MICROBIOTA AND BIOGENIC AMINES VARIATION OF CHICKEN MEAT; COMPARISON BETWEEN WHITE AND RED MEAT

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Abstract
Chicken meat freshness is in permanent attention for all partners involved in food chain. In this paper we want to highlight the variation of microbiota (psychrotrophic and total viable count) and the variation of biogenic amines in chicken red and white meat. We compared the two anatomical parts of chicken because they have different metabolism, and after cutting from the carcasses they can suffer microbial contamination in the process. The purpose of the study is the evaluation of refrigerated white and red chicken meat (breast and legs) quality using biogenic amines and microbiota. The psychrotrophic microorganisms were initially around a value of 4 log CFU/cm^2 in both anatomical parts, when total viable count were determined around a value of 5 log CFU/cm^2. The microbial load growth until the seventh day, predominant for chicken breast being the psychrotrophic microorganisms, and for chicken legs remaining the total viable count. We studied the most five well-known biogenic amines: histamine, cadaverine, putrescin, spermine and spermidine. Theirs variation during storage was as follows: histamine increased slowly, spermine decreased, spermidine decreased, cadaverine and putrescin increased. Cadaverine was not detected until the fifth day for both chicken legs and breasts and putrescin was not detected until third day and only for chicken legs.

Keywords: meat freshness, psychrotrophic, total viable count, chicken breast, chicken leg.

1.INTRODUCTION

The refrigerated chicken meat spoilage when stored for a long period is due to the microorganism action and the biochemical transformations inside the product. After chicken slaughter, the muscular tissue suffers irreversible physical, chemical and biochemical transformations that determine the muscle to convert in meat. The microbial spoilage processes occurs later. Using refrigeration temperatures for meat conservation purpose reduces microorganism activity.
The difference between white and dark meat or white and red meat is a consequence of the different muscle cell types. Muscle cells are commonly called muscle fibers. White muscle fibers are also known as "fast-twitch" muscle fibers, and are geared towards (as their name implies) quick, sudden movements like a short burst of flight. Red or, "slow-twitch" muscle fibers, by contrast, dominate in muscles that require prolonged constant effort, such as the legs of most animals. Their primary source of energy is fat stores by way of cellular respiration. Birds such as chickens or turkeys fly rarely, and only for short periods, so their breast muscles are mostly white fibers, while their legs are a combination of white and red.
Initially, chicken meat quality was evaluated by determination of microbiological and sensorial attributes. For the identification of the early signs of meat alteration, some chemical indices were proposed: volatile nitrogen basis, composites resulted after breaking the nucleotides, volatile acidity and the biogenic amine content [5]. The biogenic amine occurrence is a consequence of the enzymatic decarboxylation of the precursor amino acids because of the microorganism activities. Polyamines: spermine and spermidine are natural amines produced by the body. The biogenic amines: putrescin, cadaverine, histamine, tyramine, tryptamine, β-phenylethylamine can be formed when storing the chicken meat due to microorganism action. The biogenic amine determination is important not only because of their toxicity but also their potential use as freshness indicators [2]. Different authors' studies regarding the refrigerated chicken meat showed that some of the previously mentioned biogenic amine concentrations are increasing in time, while others are decreasing during storage [1,2,3,8].

The occurrence of these amines is dependant on different factors that vary in time. The microbial population influences the profile of biogenic amines. Spoilage responsible microorganisms might not have the capacity of amine forming. From a practical point of view, the relative simplicity and quickness identification and quantification of the biogenic amines (compared to the microbiological measurement) besides the economical advantages (for example the quick test for determining the diamines described by Hall *et.al* [6]), are reasons for using these substances as chemical indices for animal origin product freshness.
The purpose of the study is the evaluation of refrigerated white and red chicken meat (breast and legs) quality using biogenic amines and microbiota (expressed by psychrotrophic and total viable count).

2. MATERIALS AND METHODS

The chicken carcasses were purchased from a Romanian slaughterhouse that also makes chicken meat carving. We were interested in chicken legs (thigh and drumstick) and breasts that we considered as red, respectively white meat. The meat was analyzed after cooling, packaging and transported from the slaughterhouse after one day after chicken slaughter. The anatomical parts were stored aerobically for 7 days at a temperature of 4±1°C in the refrigerator. The refrigerator used was Electrolux ENB43691S. The samples were analyzed the first day when the meat was received, recorded as day 1, then at the 3rd, 5th and 7th day.
The dry matter determination was done according to Romanian STAS 9065/3-73.
Pieces of raw chicken meat and skin (16 cm^2 in area) were aseptically excised from carcasses and each piece was homogenized with 100 ml of saline water (0,8% NaCl) by using a homogenizer type Bagmixer 400. Duplicate 0,1 ml aliquots of suitable dilutions of each skin homogenate were spread on the surface of nutrient agar plates. Inoculated plates were incubated in ATICH 9082 Incubator in aerobically condition at 4ºC for 14 days for psychrotrophic microorganisms and at 30ºC for 2/3 days for total viable count. After that viable colonies were counted using Automatical Colony Counter SC6.
The measurement of biogenic amines content using high performance liquid chromatography, was performed according to the method proposed by Food Research Institute from Helsinki, Finland [4]. The method principle is as follows:
-bioactive amines are extracted from a homogenized sample with diluted perchloric acid;
-an aliquot of the extract is derivatised with dansyl chloride reagent;
-separation and quantification of dansylated amines is performed by reversed phase liquid chromatography with ultraviolet detection at 254 nm.
All the reagents used were analytic pure, for HPLC use. Te water used was deionised. The necessary reagents were purchased from the Merck and Sigma-Aldrich companies. Installations and equipment used for biogenic amine determination were: Philips 7768 food processor, homogenization device 7011S, Kern 770-60 analytical balance, Silent CrusherM homogenization device, centrifuge EBA 21, filter paper for quick filtering with 55 mm diameter, syringe filters with porosity of 0,45 µm and 13 mm diameter, Heidolph REAX control agitator, ultrasonic water tank Aquawave TM, incubator BMT INCUCELL 55, water deionising system EASY pure RoDi, filtering assembly with vacuum pump. The device for the HPLC determination was a liquid chromatograph model SURVEYOR produced by Thermo Electron company, configured with detector model PDA PLUS DETECTOR, auto-sampler model AUTOSAMPLER PLUS, pump model LC PUMP PLUS and detector UV-VIS. Chromatography column is type BDS Hipersyl C18. The biogenic amines quantification: quantitative measurement was performed depending on the internal standard using peaks for each biogenic amine. The 254nm wavelength absorbance was measured and the

resulted peaks were integrated with CromQuest software. The concentration of each biogenic amine was expressed in mg/kg d.w. (d.w. = dry weight).
The statistical analysis of the obtained data was done using Microsoft Excel features for 10 samples in each of the storage days. The results obtained are presented as the mean values. The standard deviation is a measure of the dispersion of outcomes around the mean. The differences among means were determined using the method of the smallest squares and the significance level was $p< 0.05$.

3. RESULTS AND DISCUSSIONS

3.1.Microbiota of red and white chicken meat

Our results concerning psychrotrophic microbiota and total viable counts are presented in two figures. In each figure we made a comparison for each kind of meat (red and white) for the two types of microbes studied.
In figure 1 we show the microbiota variation of red chicken meat. In first day of storage the content of the psychrotrophic microorganism are smaller than the total viable count. In the following days the number of microorganisms are increasing until the fifth day of storage with one logarithmic cycle. In the last day of storage the psychrotrophic microorganisms increase with one logarithmical cycle, and the total viable count increase from 7.5log CFU/cm^2 to 7.7log CFU/cm^2. This means that the majority of the microorganisms incline to become mostly psychrotrophic-type due to the adaptation at the new environment conditions.
In figure 2 we show the microbiota variation of white chicken meat. Initially, the psychrotrophic microorganisms content are a little bit smaller than those found in red chicken meat.

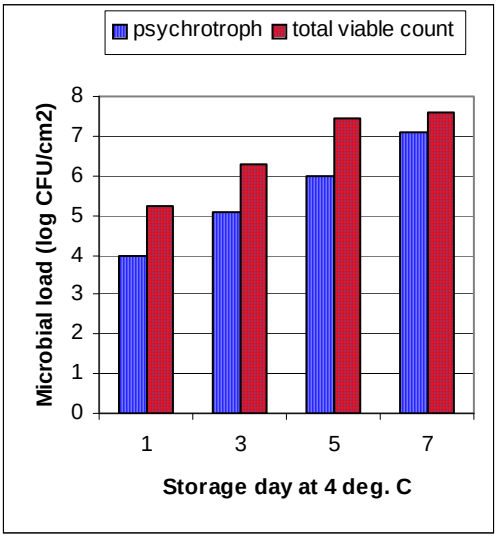

Figure 1. Microbiota of red chicken meat

Until the third day the microbiota studied increased with one logarithmical cycle. Since the fifth day the psychrotrophs content are near to total viable count, and in the last day of storage, due to microbes adaptation, the psychrotrophs are exceeding the total count. Comparing the variation of microbiota between the two kinds of meat we can say that the total viable count for white meat had a lesser growth than red meat because of the smallest initial contamination. The psychrotrophic microorganisms had in case of white and red chicken meat the same evolution.

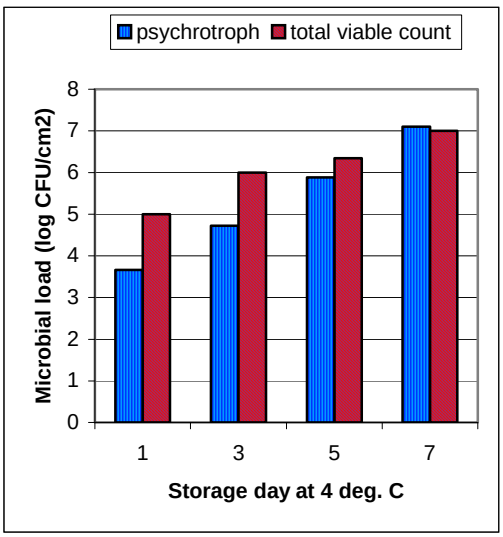

Figure 2. Microbiota of white chicken meat

3.2. Biogenic amines variation in red and white chicken meat

We studied the most five important Biogenic amines that can be found in various kinds of meats: histamine, cadaverine, putrescin, spermine and spermidine. Mietz and Karmas [7] proposed a fish meat freshness index based on the value of the considered five amines. So, those five biogenic amines are very important for the quality of animal origin products, such as, in our case, the red and white chicken meat.
In figure 3 is shown the variation of histamine at refrigeration storage of red and white meat. The initial value of histamine content is very small, not exceeding 2mg/kg. It increases in time due to microbial activity by aminoacid decarboxilation, cells biochemical and enzymatic activity. In breasts the value of histamine increased more than in legs because of microbial activity. In legs the increase of histamine is very slow, not exceeding 3mg/kg at the last day of storage. So, the histamine content in red and white chicken meat is not a threat to human health if the refrigeration conditions are respected.

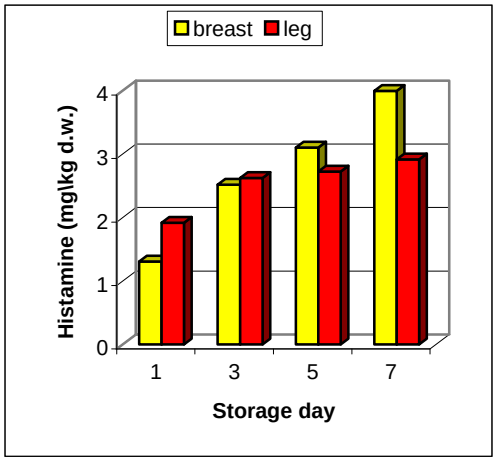

Figure 3. Histamine variation in red and white meat

In figure 4 and 5 it is shown the variation of two biogenic amines that can influence the smell of the meat. It is well known that those two amines are a particular, unpleasant smell. Those amines are not detected by our method of determination until the fifth day, with one exception- putrescin that were detected at the third day, in a small amount, in red meat. This can suggest a microbial activity that produced the amine from ornithine by decarboxilation. Putrescin were determined in the last day of storage to a value of 10.5mg/kg.
Cadaverine was detected at fifth day of storage around 2 mg/kg for red and white chicken meat. At the last day of refrigeration the content of cadaverine were higher in red meat, but in a value that not exceed 8mg/kg.
About those two unpleasant amines we can say that from the fifth day become the meat spoilage and, also, the freshness lose of chicken meat, especially of red meat, due to microbial contamination.

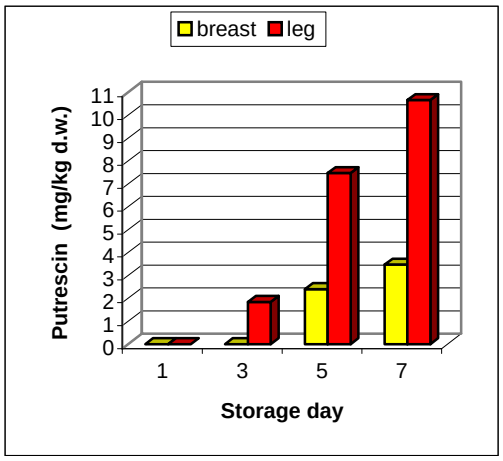

Figure 4. Putrescin variation in red and white meat

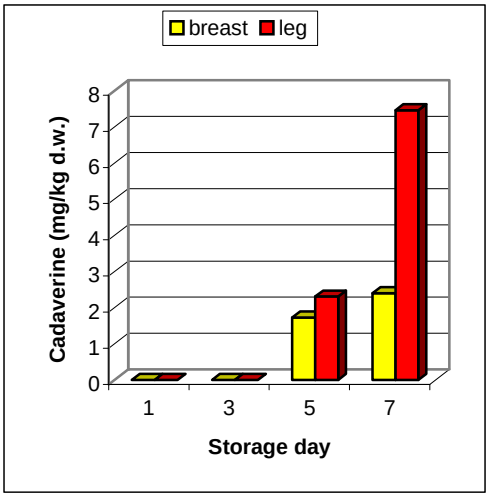

Figure 5. Cadaverine variation in red and white meat

The initial content of spermine is high (22mg/kg for white meat and 18mg/kg for red meat). It slowly decreases until the seventh day

of storage (20mg/kg for white meat and 17mg/kg for red meat). This slow decrease can be due to a use of spermine as nitrogen source of microorganisms.

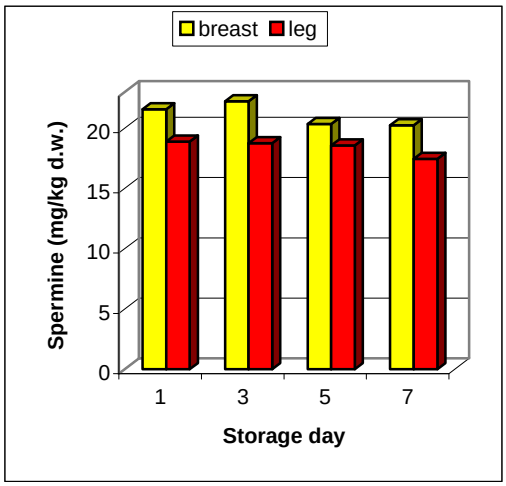

Figure 6. Spermine variation in red and white meat

Spermidine is a polyamine that has a relatively small amount (around 5mg/kg for white meat and 6.5mg/kg for red meat). The spermine content decreases in time, possibly due to its use as nitrogen source for microorganisms and also due to enzymatic action of polyaminooxidase.

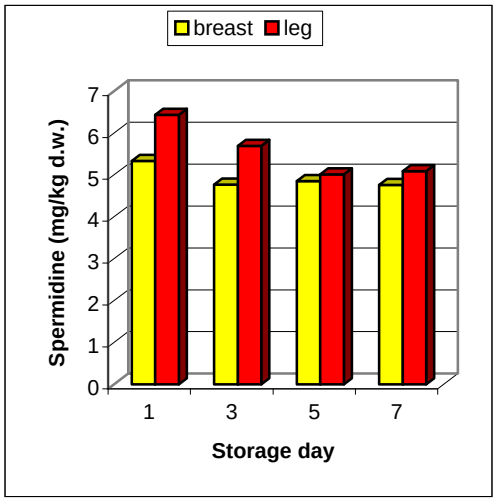

Figure 7. Spermidine variation in red and white meat

4.CONCLUSIONS

If the chicken meat is kept under right refrigerant conditions, the microbial load and biogenic amines content are in reasonable limits.

The red meat had a slight exceeding of microbial load, and cadaverine and putrescin content. This can mean that it was a more contamination at cutting operations than white meat.

5.REFERENCES

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