FRESHNESS EVALUATION OF CHICKEN MEAT USING MICROBIOTA AND BIOGENIC AMINE INDEX.

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Abstract: *The purpose of our study was to evaluate freshness of raw chicken meat using microbial and biogenic amines content. The objectives were to determine the variation of microbiota (total viable count, psychrotrophic and Pseudomonas spp.), to study the variation of some important biogenic amines and to calculate a biogenic amine index for refrigerated chicken carcasses aerobically stored for one week. Hereby, we used microbial analysis and HPLC determination for the following biogenic amines: tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine, serotonin, tyramine, spermine and spermidine. Our determinations showed that total viable count increased in value from the first to the seventh day (the last day of storage), psychrotrophic microorganisms also increased in number and Pseudomonas spp. increased from the first day to the seventh day of storage. Regarding biogenic amines variation, tryptamine had a low initial content and after a week of storage the content was below 5mg/kg, β-phenylethylamine had also a small initial value and after a week of storage its value is slightly higher than 5 mg/kg, serotonin had a similar comportment with β-phenylethylamine and tyramine the same with β-phenylethylamine only that the final value was slightly higher. Cadaverine and putrescine were detected beginning with the third day of storage and they had the highest values after a week refrigeration of chicken meat. After one week of refrigerated storage, spermine had a particular allure because it decreased permanently. Spermidine had a very slow increase, from 4.8 mg/kg to 6 mg/kg. Biogenic amines index were calculated according to the mathematical relation proposed by researchers from Barcelona University.*

Keywords: *meat, freshness, microbiota, Pseudomonas, biogenic amine index*

Introduction

The spoilage of refrigerated chicken meat when stored for a long period is due to the microorganisms' action and biochemical transformations inside the product. If the refrigerating chain from producer to consumer is not ensured, or if the seller overpasses shelf life, the consumer can have an unpleasant surprise of buying an altered product. After chicken slaughter, the muscular tissue suffers irreversible physical, chemical and biochemical transformations which determine the muscle to convert into meat. The microbial spoilage processes occur later. Microorganisms' activity is slowed down by using refrigeration temperatures for meat conservation purpose. In order to obtain products with high conservation durability and to increase the refrigeration effect, it is necessary to have as less initial microbial load as possible [1].

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 [2]. The biogenic

amine occurrence is a consequence of the enzymatic decarboxylation of the precursor amino acids because of the microorganisms' activities. Polyamines: spermine and spermidine are natural amines produced by the body. The biogenic amines: putrescine, cadaverine, histamine, tyramine, tryptamine, β-phenylethylamine can be formed when storing the chicken meat due to microorganisms' action. The biogenic amine determination is important not only because of their toxicity but also their potential use as freshness indicators [3].

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. It is difficult to establish quality limits universally accepted based on the biogenic amine content. The above mentioned reasons are partially justifying the relative dispersal of biogenic amine values for meat, in various researches. 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 all. [4], are the reasons for using these substances as chemical indices for animal origin product freshness.

The purposes of the study are:

- determination of microbiota variation from chicken meat, concerning total viable counts, psychotrophic microorganisms and Pseudomonas spp at refrigerated raw chicken meat storage;
- evaluation of freshness of the refrigerated chicken meat using biogenic amine index.

Materials and methods

Chicken carcasses were purchased from a Romanian slaughterhouse. The meat was analyzed after cooling, packaging and transportation from the plant the first day after slaughter. All the carcasses were stored aerobically, without package foil, for 7 days, at the temperature of 4±1°C in the refrigerator type Electrolux ENB43691S. The carcasses weight varied between 1.2÷1.5 kg. 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 made according to Romanian STAS 9065/3-73.

Pieces of raw chicken meat with 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 model 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 or 3 days for total viable count. Pseudomonas were determined on centrimide-fusidin-cephaloridine agar supplemented with SR 103 for the selective isolation of *Pseudomonas* spp. generally, after incubation at 25ºC for 1 or 2 days, and oxidase-positive colonies were enumerated. [5] 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 [6]. The method principle is as follows:

-Bioactive amines are extracted from a homogenized sample with diluted perchloric acid;

-An aliquot of the extract is derivated 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: 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.m. (d.m. = dry matter). Our determinations refer to the following amines: tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine, serotonin, tyramine, spermine and spermidine.

The statistical analysis of the obtained data was made using Microsoft Excel features for 10 samples in each of the storage days. Each sample was analyzed in triplicates. The results obtained are presented as the mean ± standard deviation (SD). 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 $p< 0.05$.

Results and discussion

After the chicken carcasses were refrigerated for three weeks at 4ºC, we determined the variation in time of total viable count and psychotroph count. In figure no. 1 we present the variation of refrigerated storage of total viable count. The average initial contaminations of the chicken meat were 5.11 log CFU/cm^2 (CFU – colony forming unit), it increase at the third day at 5.95 log CFU/cm^2 and in the 5th day of storage at 7.1 log CFU/cm^2. The shelf life of carcass, as stated by the food manufacturer, was the fifth day of storage. In the 7th day of refrigeration the total viable count were 7.56 log CFU/cm^2. This slow increase of microbiota is due to refrigeration temperatures that do not allow a quick spoilage of chicken carcasses and preserving the freshness for longer period of time.

Psychrotrophic count is characteristic for food that is preserved at refrigeration temperatures. We determined psychrotrophic microbiota after seven days of storage. The variations of microorganisms are presented in figure 2. The initial load were 4.00 log CFU/cm^2, increasing permanently to 6.9 log CFU/cm^2 for the 7th day of storage at refrigerated temperature. Comparing to total viable count, on the first day of storage we have a difference of more than 1 log CFU/cm^2, at the expiration date of chicken carcass (the 5th day), the difference was higher than 1.5 log CFU/cm^2 for the total viable count. In the 7th day of storage, the difference begins to decrease, being only of 0.6 log CFU/cm^2. It appears

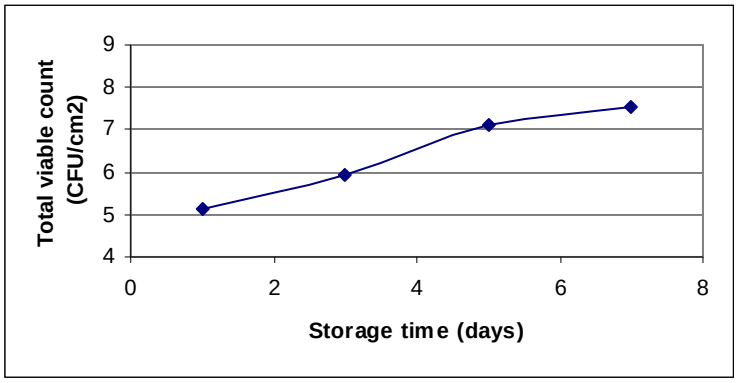

Figure 1. Total viable count variation for refrigerated chicken meat

that after the 5th day of storage, the psychrotrophic microbiota begin to prevail

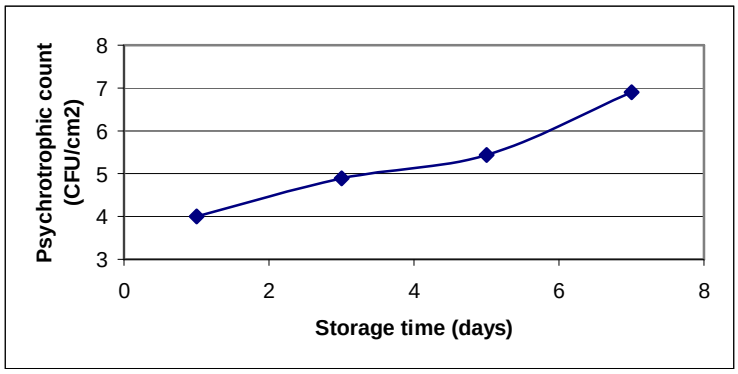

Figure 2. Psychrotrophic variation for refrigerated chicken meat

Pseudomonas spp. is very important microbiota that spoils chicken carcasses during aerobically refrigeration storage. In figure 3 we show the variation of *Pseudomonas spp.* from chicken carcasses until the 7th day of storage at 4ºC. As it can be noticed, the number of *Pseudomonas* increase in time, from 3.8 log CFU/cm^2 on the first day of storage to 6.4 log CFU/cm^2 on the 7th day. Because *Pseudomonas* can be mesophile or psychrotrophic microorganisms, we can compare the values obtained with those of psychrotrophic biota.

Thus, the initial contamination of carcasses is mostly by *Pseudomonas spp.*, the trend kept until the 5th day of storage. On the7th day, besides *Pseudomonas spp*, it appears that another biota become to increase.

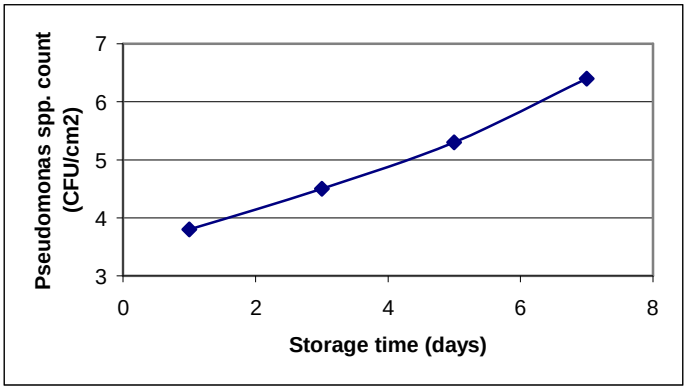

Figure 3. *Pseudomonas spp*. variation for refrigerated chicken meat

Biogenic amines can be an indicator of chicken meat freshness. Researchers from Barcelona University proposed from raw foods a biogenic amine index that has a mathematical relation of:

$$BAI = \frac{\text{hystamine} + \text{cadaverine} + \text{putrescin}}{1 + \text{spermine} + \text{spermidine}}$$

. Based on this index it can be determined allergenic risk and freshness of raw foods.
Our determined values for considered biogenic amines are presented in figure 4.

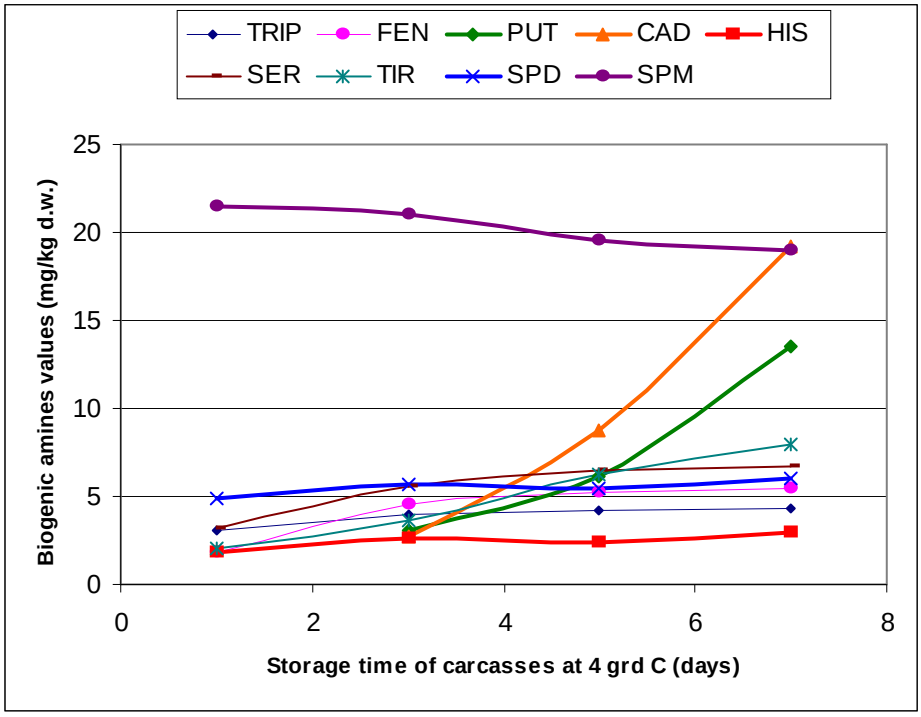

Figure 4. Biogenic amines variation for refrigerated chicken meat, were: d.w.-dry weight

As it can be seen, in time, the values of mostly biogenic amines increase, with one exception, of spermine which decreases.
On the first day of storage, the biogenic amine content is low, spermine having the highest value: 21.5 mg/kg d.w. followed by spermidine, of 4.8 mg/kg d.w. Also, putrescine and cadaverine have not been detected on the first day of storage. This is due to the fact that either our method is not sensible enough to detect smaller amounts of those biogenic amines, or they do not exist in chicken meat. Tryptamine and tyramine presence is due to their action as hormones, neurotransmitters, and serotonin being one of tryptamine derivative. β-phenylethylamine action as neuromodulator and neurotransmitter, its derivatives being also hormones (tyramine is in fact 4-hydroxi-phenylethylamine).
Spermine and spermidine are two amines involved in cellular metabolism, essential for organism growth and for development and proliferation of cells. Spermine can be synthesized from spermidine. Histamine is present in small amount (1.8 mg/kg d.w.).
The third day of storage of raw chicken carcasses is important to be highlighted as we detected small amounts of putrescine and cadaverine.
On the day of shelf life ending cadaverine and putrescine amounts are increasing, cadaverine increase being over four times, putrescine increase being of 7 times, spermine are decreasing. The spermine decrease can be due to its use by the

microorganisms as nitrogen source. Histamine has a particular variation because on the fifth day is slowly decreasing, after a slow increase from the first day of chicken meat refrigeration storage.

All biogenic amines that we determined could be made as a result of microbiota activity on amino acids chicken carcasses. It is well known that cadaverine and putrescine are two amines with characteristic repugnant smell especially in more advanced stadium of alteration. A slight modified smell was distinguished on seventh day of storage, but we cannot be sure if this was due to cadaverine or putrescine.

We calculated the BAI for the chicken carcasses and we had the following results:

-IAB for the first day were 0.068
-IAB for the third day were 0.30
-IAB for the fifth day were 0.66
-IAB for the seventh day were 1.37

Compared with the first day we can observe that IAB of the third day increased 5 times and IAB for the fifth day 10 times. We can also say that fresh raw chicken meat has an IAB below 1.00.

Conclusion

An increase in total viable count number leads to spoilage, loss of freshness and shelf life of raw chicken meat. *Pseudomonas spp*. was the prevalent microorganisms that characterized best the chicken meat spoilage and being the principal cause for freshness loss. Chicken meat freshness decreases in time and this leads to quality loss and shelf life reduction.

Chicken freshness is good if IAB is below 1.00.

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