g to normalize the distribution.

Table 1. Variation of the quantity of bacteria in the analyzed Merguez according to both trade types (Average ± Standard deviation, expressed as log_{10} cfu/g).

Type of trade		TTC	CPS	SRC	SLM
Independent butchers	IB_1	1.8±0.2	2.1±0.1	Absent	Absent
	IB_2	2.0±0.3	2.1±0.2	Absent	Absent
	IB_3	2.1±0.1	2.3±0.1	Absent	Absent
	IB_4	2.0±0.1	2.1±0.2	Absent	Absent
	IB_5	1.8±0.4	2.1±0.3	Absent	Absent
	Average	1.9±0.2	2.1±0.2	Absent	Absent
Covered markets	CM_1	1.9±0.1	2.3±0.2	Absent	Absent
	CM_2	2.1±0.3	2.3±0.2	Absent	Absent
	CM_3	2.1±0.3	2.3±0.1	Absent	Absent
	CM_4	2.0±0.1	2.3±0.1	Absent	Absent
	CM_5	1.9±0.3	2.3±0.2	Absent	Absent
	Average	2.0±0.2	2.3±0.2	Absent	Absent
Average		2.0±0.2	2.2±0.2	Absent	Absent
CI (95%)		[1.9; 2.0]	[2.2; 2.3]	Absent	Absent
NS<CR (%)		36 (60.0%)	10 (16.7%)	60 (100%)	60 (100%)
NS>CR (%)		24 (40.0%)	50 (83.3%)	0 (0.0%)	0 (0.0%)

CI (95%): Confidence interval in 95%; TTC, thermotolerant coliforms;, CPS, coagulase positive staphylococci; SRC, sulfite-reducing *Clostridium*; SLM, *Salmonella* spp., NS<CR, number of samples which present a bacterial burden lower than the criterion fixed by Algerian standards; NS>CR, number of samples which present a bacterial load higher than the criterion fixed by the standard; (%), prevalence.

RESULTS

Overall microbiological quality of the sampled Merguez

Table 1 presents the rate of contamination of the samples by the various flora according to both types of trade in M'Sila. For a given bacterial marker, this table gives the average bacterial concentration of five samples of each type of trade considered, expressed as log_{10} cfu/g. In all the positive cases, only the average concentration of CPS in the two types of trade is significantly higher than the threshold of acceptability ($p<0.01$ for independent butchers and $p<0.001$ for covered markets), that is to say at the maximum tolerated concentration (criterion fixed by the standards). Out of the 60 samples, 40.0% present a load in thermotolerant coliforms higher than the fixed standards; their average concentration is 2.0±0.2 log_{10} cfu/g.

In the same way, 83.3% of the samples show a load in coagulase positive staphylococci higher than the standards, the average number is 2.2±0.2 log_{10} cfu/g.

For the *Salmonella* spp. and the sulfite-reducing *Clostridium*, no bacterium was isolated at least during this survey. Therefore, 100% of the analyzed Merguez are in conformity.

Compared to the types of commerce, CTT and SCP were less counted in independent butchers compared to the covered markets.

The comparison of the results obtained in the two types of trade does not show a significant difference ($p>0.05$) except for the TTC.

Daily variation of the bacterial indicators

Table 2 presents the daily variation of the two bacterial indicators (TTC and CPS) per day of sampling. The loads of the Merguez in both bacterial indicators did not vary according to the day of sampling for independent butchers ($p>0.05$). However, they significantly increased from one day to another for the covered markets ($p<0.05$).

In general, the higher bacterial concentrations were recorded on weekends for the covered markets as compared to independent butchers.

Relationship between the bacterial indicators and the temperature of storage of the Merguez in the various points of sale

The temperature of storage for the Merguez, recorded in the various sites interferes with the processes of contamination. Of a minimum of 10.4°C for independent butchers and of 12.2°C for the covered markets whereas the maximum is of 12.2°C for independent butchers and of 13.6°C for the covered markets.

The average temperatures for storage, during the six weeks of the study were 11.4 ± 0.6°C for independent

Table 2. Daily variation of the two bacterial indicators counted by type of trade (Data were expressed in $\log_{10}$ cfu/g).

Thermotolerant coliforms (TTC)		
Day of sampling	**Independent butchers**	**Covered markets**
Saturday	2.1±0.1	1.8±0.3
Sunday	2.0±0.2	1.9±0.3
Monday	2.0±0.2	2.1±0.2
Tuesday	2.0±0.2	2.0±0.2
Wednesday	1.8±0.3	2.1±0.2
Thursday	1.8±0.3	2.2±0.2
Average	1.9±0.2	2.0±0.2
CI (95%)	[1.9; 2.0]	[1.9; 2.1]
Coagulase positive staphilococci (CPS)		
Day of sampling	**Independent butchers**	**Covered markets**
Saturday	2.3±0.2	2.2±0.3
Sunday	2.2±0.2	2.2±0.1
Monday	2.1±0.3	2.3±0.2
Tuesday	2.2±0.1	2.3±0.1
Wednesday	2.1±0.2	2.3±0.2
Thursday	2.0±0.2	2.4±0.2
Average	2.1±0.2	2.3±0.2
CI (95%)	[2.1; 2.2]	[2.2; 2.3]

CI (95%), Confidence interval in 95%.

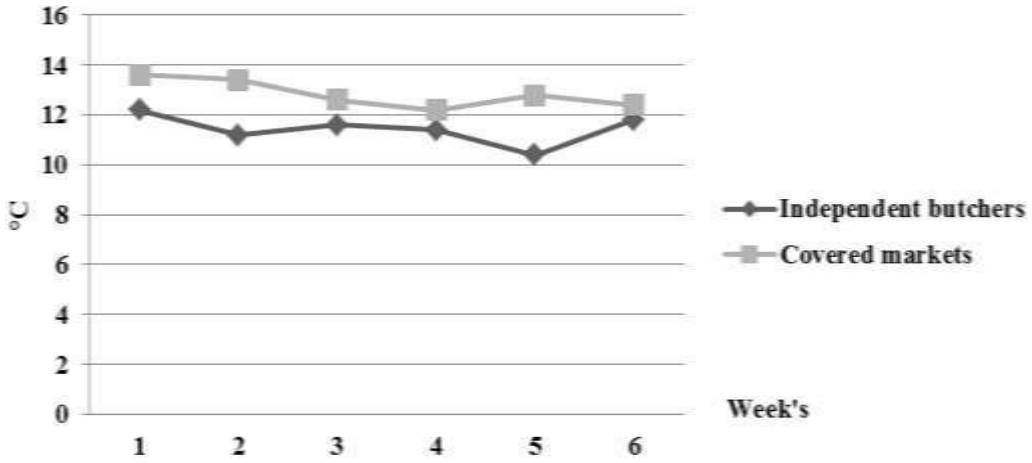

Figure 1. Weekly variation of storage temperature according to meat retailing type (independent butchers and covered markets).

butchers and 12.8±0.5°C for the covered markets (Figure 1).

For the two types of trade, the temperature reduced irregularly during the six weeks but with values higher in covered markets.

The bacteria for which the Pearson's coefficients of correlation were high (r>0.5) were that between thermo-tolerant coliforms and the temperature of storage in covered markets (r=0.7) and the coagulase positive staphilococci which appeared more correlated with the temperature of storage (r=0.8) in independent butchers. In this last case, one notes that the coefficient of determination (R^2=0.7), remains higher, which indicates a real correlation.

Moreover, there were two slightly weak correlations, the first one was negative (r=-0.4) between the coagulase positive staphylococci and the temperature for storage in covered markets and the other positive one (r=0.5)

Table 3. Correlation between the results of numerations of the two bacterial indicators and the storing temperature.

Independent butchers		
Relation between the parameters	**COC (r)**	**COD (R^2)**
TTC- TS	0.5	0.2
CPS-TS	0.8	0.7
Covered markets		
Relation between the parameters	**COC (r)**	**COD (R^2)**
TTC- TS	0.7	0.5
CPS-TS	-0.4	0.1

CPS, Coagulase positive staphylococci; TTC, thermotolerant coliforms; TS, temperature of storing; COC = coefficient of correlation (r); COD, coefficient of determination (R^2).

between the thermotolerant coliforms and the temperature of storage in independent butchers (Table 3).

DISCUSSION

The main aim was to evaluate the microbiological quality of the Merguez sold in both meat retailing types in M'sila, as well as investigating the factors involved in the bacterial contamination. The microbiological quality of the Merguez was assessed according to Algerian criteria related to the microbiological specifications of these food products. In almost all cases, these meat products were strongly contaminated.

The majority of our samples did not meet the criteria fixed by the Algerian standards recommended in this field, which signs bad conditions of hygiene at the time of manufacture and conservation of the Merguez. Only ten samples met the relevant criteria in Algeria at least in this study.

The thermotolerant coliforms indicate a fecal contamination in general and their number is generally proportional to the degree of pollution by the faeces. They can judge the hygienic condition during or after the transformation of these foods (Giraud and Galzy, 1980). In 40% of our samples, their concentration is above the standard (Arrêté interministériel, 1998). The average of the enumerations of these fecal originated bacteria is 2.0 $\log_{10}$ cfu/g. These results remain however very inferior than those reported by El Allaoui et al. (2012) in the town of Meknès in Morocco and Cohen and Karib (2006) in Casablanca which present averages of 4.6 and 3.7 $\log_{10}$ cfu/g respectively. According to Mescle and Zucca (1998), the contamination occurs during the chopping and the manufacturing. These operations lead to a homogenisation of the flora of the various ingredients and to a modification of the structure of the products. That makes it possible the contamination of surface to be introduced into the mass.

The coagulase positive staphilococci are regarded as pathogenic bacteria and their presence in the foodstuffs denotes the bad conditions of handling during the preparation as well as the bad hygienic quality of material used (Salihu et al., 2010). The detection and the enumeration of these bacteria make it possible to evaluate the risk of the Merguez for the consumers, since they are the principal species which can possibly produce enterotoxins responsible of food poisoning (Joffin and Joffin, 1999). Our results (2.2 $\log_{10}$ cfu/g) are higher than those of Scagna et al. (2000) who reported an average level of contamination of 1.1 $\log_{10}$ cfu/g. Besides, they remain clearly inferior than those obtained by El Allaoui et al. (2012) in Morocco with an average of 4.3 $\log_{10}$ cfu/g. The majority of our samples (83.3%) are of bad quality giving that the Algerian criteria fix the threshold of contamination at 2.0 $\log_{10}$ cfu/g (Arrêté interministériel, 1998). These results reveal a lack of respect of the good practices of hygiene and manufacture as well as temperature of storage. Moreover, the medical status of staff plays a fundamental role in the contamination of the foodstuffs by the coagulase positive staphilococci (International Biological Standards Commission Relating To Food, 1974).

Sulfite-reducing *Clostridium* is bacteria which exist in two forms: Vegetative and sporulated form, very resistant bacteria which indicate a fecal contamination. Their research and enumeration in foodstuffs are recommended by the standards applicable to foodstuffs (Delarras, 2007). The microbiological criterion in Algeria concerning these bacteria being fixed at 1.5 $\log_{10}$ cfu/g (Arrêté interministériel, 1998). The totality (100%) of our samples is deprived of these bacteria. Therefore, they are in conformity as compared to the standards. Our results are close to those of Tapounie (1977) which found a rate of conformity of 99.2%. On the other hand, they are clearly inferior than those of El Allaoui et al. (2012) in Morocco with an average concentration of 4.9 $\log_{10}$ cfu/g. According to Roua (1988), sulfite-reducing *Clostridium* is not toxic except when they are introduced in great number. Moreover, they are usual hosts of the digestive tract of the man and animals. The concerned food is

meat and of meat products.

With regard to *Salmonella* spp., all samples were in conformity with the microbiological Algerian standards, namely the total absence of this bacterial genus in Merguez. Our results were similar to those obtained by Seydi and Sylla (1996) in Dakar, but were clearly inferior to those of Elhag et al. (2014) in Khartoum who revealed that 97.5% of the studied sausage samples were contaminated by these bacteria. Many studies showed than the *Salmonella* can be isolated from various types of sausages (Abrahim et al., 1998; Mattick et al., 2002; Özbey et al., 2007). The high incidence of these bacteria could be due to the faeces contamination of the chopped meat used for the production of pork-butchery, contaminated water, the environment and bad hygiene of workers (Reid et al., 2002). It worth note that human is a natural host of *Salmonella typhimurium* (Karib et al., 1994).

Comparing the two types of trade, no significant difference ($P>0.05$) was observed between independent butchers and the covered markets for the TTC. The daily loads concerning these two bacterial indicators did not vary from one day to another in independent butchers. But in the covered markets, these loads significantly varied from one day to another, it is because the conditions of hygiene fluctuate from one day to another. This variation proves an instability in the working method in the covered markets. In the same way, these strong bacterial concentrations are due to a failure of the cycle of cleaning-disinfection of the equipment of manufacture. In the majority of our markets, the material is only rinsed once at the end of day (Collobert et al., 2007).

Moreover, these concentrations observed at the covered markets, slightly higher as compared to those counted in independent butchers, could be explained by the observations raised at the time of sampling. Indeed, one noted the bad respect of the conditions of hygiene in the covered markets in comparison to independent butchers. In the latter, the personnel wears gloves, the rooms of cutting and the schemes of work clean and are equipped with a wash-stand. The products are preserved at cold. Moreover, the case is separated from the place of preparation, which decreases certainly the likelihood of contaminations. On the other hand, the increase in the load in micro-organism in the samples taken at the covered markets testifies a bad condition of hygiene during manufacture of the Merguez.

The storage temperatures of the Merguez exert a crucial effect on bacterial growth. Among the 60 samples, no one met the Algerian standards (+4°C) (Arrêté interministériel, 1998). These results reflect non respect of the chains of cold in these sale sites. This temperature (+4°C) is regarded as a threshold from which the bacteria can start to produce the toxins responsible of food borne diseases. So, the consumer is exposed at the real risk of intoxication and poisoning especially if the level of initial contamination is high and the time of consumption is long (Hennekinne, 2009).

According to Christieans (2003), the temperature of storage of the Merguez presents an undeniable effect. A chain of cold well controlled prevents the growth of the pathogenic microorganisms and maintains constant a possible contamination.

The correlation between the two bacterial indicators concerning the 60 samples and the storage temperature of Merguez showed the existence of two high positive correlations, one between the thermotolerant coliforms and the temperature of storage in covered markets ($r=0.7$) and the other between the coagulase positive staphilococci which appears correlated better with the temperature of storage ($r=0.8$) in independent butchers.

According to De Buyser (1996), the coagulase positive Staphylococci multiply at temperatures ranging between 6 and 46°C with an optimal temperature of 37°C and the toxins synthesis intervenes under conditions a little more restrictive than those necessary for the growth. The dejections of the bovines constitute the principal tank of the thermotolerants coliforms in particular of the *Escherichia coli* species which multiplies at temperatures ranging between 4 and 46°C, with an optimum of growth at 37°C, a pasteurization at 72°C during 15 s is sufficient to eliminate this bacterium.

According to Daelmann and Van hoff (1975), the temperature of storage greatly influences the bacterial proliferation. Conservation at 10°C is not appropriate, but a storage in 2°C is possible during two to three weeks. Storage in 4°C is misadvised when the product contains the enterococci ones; because the proliferation of these bacteria is especially favored by these foodstuffs. The compliance with the rules of hygiene, preparation and conservation of the foodstuffs, medical education and monitoring of the personnel, constitute the main preventive actions (CMIT, 2014).

Conclusion

The present study reveals that in this topic the Algerian standards were not met. The variation of the bacterial load according to the day of sampling proves an instability in the working method in the covered markets, the most significant loads being recorded in weekend and are proportional to the quantity of the Merguez manufactured. The noted high bacterial burdens testify the bad hygiene at the retailing points and bad handling of the Merguez during and after manufacture. They constitute for the consumer a potential risk which will become real risk if errors are made during the preparation, in particular with regard to the temperature and the time of cooking. Moreover, the Algerian culinary practices imply intense torrefaction of the Merguez. However, it can be noticed a change in practice food and a development of the sector of the fast food. The hygienic quality of Merguez for sale could be improved by setting-

up of a policy of traceability of the meat in order to ensure healthiness throughout the production, storage and retailing chain. Moreover, the authorities in charge of the control of these foodstuffs should set up programs of health education and good practices of hygiene.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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