REFRIGERATED CHICKEN MEAT FRESHNESS. CORRELATION BETWEEN EASILY HYDROLISABLE NITROGEN, *p*H VALUE AND BIOGENIC AMINE CONTENTS

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Received 24 June - Accepted 10 August

Abstract
In this study we evaluated the variation of highly hydrolysable nitrogen, pH and the biogenic amines from the refrigerated chicken meat and we calculated the freshness indices based on biogenic amine content. We correlated the obtained results (pH and ammonia content) with Romanian legislation in order to determine the storage time limit for the refrigerated chicken that can be acceptable for consumption. Finally we determined the freshness indices proposed by different authors for the refrigerated chicken meat based on the biogenic amine content. The meat pH increased constantly from the first (5.92) up to the 20th day of storage in refrigerated state (7.33). Also, the easily hydrolysable nitrogen increased from 20.5 to 39.68mg NH_3/100g. From the biogenic amine content point of view, the first day spermine is preponderant (50% of the total amines). The 20th day, cadaverine is the preponderant amine followed by putrescin (17%) and β-phenylethylamine (13%). Based on the biogenic amine content, three freshness indices (FI) were calculated as per the relationships proposed by: Mietz and Karmas, Veciana-Nogues et.al., and by Silva and Gloria.
Correlating the obtained results for easily hydrolysable nitrogen, pH and Romanian legislation norms, we determined that the maximum freshness limit for chicken meat is at the 3rd day of storage, hereby FI are as follows: FI (Mietz and Karmas) = 0.27, FI (Veciana-Nogues) = 12.2, FI (Silva and Gloria) = 0.24.

Keywords: biogenic amines, spoilage, quality index, freshness index, chicken meat, refrigerated meat, *p*H, easily hydrolysable nitrogen.

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. If the refrigerating chain from producer to consumer is not ensured, or if the seller overpasses the 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 in meat. The microbial spoilage processes occurr later. Microorganism activity is reduced 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 (Tofan, 2005).

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 (Halsz *et.al*, 1994).

The biogenic amine occurrence is a consequence of the enzymatic decarboxylation of the precursor

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aminoacids 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 (Balamatsia *et.al*, 2006)

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 (Vinci et Antonelli, 2002, Apostolos *et.al*, Sarinen *et.al*, 2002, Balamatsia *et.al*, 2006, 2007). The freshness index of meat calculated on the basis of biogenic amines is a topic to be debated. 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 micro-biological measurement) besides the economical advantages (for example the quick test for determining the diamines described by Hall *et.al*, 1999), are reasons for using these substances as chemical indices for animal origin product freshness.

The purpose of the study is evaluation of refrigerated chicken meat freshness using biogenic amine index.

2. Materials and Methods

The chicken carcases were purchased from the Agricola International Bacau company slaughterhouse. The meat was analysed after cooling, packaging and transportation from the plant the first day after slaughter. The carcases were stored aerobically for 20 days at a temperature of 4±1°C in the refrigerator. The refrigerator used is Electrolux ENB43691S. The carcases weight varied between 1.2÷1.5 kg. Sampling was done as per Romanian Recommendation Norm 24/01/2005 (***, 2005).

The samples were analysed the first day when the meat was received, recorded as day 1, then the 3rd, 5th, 7th, 13th, and 20th day. The dry matter determination was done according to STAS 9065/3-73. The pH was measured using a standardized method (STAS 9065/8-74) with a WTW Ino Lab pH 730 *p*H-meter.

The easily hydrolysable nitrogen was determined according with STAS 9065/7-74. The purpose was to determine the ammonia quantity that is formed in the product after protein degradation by the spoilage microorganism activity.

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 (Eerola *et.al*, 2001). 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, homogenisation device 7011S, Kern 770-60 analytical balance, Silent CrusherM homogenisation 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.

The statistical analysis of the obtained data was done using SPSS 13 software for 10 samples in each

of the storage days. 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 $p< 0.05$.

3. Results and Discussion

3.1. Evaluation of the *p*H during chicken meat storage

The *p*H values are shown in table 2. We obtained an initial *p*H value of 5.82, that is fitting well in the limits of 5.8…6, which indicates that the product is fresh in accordance with Romanian Directive 86 (***, 2002). In time, it can be noticed that the chicken meat *p*H increases. Starting with the 3rd day, the meat *p*H is over 6.0, meaning that it starts spoiling. The spoilage continues up to the last day of measurements.

The *p*H increase is due to the bio-chemical reactions in post-mortem chicken meat. There are many factors that influenced the *p*H initial chicken meat value. The chicken meat was evaluated the second day after slaughter. The first day of slaughter the *p*H value is not yet constant, it decreases within the first few hours and then increases in time (Debut *et.al*, 2003, Duclos *et.al*, 2007). During the ageing process, the *p*H rises, due to proteolysis.

The sampling has an important influence concerning the results because the global *p*H was influenced by the skin *p*H, meat/skin ratio and the type of meat from the anatomical parts where the sampling was performed.
Only the *p*H value as a freshness indicator is controversial because its value varies depending on many factors. Yet, according to this factor, and in concordance with Romanian regulations (*p*H=5.8…6.0), good quality fresh meat is up to the 3rd day of storage.

3.2. Evaluation of easily hydrolysable nitrogen content of refrigerated chicken meat

In Table 3, there are shown the easily hydrolysable nitrogen values in the refrigerated chicken at 4ºC for 20 days.

In Table 3, it can be noticed that the first day of evaluation, the easily hydrolysable nitrogen value is 20.5 mgNH_3/100g, increasing up to the 20th day to 39.685 mgNH_3/100g.

Table 1. Operating conditions of HPLC instalation

Time, min	Gradient		Flow, ml/min	Wave length, nm	Column pressure, bar	Column temperature, ºC	Sample room temperature, ºC	Injected sample volume, µl
	Ammonia acetate, %	Nitrile acetate, %						
0.01	40	60	1.00	254	min. 70	40	7	20
15	40	60						
20	30	70						
25	5	95						
30	40	60						

Table 2. *p*H variation of chicken meat stored at refrigerated conditions

*p*H value	Storage time (days)											
	1		3		5		7		13		20	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	5.82	0.1	6.05	0.08	6.41	0.09	6.52	0.08	6.81	0.06	7.33	0.19

SD - standard deviation of 10 determinations.

Table 3. Easily hydrolysable nitrogen content variation during storage of refrigerated chicken

Easily hydrolysable nitrogen content, mg NH_3/100 g	Storage time (days)											
	1		3		5		7		13		20	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	20.5	1.26	22.2	0.68	24.9	1.11	25.7	0.87	33.7	0.75	39.68	0.40

SD - standard deviation of 10 determinations.

Correlating the obtained values with the imposed limits by the Romanian Recommendation Norm (***, 2002) of 25 mgNH_3/100g, the 5th day of storage refrigerated chicken meat is considered to be fresh. The accumulation in time of easily hydrolysable nitrogen is due to protein hydrolysis catalysed by meat enzymes. Also, microorganisms with proteolytical activity can act on proteins transforming them into smaller compounds, such as free aminoacids. The aminoacids can suffer oxidative deamination, decarboxylation and desulphuration resulting gases as ammonia, carbon dioxide, hydrogen sulphide etc. Meat itself contains free aminoacids or they can occur from proteins by hydrolysis. Subsequently, they can be degraded partially or totally to simple compounds as CO_2, H_2O, NH_3, H_2S etc. Also, easily hydrolysable nitrogen in food Romanian legislation is a very important marker for raw meat freshness.

3.3. Biogenic amine accumulation in refrigerated chicken meat

Table 4 shows the biogenic amine content, measured from the raw chicken meat, refrigerated and stored for 20 days.
In the table it can be noticed that: the first day of measurement of the biogenic amines, cadaverine and putrescin were not detected in any of the analysed samples. Tryptamine content is increasing in time having a small variation during the 3rd day of storage. Phenylethylamine is increasing in time, mainly after the 7th storage day the quantity is highly increasing (approximately threefold after the first day). Putrescin has a similar variation as the phenylethylamine. Cadaverine is increasing in time and after the 7th day has a very high increase from 3.15 mg/kg (day 7) up to 30.07 mg/kg (day 13) and 57.98 mg/kg in the 20th day. Histamine during the first day of storage has the lowest content of the all studied biogenic amines in chicken meat. It increases slowly in time; the 20th day the quantity is 6.1 mg/kg. Serotonin is increasing in time, thus its accumulation is low: 5,16mg/kg during the first day and 7.93 mg/kg during the 20th day. Initially, tyramine is not so high (2.99 mg/kg) as compared with the other biogenic amines. However the concentrations increase in time up to 13.67 mg/kg. Spermidine values are limited approximately between 5 and 6 mg/kg for all the 20 days of chicken meat storage. Spermine decreases in time, but during the first 7 days has the highest values compared with the other amines.
Calculating each amine ratio out of the total amount (table 5), it can be noticed that from the first day up to the 7th day spermine was the dominant amine, its ratio decreasing gradually (from 50% down to 38%). The first day, cadaverine, putrescin and histamine had the lowest content out of the total amines. The 20th day, tryptamine, serotonin and histamine had the lowest level of amines. The first day, spermine had the highest percentage of the biogenic amines while during the 20th day the first place belongs to cadaverine followed by putrescin and phenylethylamine. These three amines have a negative influence on the food product odour.

The biogenic amine occurrence is mainly a consequence of the activity of decarboxylases, enzymes produced by microorganisms. These enzymes act on the free aminoacids or on the aminoacids resulted from protein hydrolysis. Polyamine spermine and spermidine are amines existing in the body and are naturally produced by it. The biogenic amines: putrescin, cadaverine, histamine, tyramine, tryptamine,β-phenylethylamine are formed during storage of chicken meat due to microorganism activity. The decrease in time of spermidine and spermine is due to their use as nitrogen sources by microorganisms (Balamatsia *et.al*, 2006, 2007).

3.4. Raw chicken meat freshness index evaluation

In a series of researches, the limits of freshness meat food products were determined with index calculated based on the biogenic amine content. The researchers proposed some biogenic amines as an index or freshness index based on biogenic amine content for meat evaluation (Apostolos *et.al*, 2006, Balamatsia *et.al*, 2006, 2007, Silva et. Gloria, 2002).

The advantage is the small concentration of biogenic amines that can be detected with HPLC long before they can be sensorially identified especially by smell. That's why biogenic amines can be chemical indicators of meat spoilage, and therefore can be used for evaluation of the freshness status of the animal origin products. Initially, those freshness indicators were used on fish meat.

Table 4. The biogenic amine content variation during storage of refrigerated chicken

Biogenic amine content, mg/kg	Storage time (days)											
	1		3		5		7		13		20	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tryptamine	4.04	0.88	3.98	0.56	5.40	1.21	5.94	1.17	6.50	1.89	10.34	0.85
Phenylethylamine	4.19	0.36	4.85	0.90	5.21	2.64	5.40	3.39	17.62	1.36	23.14	3.48
Putrescin	nd	0.00	3.05	4.86	3.19	0.19	3.47	2.63	16.67	7.72	28.84	10.59
Cadaverine	nd	0.00	2.74	3.63	2.75	0.79	3.15	2.23	30.07	10.47	57.98	12.33
Histamine	2.36	0.12	2.64	0.78	2.39	0.81	3.00	0.84	2.91	0.13	6.10	0.44
Serotonin	5.16	0.84	6.57	1.98	6.50	1.54	6.72	0.46	7.65	1.23	7.93	0.28
Tyramine	2.99	1.52	3.79	1.82	3.24	1.80	3.93	1.57	8.08	2.84	13.67	2.12
Spermidine	4.84	0.33	5.67	1.15	4.89	2.91	6.01	1.10	5.92	1.72	5.68	2.38
Spermine	23.01	2.55	24.00	2.93	23.55	1.42	22.94	1.36	20.52	1.10	17.93	3.74

nd-not detected, SD - standard deviation (10 determinations).

Table 5. Storage variation percentage of each biogenic amine from the total content

Biogenic amine content, %	Storage time (days)					
	1	3	5	7	13	20
Tryptamine	9	7	9	10	6	6
Phenylethylamine	9	8	9	9	15	13
Putrescin	0	5	6	6	14	17
Cadaverine	0	5	5	5	25	34
Histamine	5	5	4	5	3	4
Serotonin	11	11	11	11	7	5
Tyramine	6	7	6	6	7	8
Spermidine	10	10	9	10	5	3
Spermine	50	42	41	38	18	10

The freshness index (FI) most used in the literature for refrigerated chicken meat are:

a) $\frac{\text{histamine} + \text{cadaverine} + \text{putrescin}}{1 + \text{spermine} + \text{spermidine}}$, proposed by Mietz and Karmas (Balamatsia et.al, 2006, Silva et Gloria, 2002)

b) Cadaverine + Putrescin + Tyramine + Histamine, expressed in mg/kg, and proposed by Veciana-Nogues and other (Balamatsia *et.al*, 2006, Silva and Gloria, 2002)

c) $\frac{\text{spermidine}}{\text{spermine}}$ proposed by Silva and Gloria (Silva et Gloria, 2002)

We made the necessary calculations for the freshness index expressions mentioned above in order to establish the variation curve for the refrigerated chicken meat. The results are presented in figure 1, figure 2 and figure 3.

We know that meat freshness is a quality indicator that decreases in time because of meat spoilage.

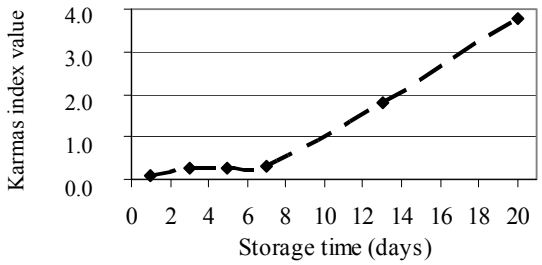


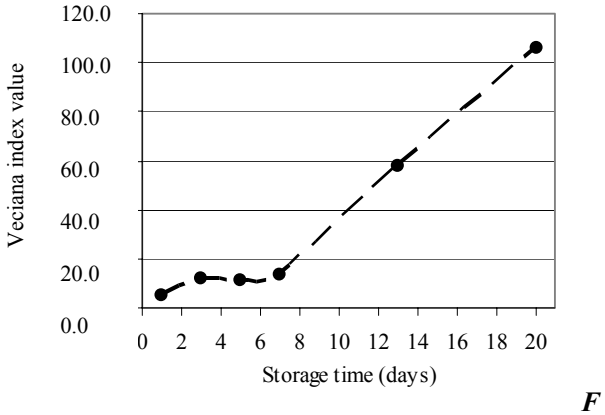
Figure 1. Mietz and Karmas freshness index variation for refrigerated chicken meat

The refrigerated meat freshness is reduced in time as a result of biochemical, physico-chemical and microbiological transformations. The loss of freshness indicates that meat has started spoiling.

3.5. Correlating the experimental values and establishing the freshness limit

Based on the performed chemical measurement, and taking into account the limit imposed by the

Romanian standards regarding *p*H and the easily hydrolysable nitrogen, we can asses that the 3rd day is the maximum limit for the refrigerated chicken meat best quality (freshness).



F igure 2. Veciana-Nogues freshness index variation for refrigerated chicken meat

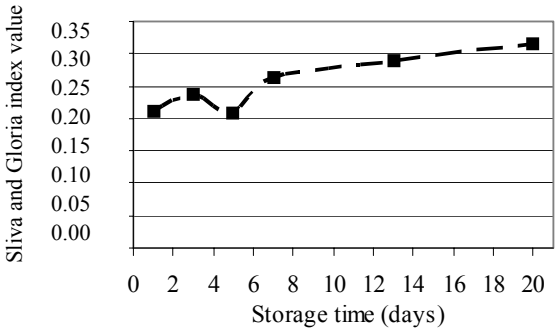


Figure 3. Silva and Gloria freshness index variation for refrigerated chicken meat

Correlated with the biogenic amine content expressed by the values of freshness index, during the 3rd day of storage, the refrigerated chicken meat FI are as follows:

- the FI proposed by Mietz and Karmas is 0.27.
- the FI proposed by Veciana-Nogues is 12.2.
- the FI proposed by Silva and Gloria is 0.24.

4. Conclusions

Based on the evaluation quality indicators of (the easily hydrolysable nitrogen and *p*H) the freshness limit of the raw refrigerated chicken meat is three days after slaughter.

Correlating the *p*H variation and the easily hydrolysable nitrogen with the quality indices measured based on the biogenic amines, we consider that all three freshness indices can be used as quality indicators for the refrigerated chicken meat.

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