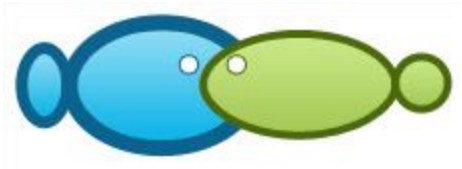

Hygienic control of fresh and semi-preserved anchovies in Rabat city in Morocco

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Abstract. In Morocco, the fishery sector and the industry of transformation and promotion of fishery products hold a position of privilege in the economy of the country. However, fishery products may contain microorganisms which may threaten the health of consumers. This study comes within this context, with the main objective to assess the hygienic quality of fresh and semi-preserved anchovies sold in the city of Rabat. A monthly sampling was carried out between April and July 2018 to probe for the presence of the following pathogenic germs: *Staphylococcus aureus,* sulfite-reducing *Clostridium, Salmonella, Pseudomonas aeruginosa* and *Listeria monocytogenes*. The results obtained show a very high rate of microbiological non-conformity for the samples of fresh anchovies vis-à-vis pathogenic germs (*S. aureus,* sulfite-reducing *Clostridium* and *Salmonella*), while semi-preserved samples showed weak bacterial growth for the *S. aureus* and sulfite-reducing *Clostridium* organisms, probably due to their insufficient treatment. These results show the presence of health risks linked to the consumption of anchovies in Rabat city.
Key Words: anchovies, bacteria, contamination, hygiene, health.

Introduction. The main cause of spoilage is microbial growth. Sporulating bacteria can be a problem in the food industry, especially the canning industry. The presence of spores in the ingredients or in the processing environment severely defies the preservation process since their thermal resistance can be very high (Oomes et al 2007). The destruction of thermosensitive microorganisms in vegetative forms and sporulated by heat is done as a function of time, for a given processing temperature. The speed of this thermal destruction is itself dependent on the temperature according to the classical Arrhenius law (Zuber et al 2008).

The fashion for raw, minimally processed and reputable products has led to the search for new preservation methods to keep food longer. Reducing the intensity of preservative processing and increasing the shelf life of products are important risk factors (Tauxe 2001). Recent trends in environmental protection have led to the reduction of food packaging to the least minimum possible. These initiatives can however have a perverse effect, since the primary function of packaging is to protect the product from external contamination. The food we consume, including a large part of imported products, in particular canned food, may therefore contain substances or pathogens dangerous to health (Buisson et al 2008).

With globalization imposing changes in eating habits, we are currently eating everything everywhere. Consumption of canned and semi-canned food has seen a great evolution in recent years, with tens of billions of boxes of food produced annually worldwide and 20 to 90% of the harvest will probably be preserved by canning (Trirach et al 2010). Depending on the country, the type of food and the type of processing, these products can often be contaminated with mycotoxins and spores, many of which are

thermo-tolerant (Ababouch 2014). Although the impacts are rare, they have serious health consequences due to the pathogenic microorganisms which can multiply there. Poisoning by canned tuna has been reported (Boyer et al 1956; Leuschner & Hammes 1999) and the poor quality of canned fish has high histamine levels and organoleptic defects (Nerisson 1976; Fletcher et al 1998; Kanki et al 2004).

In Morocco, the safety of fishery products including anchovies, whether fresh or processed, depends on maintaining good hygienic practices while respecting the cold chain during the various stages before consumption, namely: capture, transport, processing, packaging, distribution and storage. Indeed, several factors can intervene in their alterations. The temperature should be as close as possible to 0°C for fresh anchovies, below 15°C for semi-preserved foods and below 4°C for pickles (Ababouch & El Marrakchi 2009). The pH should be close to 7 because it is neutral in the flesh of live fish and it normally decreases during the first days after capture due to the formation of lactic acid in anaerobiosis, thus facilitating its alteration (Ndiaye 2013). Otherwise, the rise in temperature and the decrease in pH lead to the proliferation of different germs (GDCCFC 2016). As a result, non-compliance with good hygiene practices, the manufacturer's recommendations and the cold chain during the storage or conservation of anchovies, give rise to microbial contamination, which can lead to food poisoning.

The latest data from the food analysis activities of the National Institute of Hygiene in Rabat show a drawback in the compliance of canned food. Knowledge of the hygienic quality of food is therefore necessary to form a judgement about the notion of biological stability, the different means of processing and the related inspections. For this reason, we are interested in this work in the hygienic control of fresh and semi-preserved anchovies intended for consumption in the city of Rabat, in order to shed light on the microbiological quality of these products. The focus here is on the identification of the following pathogenic germs: *Staphylococcus aureus,* sulfite-reducing *Clostridium, Salmonella, Pseudomonas aeruginosa* and *Listeria monocytogenes*.

Material and Method. The study involved a total of 65 samples of anchovies, including 10 fresh and 55 in semi-preserved, taken randomly on a monthly basis between April and July 2018 from the city of Rabat from 16 different sites (Table 1).

Table 1

Distribution of samples according to sampling sites

Category of anchovies	*Sampling site*	*Number of samples taken*
Fresh anchovies	SD	2
	P1	2
	P2	2
	P3	2
	P4	2
Semi-preserved anchovies	A	3
	B	3
	C	3
	D	3
	E	3
	a	5
	b	5
	c	5
	d	5
	e	5
	S	15
Total	16 sites	65

SD = landing site; P1, P2, P3 and P4 = fishmonger; A, B, C, D and E = supermarkets; a, b, c, d and e = grocery stores; S = company preparing semi-preserved anchovies. S is a Moroccan food company that wants to remain anonymous, specializing in the production and distribution of a variety of canned and semi-canned seafood, as well as vinegar and jams.

Once collected, each sample is immediately put in a sterile plastic bag, then in a cooler at 4°C and transported to the Laboratory of Microbiology and Food Hygiene of the National Institute of Hygiene in Rabat (NIH) to perform the microbiological analyzes. In this study we looked for the following pathogens:

- *Staphylococcus aureus* according to standard NM 08.0.112 (2009);
- sulfite-reducing *Clostridium* (SRC) according to standard NM 08.0.125 (2012);
- *Salmonella* according to standard NM 08.0.103 (2006);
- *Pseudomonas aeruginosa* according to standard ISO 13720 (2010);
- *Listeria monocytogenes* according to standard NM 08.0.172 (2017).

In our samples, the search for the above pathogens was carried out in four stages: pre-enrichment, enrichment, isolation and identification.

Results and Discussion

Detection of Staphylococcus aureus germs. Out of all the samples analyzed (Figures 1 and 2), 40% of fresh anchovies and 4% in semi-preserved show the presence of *S. aureus* germs. A dominance in the case of fresh anchovies was noted, which is due to the fact that several factors can intervene. In the case of fresh anchovies, it may have arisen due to lack of hygiene, by direct contact via the hands of fishmongers, as well as by storage on contaminated surfaces. Indeed, *S. aureus* are commensal bacteria, present on the skin, mucous membranes, the kitchen, the refrigerator, the soil, water and everywhere in the environment (Evenson et al 1988). Some of them produce toxi infections which can trigger nausea followed by projectile vomiting, abdominal pain, diarrhea, dizziness, chills, general weakness accompanied by moderate fever and even headache and hypotension in the most severe cases when they are present in sufficient quantity in food (Uçkay et al 2012).

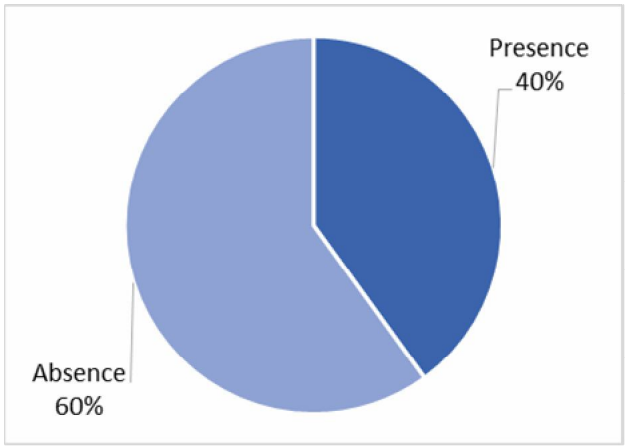

Figure 1. Contamination rate of fresh anchovies with *Staphylococcus aureus* germs.

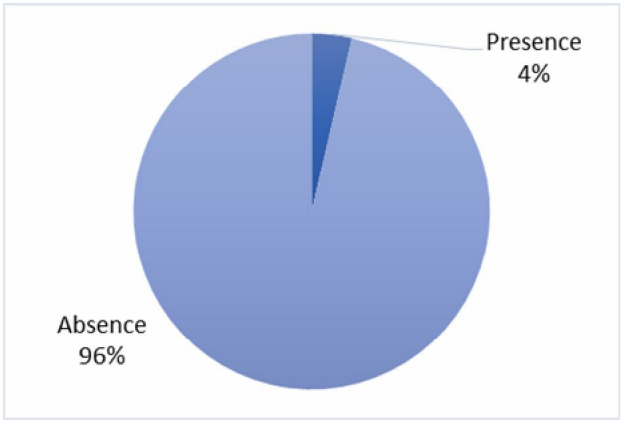

Figure 2. Rate of contamination of semi-preserved anchovies with *Staphylococcus aureus* germs.

In the case of semi-preserved anchovies, it is possible that it occurred due to non-compliance with the cold chain, since the growth of germs takes place at a temperature between 7 and 48°C. Therefore, there are vendors who store the boxes of semi-canned goods on shelves at room temperature.

In comparison with hygiene standards (NM 2009), these results are not in conformity. Contrary to the results found by Assogba et al (2018), who worked on smoked *Trachurus*.

Detection of sulfite-reducing Clostridium (SRC) germs. Out of all the samples analyzed, 50% of fresh anchovies (Figure 3) and 11% of semi-preserved anchovies (Figure 4) show the presence of SRC germs, with dominance always in the case fresh anchovies. This could be explained in the case of fresh anchovies by the conditions of storage and by the non-observance of elementary rules of hygiene. These conditions are very favorable for the rapid growth of SRC germs in agreement with the results found by Micha et al (2018) in Chad. So, in the case of semi-preserved foods, this contamination could be explained by the probable break in the cold chain during transport or storage. Because, the SRC germs grow quickly at room temperatures and produce neurotoxic substances. These substances cause a serious illness, botulism, which leads to paralysis of the muscles, leading to the death of individuals if they are not taken care of in time (Campello & Colas 1982; Lehane & Olley 2000; Nciri 2005).

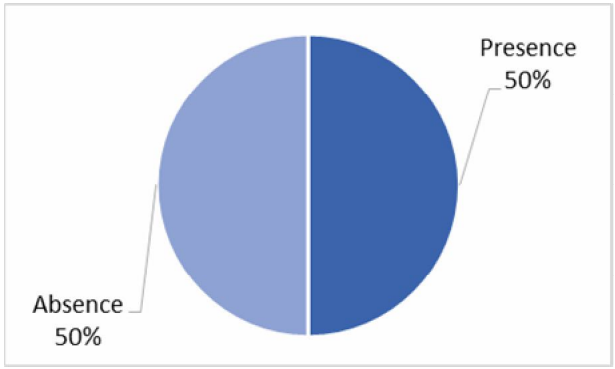

Figure 3. Rate of contamination of fresh anchovies by sulfite-reducing *Clostridium* germs.

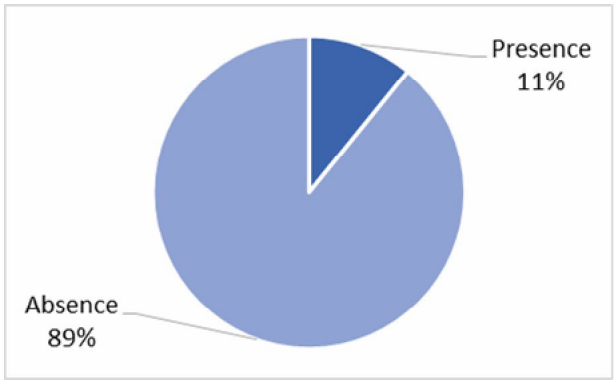

Figure 4. Rate of contamination of semi-preserved anchovies by sulfite-reducing *Clostridium* germs.

Detection of Salmonella germs. Bacteriological analyzes showed the presence of *Salmonella* germs in the case of the fresh anchovies samples only, with a percentage of 40% (Figure 5). This could be explained by direct contact and the lack of hygienic conditions. Meanwhile, their absence in semi-preserves samples could be explained by the large amount of salt in these products, because salt can be used alone as a preservative without any heat treatment or the addition of additives (Taylor 1986). These results are in agreement with those of Degnon et al (2013) and those of Ayessou et al (2014), who explained the absence of these germs in smoked *Arius* spp., where smoking has an antimicrobial effect.

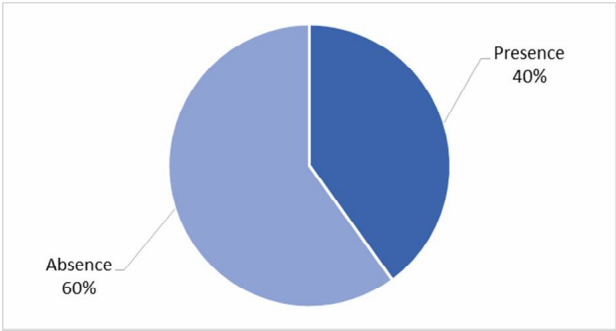

Figure 5. Rate of contamination of fresh anchovies with *Salmonella* germs.

Detection of Pseudomonas aeruginosa and Listeria monocytogenes germs. Of all the samples analyzed, no contamination was detected for the germs of *P. aeruginosa* and *L. monocytogenes*, neither in the case of fresh anchovies, nor in the case of semi-preserved anchovies. This could be explained by the absence of these germs in the preparation and exposition environments of these products. While the germs of *P. aeruginosa* are mesophilic and able to use nitrates under anaerobic conditions (Genestet et al 2012), this has not been the case in our study. As to the germs of *L. monocytogenes*, they are quickly destroyed at high temperatures (Rosset 2001).

Conclusions. In summary, the present study is ultimately concerned with preventing the health risks that could be caused by presence of germs in the anchovies of the city of Rabat. The results of the evaluation of the anchovies' bacteriological quality shows a prevalence of *Staphylococcus aureus*, *Clostridium* and *Salmonella*, with respectively 40%, 50% and 40% in the samples of fresh anchovies and 4%, 4% and 0% in semi-preserved anchovies. This shows that anchovies sold in Rabat city present a health risk to the health of consumers.

To remedy this problem, we recommend a set of preventive measures which must be adopted and applied at different levels, such as good hygiene practices from capture through preparation to sale (cleaning, disinfection, etc.); compliance with and the enforcing of the cold chain; application of good hygiene practices along the chain in order to keep the product clean as soon as possible.

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