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MICROBIOLOGICAL QUALITY OF FROZEN CHICKEN FILLETS IMPORTED IN MACEDONIA DURING COLD AND WARM SEASONS

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Abstract

There is an increase in the consumption of poultry meat, which leads to a high influx of imported frozen chicken fillets into the Macedonian markets and there is little information on their microbial qualities. Our study examined the microbiology quality of imported frozen chicken fillets from Brazil in Macedonia in warm and cold seasons.

A total of 47 samples (frozen chicken half breast, skinless boneless without inner fillet) were analyzed in an accredited laboratory, from which 25 samples were in warm and 22 samples in the cold season. The total bacteria count (TBC), *Staphylococcus aureus* count, and the presence of *Salmonella* spp. and *Campylobacter* spp. were determined by plate count technique using the appropriate media.

TBC mean counts that were found in the warm season (6.17 log CFU/g), and in the cold season (5.95 log CFU/g) meet the international criteria. There wasn't a significant difference between the level of TBC counts in the warm and cold seasons. *Staphylococcus aureus* was found only in 6 samples, and all of them meet the international criteria. *Salmonella* spp. and *Campylobacter* spp. were not detected. But in our study as part of the Enterobacteriaceae family on XLD agar, *Proteus* spp. was isolated. *Proteus* spp. was found in 19 of 25 samples analyzed during the warm season, and in the cold season, it was found in 5 of 22 samples analyzed. There was a significant difference between the number of positive samples for *Proteus* spp. in warm and cold seasons. We suspect that this difference originates from temperature abuses that occur during transport, which are greater in the summer period.

The frozen chicken fillets in Macedonia are contaminated with *Proteus* spp. This should not be ignored because some *Proteus* strains are opportunistic pathogens and can endanger people's health.

***Key words**: Frozen chicken fillets, Total bacterial count, Staphylococcus aureus, Salmonella spp., Campylobacter spp., Proteus spp.*

1. Introduction

Meat can be defined as all parts of warm-blooded animals, in fresh or processed form, which are suitable for human consumption [1]. We can divide meat based on the muscle color of red meat and white meat. The difference between meat colors is caused by a different content of myoglobin in muscles. Red muscles tend to have a greater proportion of narrow myoglobin-rich fibers and white muscles tend to have a greater proportion of narrow myoglobin-poor fibers [2]. In recent years, the demand for poultry meat which is part of white meat has increased due to low-fat, low-cholesterol, low-calorie, and high-protein food [3].

The shelf-life of foods is improved by storage mainly under a very low temperature by refrigeration (4.4 ^{0}C) or freezing (-17 ^{0}C) [4]. Freezing is considered an excellent method for keeping the quality of chicken meat for a long period (9 - 12 months) at a temperature below -18 ^{0}C, as during freezing, the growth of many types of microorganisms will be ceased due to metabolic injury while others especially psychotropic bacteria can grow until the medium freezes [5]. However microorganisms are ubiquitous, and they can cause spoilage of food products if the necessary precautions are not taken [6].

The quality of frozen chicken fillets is a critical issue for consumers, retailers, and producers. Deterioration can be caused by chemical and physical factors that can

occur depending on the microbiological conditions of poultry carcasses which are directly affected by slaughter, sanitization, and storage conditions [7].

Microbiological quality refers to the level of microorganisms present in a food product, and it is an essential factor in determining the safety and shelf life of frozen chicken fillets. Bacteria are the primary cause of spoilage in chicken fillets, and they can produce toxins that cause foodborne illnesses [8]. Poultry and poultry meat are often found contaminated with potentially pathogenic microorganisms such as *Salmonella, Campylobacter, Staphylococcus aureus, E. coli*, and *Listeria* [9]. Some of these bacteria can survive in frozen chicken fillets and can continue to grow when the product is thawed, leading to spoilage and potential health risks. Also, number of total bacteria count (TBC) is a good indicator of meat quality and gives information about hygienic measures during processing [10].

During winter and summer periods, the temperature and humidity conditions can vary significantly, affecting the microbiological quality of frozen chicken fillets. Therefore, this study aims to understand the impact of seasonal changes on the microbiological quality of frozen chicken fillets imported in Macedonia which is crucial for ensuring the safety and quality of the product. The results suggest that imported frozen chicken meats in the Macedonian market have moderate quality with potential opportunistic pathogen *Proteus* spp.

2. Materials and Methods

Frozen chicken half breast, skinless boneless without inner fillet, transported and stored at about -18 ^{0}C were analyzed once a week for 1 year. Analyses were divided into two seasons: the warm season (from March to August) and the cold season (from September to February). The laboratory where all the analyses were done was not responsible for the transport of the samples. The half breasts were of Brazilian origin, vacuum packed in a 2 kg package. All the samples were taken from one distributor.

The samples arrived in the laboratory in frozen form, then they were defrosted overnight at controlled temperature conditions in a refrigerator at 5 - 6 ^{0}C according to ISO 6887-2:2017 [11]. The next day, packages were sanitized with 70% ethanol before opening and one piece weight of about 250 - 300 g was analyzed for microbiological quality.

For TBC and *Staphylococcus aureus* 10 g of the sample was cut in sterile condition and transferred to a filtered stomacher bag, then was added 90 mL sterile peptone water (0.1% w/v), and the content was homogenized for 1 minute using Stomacher (BagMixer 400 CC, Interscience, France). After that samples were analyzed according to international standards.

For TBC, 1mL of the serial dilutions were pour-plated as follows: on plate count agar (Himedia, Mumbai, India) and incubated at 30 ^{0}C for 72 h (ISO 4833-1:2013) [12]. *Staphylococcus aureus* enumeration was performed by spread-planting (0.1 mL) on Baird Parker agar (HiMedia, Mumbai, India) and incubation at 37 ^{0}C for 48h (ISO 6888-1:1999) [13].

For *Salmonella spp.* and *Campylobacter* spp., an amount of test portion was mixed in a filtered stomacher bag with a quantity of suitable enrichment medium to obtain tenfold dilution. The content was homogenized for 1 minute using Stomacher (BagMixer 400 CC, Interscience, France) [14].

After that samples were analyzed according to the following ISO standards:
- Detection of *Salmonella* spp. (ISO 6579-1:2017) [15].
- Detection of *Campylobacter* spp. (ISO 10272-1:2017) [16].

Proteus spp. was isolated during analyses of *Salmonella* spp. on XLD agar. Salmonella-like colonies (black on XLD) were examined for swarming phenotype of the isolates and were made detailed biochemical properties to identify presumptive *Proteus* spp. Biochemical tests were made according to ISO 6579-1:2017, and *Proteus* spp. was distinguished from *Salmonella* spp. based on the ability of Proteus to produce urease constitutively. The enzyme was detected by the degradation of urea by the inoculum, thereby creating an alkaline reaction on pH indicator paper [17].

3. Results and Discussion

TBC log levels in the warm season were reported between 3.32 log CFU/g and 6.17 CFU/g, and between 3.11 log CFU/g and 5.95 CFU/g, in the cold season (Table 1).

Table 1. Levels of TBC and *Staphylococcus aureus* in frozen chicken half breast imported in Macedonia in warm and cold seasons (log CFU/g)

Microorganisms	Warm season		Cold season		ICMSF criteria
	min	max	min	max	
Total bacterial count	3.32	6.17	3.11	5.95	7.0
Staphylococcus aureus	2.00	2.30	2.00	2.85	4.0

According to the microbiological criteria set by the International Commission on Microbiological

Specifications for Foods (ICMSF), all samples were accepted for TBC, they had TBC counts below 7.0 log CFU/g [18]. There wasn't a significant difference between the level of TBC counts in the warm and cold seasons.

Staphylococcus aureus was detected only in 2 samples in the warm season with results of 2.0 log CFU/g and 2.30 log CFU/g and was detected only in 4 samples in the cold season. As shown in Table 1 all the samples were below the maximum allowed limits set for *Staphylococcus aureus* by ICMSF.

All the samples were negative for *Salmonella* spp. and *Campylobacter* spp. and thus acceptable according to National regulation criteria (Table 2) [19].

Table 2. Pathogens present in frozen chicken half breast imported in Macedonia in warm and cold seasons

Microorganisms	Warm season	Cold season	National regulation criteria
***Salmonella* spp.**	Not detected	Not detected	Not detected
***Campylobacter* spp.**	Not detected	Not detected	Not detected

As part of the *Enterobacteriaceae* family on XLD agar was isolated *Proteus*, which was differentiated from *Salmonella* spp. by biochemical tests. In the warm season *Proteus* was found in 19 of 25 samples analyzed and in the cold season was found in 5 of 22 samples analyzed (Table 3).

Table 3. *Proteus* spp. present in frozen chicken half breast imported in Macedonia in warm and cold seasons

Microorganism	Warm season		Cold season	
	Positive tested	Total tested	Positive tested	Total tested
***Proteus* spp.**	19	25	5	22

In this study, the microbiology assessment in both warm and cold seasons does not exceed the upper limits set by international and national regulations.

The maximum value of TBC was found in the warm season at 6.17 log CFU/g, similar values of 6.14 log CFU/g for frozen chicken fillets of Brazilian origin were found in another study [20]. What we have found was that values of TBC vary every week with a mean value of 4.32 log CFU/g during the warm season and 4.18 log CFU/g during the cold season. Studies have shown the correlation of food spoilage with the total bacterial count on the surface part of the carcass: off-odor and sour are noticeably evident when the bacterial count on the carcass reaches about 7.0 log CFU/g and visible slime formation can be observed when the bacterial count reached approximately 8.0 log CFU/g threshold [21].

It should be mentioned that TBCs can predict the shelf life of food products and are used mainly as indicators of process hygiene and quality but not of safety.

S. aureus contaminations remain hazardous as this bacterium can produce toxins that cannot be destroyed by heat and cooking procedures [22]. *S. aureus* was found in 6 samples of 47 total samples tested. In the warm season, two samples were positive for *S. aureus* with values of 2.00 and 2.30 log CFU/g. In the cold season, four samples were found positive with values of 2.85, 2.30, 2.30, and 2.00 log CFU/g. These values were in agreement with the previous study of Maharjan *et al.*, [23] (1.99 log CFU/g), but lower than other studies by Yar *et al.*, [20], Kozacinski *et al.*, [24], and Bhandari *et al.*, [25] (> 4 log CFU/g). The presence of *S. aureus* in meat reflects unsanitary conditions, cross-contamination between the processing phase, and the surrounding environment, processing temperature, and personal contact. Generally, chicken meat becomes contaminated with *S. aureus* when an infected person coughs, sneezes, takes, or breathes inside the plant [21]. The results that were obtained during this study have shown that in both seasons the recorded data of *S. aureus* meet the criteria of ICMSF (4.0 log CFU/g).

The avian species are the most common host for Campylobacter, probably because of their higher body temperature [26]. Studies carried out in slaughterhouses have shown that the main source of the spread of *C. jejuni* on poultry carcasses is their intestinal contents [27].

Campylobacter spp. was not found in our investigation and thus acceptable according to National regulation criteria which were in accordance with the results of other studies by Kozacinski *et al.*, [24], Roasto *et al.*, [28], and Kozačinski *et al.*, [29]. One of these studies reported negative findings of bacteria in chicken breasts and legs, although bacteria were determined in carcasses (28.0%) and chicken wings (31.3%).

Another important pathogen of meat contamination is *Salmonella spp.* which habitats in the intestinal tract of animals and sheds along with feces of the animals that make its presence in the surrounding environment [21]. In this study, *Salmonella* spp. was not detected in frozen chicken fillets imported in Macedonia. There could be viable but non-culturable cells of the strain. Similar studies have been reported by Vaidya *et al.*, [30].

The presence of *Salmonella* spp. in market meat of chicken can indicate the poor hygienic status of the meat processing plant during slaughtering, and cross-contamination between machines, scalding tanks, and workers. During the slaughtering and manual

evisceration process intestinal contents may spill and contaminate the muscle and organs of the chicken which is an important source of *Salmonella* spp. contamination in meat [31].

But during the analyses for *Salmonella* spp., we have isolated *Proteus* spp. on XLD agar. A similar study by Nahar *et al.*, [32], modified the traditional standard method for Salmonella to isolate *Proteus* spp. The results have shown that *Proteus* spp. was found in 76% of tested samples in the warm season and in 22.7% of tested samples in the cold season, which led us to conclude that this opportunistic pathogen is more present in warm seasons. This may be due to temperature abuses during transport which occur more during warm seasons [33]. The rising temperature led to an increase in bacterial count. *Proteus mirabilis* is the most frequently isolated member of the Proteus genus and is also the Proteus species most often associated with infection [34]. *P. mirabilis* is a zoonotic human pathogen of urinary tract infection, nosocomial infection, and wound infection, therefore, a potential threat to public health. According to ICMSF, it is considered unnecessary to have microbiological criteria for other indicator organisms (*Enterobacteriaceae*, coliforms, fecal coliforms, *Escherichia coli*, and enterococci) because they are part of the normal intestinal flora of poultry. Some of them are spread among carcasses even under good processing practices, and some of them can multiply on refrigerated raw poultry carcasses and products. Also, Macedonian's regulation has not prescribed criteria for *Enterobacteriaceae*. However, the positive isolation of *Proteus* in poultry should not be ignored. Subsequent investigations should focus on finding what kind of Proteus is present in poultry imported into Macedonia, especially focusing on finding *P. mirabilis* which is an opportunistic pathogenic organism for people.

4. Conclusions

- Our study has shown that the imported frozen chicken fillets in Macedonia meet the criteria of International and National Regulations.
- We suspect that the differences in the detection of *Proteus spp.* in warm and cold seasons might be linked to hygienic conditions during slaughtering, processing, packaging, storage, and transport conditions.
- We believe the data this study generated will encourage further investigation about the quality of imported chicken meat in Macedonia, and that will encourage improved regulation of the presence of *Enterobacteriaceae,* as they can be a potential threat to public health.

5. References

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