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Evaluating red meat putrefaction in long term storage in freezing condition based on co-variation of major biogenic amines and Total Volatile Nitrogen

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Abstract

Nearly all of the rejected frozen carcasses in Asian countries are due to their high amount of total volatile nitrogen (TVN). The main aim of this study was evaluating red meat putrefaction based on putrescine and cadaverine content in red meat as major biogenic amines that contributed in TVN content during long-term storage in freezing condition. For this, 48 samples were collected from local slaughterhouse from neck, flank, sirloin and rib set of beef meat and analyzed for the biogenic amine and TVN content during 12 months storage in freezing condition. The results showed no sensible organoleptic changes but the amount of cadaverin and putrescine had highly-significant ($p < 0.05$) and no significant ($p > 0.05$) changes after fifth month of storage, respectively. The mean concentration of putrescine and cadaverine were increased from zero up to 118.98 and 1121.48 μg/gr after five months storage, respectively. Increasing the cadaverine content might be a good index for red meat deterioration and used as quality index after long time storage in freezing condition.

Keywords: cadaverine; frozen meat; putrefaction; putrescine; storage time.

Practical Application: Using control of major biogenic amines instead of time consuming microbial tests for control of red meat quality during long-term storage in freezing condition and introducing the best storage time for various meat cuts.

1 Introduction

Among important protein sources in human diets are meat and meat products such as bacon, ham, and sausages that are the most popular with their unique flavors (Hung et al., 2016). Although, raw meat quality actually can be varied and this depends on pre- or post-slaughter and affected meat product taste, some high pressure processing can affected the meat taste and it can be demonstrated the applicability of commercially processed meat products by developing methods like loop-mediated isothermal amplification or LAMP (Hygreeva & Pandey, 2016; Lee et al., 2016). There are lots of conventional methods for measuring chemical meat quality including measurement of pH, thiobarbituric acid reactive substances (TBARS), trimethylamine content (TMA), formation of biogenic amines, adenosine triphosphate (ATP) degradation and total volatile basic nitrogen (TVB-N), where the TVB-N and biogenic amines content are the most important factors for freshness of meat in long terms storage (Cheng et al., 2016). There are many efforts for controlling the quality of meat during storage in freezing condition however decreasing in its quality and increasing the rate of chemical and microbial reactions is inevitable for the long time storage (Alonso Lomillo et al., 2010). Putrescine and cadaverine are the most important diamines which can be noticed in meat during storage. These compounds are produced through decarboxylation of ornithine/arginine and lysine, respectively. They are more popular in red meat and so considered more than the other biogenic amines (BAs) (Cheng et al., 2016). Histamine, putrescine, cadaverine, tyramine, tryptamine, spermine and spermidine are among the most abundant BAs in food stuffs that their presence can be considered as the safety and quality indices in food health control. According to freshness, carcinogenicity and allergic factors in meat and it's by products, the toxicity and allergic effects of BAs were considered (Papageorgiou et al., 2017). Detection of BAs derived from meat protein is depended upon chemical structure and their ability for making bonds in complex reactions. In fresh meat a few BAs are considerable but during its storage due to bacterial activity they may be increased gradually and so they can be noticeable and considered as a freshness and quality index. The accumulation of cadaverine and tyramine in meat and especially in cheese were well demonstrated and the accumulation of tyramine density in meat was reported up to 38 mg/kg before (Ladero et al., 2010). Any polyamine intake by human body even in very low levels causes decreasing in cell growth but in high levels it causes inhibition in cells growth (Deloyer et al., 2001). The presence of BAs in red meat as a quality index can be considered so that how much of them might be safe, hazardous or risky for human health. Actually, the toxicity of BAs depends on the quality and quantity of consumed food stuffs and most of all the individual sensitivity and the health condition of consumers (Ruiz-Capillas & Jiménez-Colmenero, 2004). In the middle of this, histamine is the most studied biogenic amine in fish products which is effective in sensitive people who have taken 5 to 10 mg/kg of histamine in daily food consumption. In normal condition its threshold is 10 mg/kg and doses more than 100 to 1000 mg/kg induces mild to severe

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food poisoning, respectively (Doeun et al., 2017). Tyramine and β- phenylethylamine toxic dose limitation are varied between 100-800 mg/kg and 30 mg/kg, respectively. This may be due to their accumulation at the surface of meat which can be washed out easily (Paulsen et al., 2006). Putrescine, spermine and spermidine oral toxic dose are about 2000, 600 and 600 mg/kg, respectively, but the acute toxic dose of tyramine and cadaverine actually are about 2000 mg/kg and more (Til et al., 1997). The most important BAs in meat and meat products are tyramine, cadaverine and putrescine (Lázaro et al., 2015). Tyramine, cadaverine and putrescine may be increased during storage and processing while some of them such as spermine and spermidine may be decreased (Wu et al., 2016). Actually there are few reports about putrescine and cadaverine accumulation in meat and other meat based products. Cadaverine and putrescine in meat high lights two factors: first the potential toxicity and second their role as quality index for good storage condition (Önal, 2007). Recently we reported an ELISA (Akbari-Adergani et al., 2010) and a cyclic voltametric method (Akbari-Adergani et al., 2012) for the determination of histamine as a concerning BA in tuna fish having sensitivity range of parts per billion. During raw meat storage, its protein deteriorates to amino acids and peptides, where TVBN contents would be used to evaluate meat quality as an important factor (Maktabi et al., 2016). In this study variation of two major biogenic amines i.e. putrescine and cadaverine in calf meat during production and storage in freezing condition was evaluated and its correlation with microbial putrefaction was investigated.

2 Materials and methods

2.1 Materials

BAs, namely cadaverine dihydrochloride (CAD, $C_5H_{14}N_2$.2HCl, CAS.No.1476-39-7) and putrescine dihydrochloride (PUT, $C_4H_{12}N_2$.2HCl, CAS.No.333-93-7) were supplied by (USA St Louuis, MO, USA) and (Darmstadt Germany), respectively. Perchloric acid, sodium hydroxide, sodium bicarbonate and ammonium hydroxide, all from Merck (Darmstadt, Germany) and dansylchloride from Sigma (St Louuis, MO, USA) were used as received. All solvents used in chromatography analysis were HPLC grade and supplied by Merck. All solutions were prepared with de-ionized water prepared by purified water equipment (UHQ Elga, England).

2.2 Sample collection and preparation

The meat samples were collected during August 2017 to July 2018 from an abattoir in industrial zone in western part of Tehran in Robaat Karim and considered for a twelve months monitoring in freezing condition. Each sample batch were selected from one carcass of young bull with average 18-24 months old from four parts of the carcass i.e. neck, rib set, flank and sirloin. After initial cooling and hygienic packaging, 50.0 g representative meat sample was weighed by an analytical balance (ED423S-CW, Sartorius, Germany) and sent to the food control laboratory in cooling condition for subsequent putrescine and cadaverine extraction. All meat cuts held separately in freezing condition for a year at -18 °C. At each analysis run, exactly 50.0 g from each part of meat cuts were minced with a laboratory meat grinder, separately after 24 hours de-freeze period. Then 5.0 g of each defrosted sample were mixed with 20 mL of 0.4 M $HClO_4$ (Merck, Darmstadt, Germany) and homogenized (IKA-WERK homogenizer, Staufen, Germany) for 5 minutes. The solution was centrifuged for 10 minutes at 5000 rpm (CEN01C20, Biofuge, Germany) to sediment solid particles. The supernatant phase were separated from precipitate solid portion by pouring it in other vessel and then were reconstitute with 20 mL of 0.4 M $HClO_4$ and homogenized and centrifuged as before. Finally the two supernatant were mixed together and diluted up to50 mL in a volumetric flask for subsequent analysis.

2.3 Measurement of biogenic amines

Each 10 mg of the BAs were added to10 mL $HClO_4$ (0.4 mmol/mL) separately then stored in test tube at 4 °C. After all by mixing the two prepared BAs together, 6 portions from 5 to 250 µg were made as working standard solutions. Exactly, 1.0 mL of each standard solution and 1.0 mL of each supernatant extracts were mixed with 200 µL of NaOH (2.0 mmol/mL) and buffered by adding 300 µL saturated $NaHCO_3$. To this solution, 2.0 mL of dansylchloride was added then vortexed and incubated for 10 minutes at 60 °C. Finally 100 µl of NH_4OH solution (28% v/v) was added drop wise for 15 minutes to stop and remove dansylation reaction and residual of dansylchloride, respectively. After all they were fixed by 5 mL acetonitrile and an appropriate volume of their aliquot was injected into the HPLC (Önal, 2007).

2.4 Determination of TVBN

Test method

TVBN determination according to modified Conway's micro-diffusion method was performed (Food Safety and Standards Authority of India, 2012). 5 g of acid boric dissolved in 100 mL of alcohol 95% and the volume reached to 450 by adding water then added 5 mL of indicator include 0.066% methyl red and 0.33% bromocresol green in alcohol. Added sodium hydroxide 40% till produced a reddish color then reached the volume to 500 mL with alcohol.

Preparing reagents

5.0 gr of boric acid was mixed with 100 mL 95% alcohol and then added to 350 mL of distilled water, gently. Then 5 mL of indicator added to acid based solution (100 µL, 0.066% methyl red and 0.066% bromocresol green). For titration of acid, added sodium hydroxide 40% to produce a faint reddish colour. After all make the volume with alcohol up to 500 mL.

Preparing meat extract

Blend 10 gr of each meat cuts monthly with 90 mL of distilled water for 2 min. Then added 5 mL of homogenate extract with equivalent volume of trichloroacetic acid 10% (TCA). Then extract filtered with Whatman's filter paper no.1 after 15 min. The transparent and clear liquid of TCA extract, used to determine TVBN.

Analysis and calculation method

Exactly 1.0 mL of 2% boric acid solution added to the Conway's outer chamber and the Conway's indicator (Methyl red + Bromocresol green) added in the inner chamber. Then 1 mL of filtered dilution added to 1 mL 50% K_2CO_3 and injected to outer chamber and incubated for 120 minutes in 37 °C. After all, some amounts of a 0.01 N HCl was added to inner chamber till pale pink color appears and calculated as mg%. The TVBN contents were calculated by Equation 1 below:

$$TVBN\left(\frac{mg\%}{100g}\right)=\frac{14.007\times(a-b)\times f\times 100\times c}{s} \quad (1)$$

where a and b are titration of tested and blank samples respectively; f is the factor of HCl; c is dilution ratio; and s is sample weight.

2.5 Statistical analyses

The One-Way ANOVA statistical test was used for calculation of independent measurements of putrescine and cadaverine in each group of meat cuts. Means was separated by Kruskal Wallis test at the significant level of $p < 0.05$ for cadaverine but not significant for putrescine $p > 0.05$.

3 Results and discussion

The total content of biogenic amines especially putrescine and cadaverine in red meat has a potential to be one of the most important indicators at commercial trade marks for measuring meat quality. The shelf life of frozen meat samples which were stored at -18 °C for 12 months showed significant differences between putrescine and cadaverine content as major BAs in chemical properties. The average amount of putrescine and cadaverine in rib set were 56.84 and 811.14 µg/g, respectively (Figure 1A). The similar results were obtained for neck meat samples. The results for average amount of putrescine and cadaverine was 56.41 and 653.67 µg/g, respectively (Figure 1B). Flank specimens were also showed the same profile and an increasing in peak constitute after 5 months was appeared and the average amount of putrescine and cadaverine were recorded as 103.83 and 676.46 µg/g, respectively (Figure 1C). According to Figure 1D it is obvious that similar profile exists for sirloin variation during shelf life after 5 months. The average amount of putrescine and cadaverine was 54.65 and

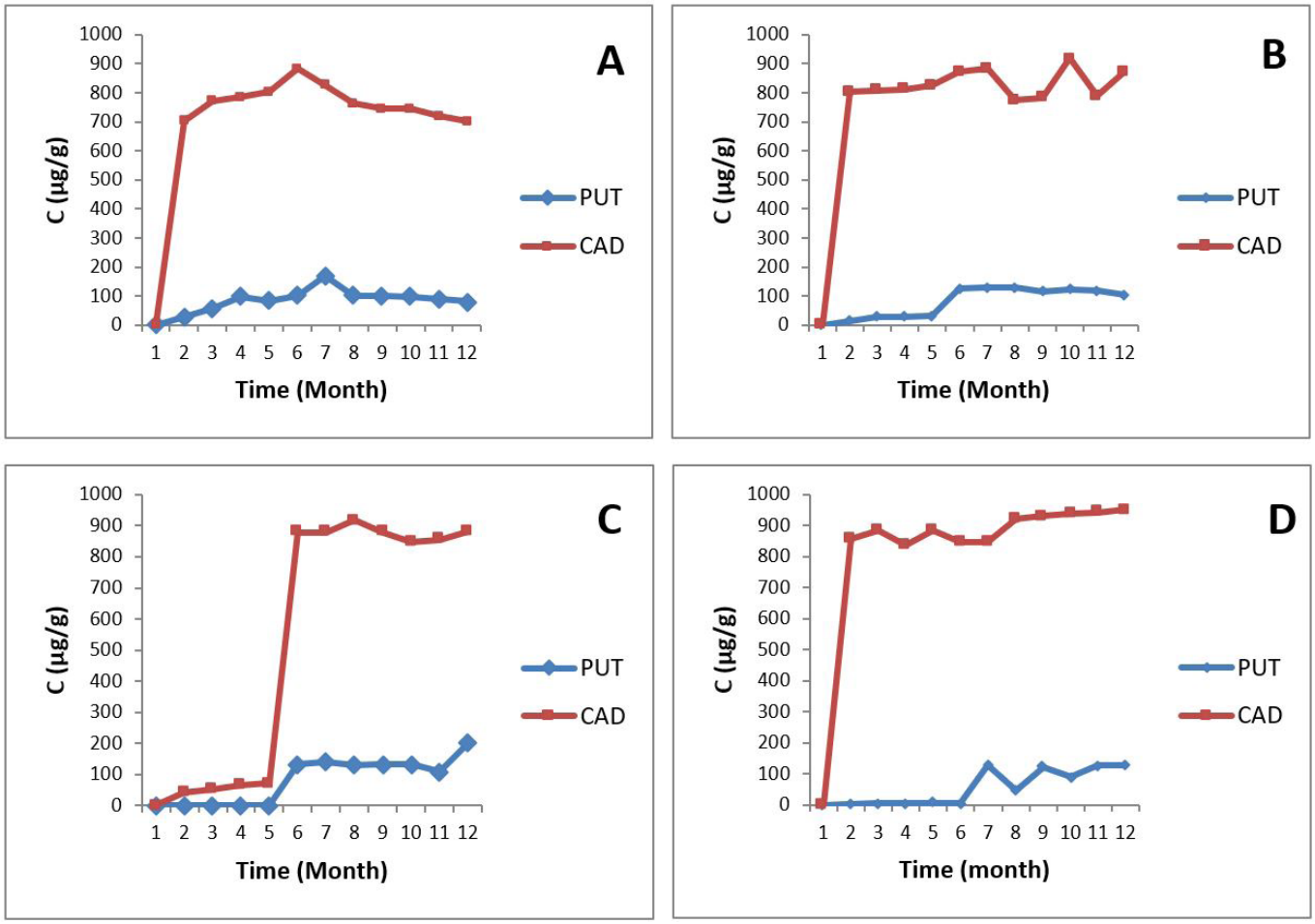

Figure 1. Variation of average amount of putrescine (PUT) and cadaverine (CAD) in (A) rib set, (B) neck, (C) flank, (D) sirloin during long storage at -18 °C.

824.01µg/g, respectively in sirloin meat samples (Figure 1D). Totally, the average amounts of putrescine in frozen rib set, neck, sir loin and flank meat samples during 12 months of shelf life were 56.85, 56.42, 54.66 and 103.84 µg/g, respectively and for cadaverine were 811.14, 807.36, 824.01 and 676.46 µg/g, respectively. These results revealed that the average amount of putrescine and cadaverine was increased up to 67.94 and 779.74 µg/g, respectively during shelf life (Figure 2A and 2B).

Totally, the mean concentration of putrescine and cadaverine in all of the meat cuts was increased from nearly 0 at slaughtering time up to 118.98 and 1121.48 µg/gr, at the end of the fifth month, respectively. These results was *not* significant at $p > 0.05$ for putrescine but it was significant for cadaverine ($p < 0.05$). There was no significant increasing in putrescine during the shelf life ($p > 0.05$) however, the obtained data showed that the increasing in cadaverine content was significant during the frozen meat shelf life ($p < 0.05$). The results were also showed that the level of putrescine and cadaverine in rib set up to 5 months after shelf life doesn't changed significantly however after fifth month from initial freezing, both of the BAs had an increasing in peak height in their chromatograms and after that reach to nearly an steady state.

The obtained values for putrescine and cadaverine showed a normal distribution and descriptive statistic (mean, standard deviation, etc.) of cadaverine and putrescine introduced in Figure 2. The results showed highly-significant difference in increment of cadaverine ($p < 0.05$) and no significant difference for increasing putrescine ($p > 0.05$) during storage time in the first five months freezing storage. Any chemical and structural change that may be occurred in raw meat during freezing and frozen shelf life depends on kind of meat and storage condition including temperature duration, temperature fluctuation, etc. (Hui & Sherkat, 2006). Actually most of all enzymatic activity in red meat decreases after 8 to 15 days storing raw meat at -18 °C. Putrescine content as a major biogenic amine in frozen raw meat at initial freezing time was almost negligible which was in accordance with all that reported by some previous reports (Custódio et al., 2018). This report revealed that freezing will not be affected biogenic amines amount even after 150 days. Although the effect of freezing and frozen storage time directly depends on initial condition of microbial and enzymes activity of raw meat and their intensity generally cause the formation of biogenic amines. The obtained results in this study were also revealed that cadaverine content increased up to 600 µg/g after five months from initial storage in each frozen meat cuts. This finding concurs with some studies which reported that cadaverine and putrescine may be increased during freezing storage so, it can be concluded that the concentration of cadaverine may come mainly from fresh meat and even more it may be influenced by pre-freezing process during storage time (Hung et al., 2016). This is compatible with the results that recorded during twelve months and revealed that the increment in the amount of putrescine is not significant during storage time but it is significant for increment in cadaverine content in frozen meat. Freezing rate is other factor that can affects the quality of meat. During slow freezing process, formation of large amount of water crystals causes serious damage in to the cells and subsequently protein denaturation may occur especially through accumulation of enzymes in the presence of other components. After slaughtering and performing a cold initial treatment, the physical, chemical and biochemical reactions do not stop however stopping in microbial growth at temperatures lower than -18 °C is in agreement with Zhou et al. (2010) report. In the mentioned study, freezing at -55 °C temperature was considered which prevent meat from qualitative changes, as an ideal storage condition so enzymatic reactions, oxidative rancidity and ice recrystallization will be at optimal qualification and few deterioration will be happened (Zhou et al., 2010). In frozen meat, low temperature condition actually restricts the bacterial growth and its population decreases about one log after each months of storage of the frozen meat. These results showed

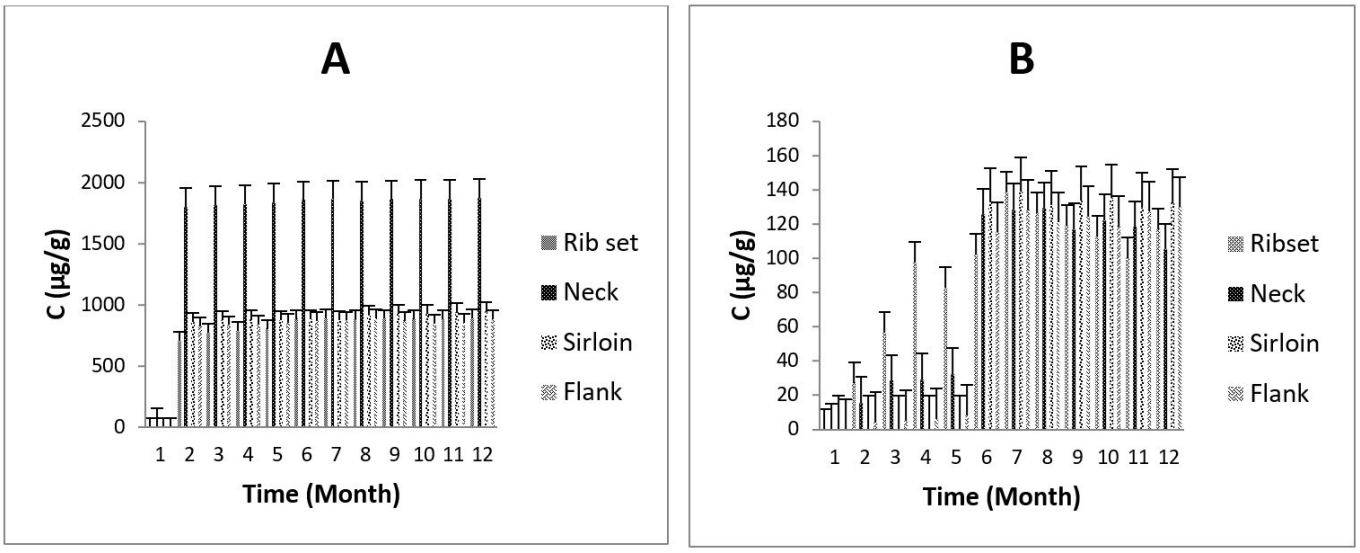

Figure 2. Comparison of average amount of putrescine (A) and cadaverine (B) in all parts of the collected red meat cuts during shelf life upto 12 months with error bars and standard deviation.

Table 1. The result of TVBN (mg/100 g) at various storage times in -18 °C for various red meat cuts.

Meat Cuts	Storage Time (month)											
	1	2	3	4	5	6	7	8	9	10	11	12
Neck	6.13 ± 0.20^a	6.50 ± 0.22^a	6.49 ± 0.20^a	6.45 ± 0.21^a	6.51 ± 0.20^a	8.46 ± 0.21^a	11.65 ±0.21^b	15.25 ±0.20^b	18.26 ± 0.20^c	21.94 ± 0.22^c	28.64 ± 0.20^c	36.42 ±0.22^c
Rib set	7.33 ± 2.22^a	7.45 ± 2.21^a	7.55 ± 2.22^a	7.65 ± 2.22^a	7.52 ± 2.20^a	11.49 ±1.20^b	12.24 ±0.20^b	16.21 ± 0.20^c	19.35 ± 0.23^c	23.82 ± 0.21^c	30.86 ± 0.22^c	36.52 ±0.23^c
Sirloin	6.54 ± 2.19^a	7.52 ± 2.20^a	7.65 ± 2.20^a	7.75 ± 2.22^a	8.12 ± 2.22^a	8.32 ± 1.22^a	11.56 ±0.21^b	15.16 ±0.21^b	17.85 ± 0.22^c	20.86 ± 0.24^c	27.96 ± 0.24^c	36.31 ±0.23^c
Flank	6.25 ± 2.22^a	6.45 ± 2.22^a	6.57 ± 2.20^a	6.66 ± 2.21^a	6.94 ± 2.21^a	8.29 ± 1.19^a	11.48 ±0.20^b	16.20 ± 0.22^c	18.23 ± 0.21^c	21.86 ± 0.22^c	29.64 ± 0.24^c	36.38 ±0.22^c

The values represent Mean ± SD of four experiments. a, b and c are significantly different.

that about 60 percent of the microbial population in the red meat samples destroyed. This is thoroughly compatible with Rahman (1999) findings. Cadaverine and putrescine formation is actually depends on the presence of endogenous enzyme and even rate and condition of meat freezing. This is mainly due to producing endogenous decarboxylase enzyme which released from destruction of muscle cells during freezing or thawing the meat especially at -18 °C which enzymatic reaction continued even though bacterial activity prevented. These findings are in agreement with Maijala et al. (1995) that mentioned biogenic amine contents may affected the microbial flora, raw meat putrefaction and even free amino acids availability as precursors causes the formation of biogenic amines. This is also in agreement with Ordóñez et al. (1999) study that reported a complicated proteolysis process during meat spoilage with several types of involved endogenous enzymes. They reported the activity of endogenous proteolytic enzymes specially cathepsins realises and increases peptides and other non-protein nitrogen compounds for this spoilage that are compatible with increasing TVN in this study (Table 1).

Freezing in long term mainly affected the TVBN in frozen beef cuts. In contrast to control fresh meat (3.07 mg/100 g), all freezing beef cuts had high amount of TVBN (Table 1) which is significantly different from control samples ($p < 0.05$). In this study a significant increasing of TVBN after freezing and storage up to 12 month from 7.23 up to 36.52 in freezing beef cuts (mg/percent) was obtained ($p < 0.05$).

The results showed that after five months the TVBN content in each meat cuts has increased noticeably (Table 1). The second impression from the obtained results is related to the important factor that cadaverine and putrescine are significant compounds in meat safety that contribute in TVN and raw meat quality determination. These compounds have a significant role as indicator of freshness and spoilage. The amounts of cadaverine was quite low during initial five months storage and after that a significant increase in the biogenic amines concentration and the raw meat putrefaction was observed. However, there was no significant difference in the amounts of putrescine after initial five months storage. The sum of these two diamines for total carcass reached to the 67.94 and 779.74 µg/g in average values for putrescine and cadaverine, respectively which is in accordance with Dadáková et al. (2011) report that seems the content value for putrescine had a little increasing. There was few published works to compare the obtained results for biogenic amine content in this study with other reported ones. This is mainly due to most of the reported cases does not specify whether they use frozen meat and actually all the surveys was based on the experiments in the upper zero temperatures. In all meat cuts the amount of cadaverine was more than putrescine (about 80 times). During freezing condition cadaverine increased after first month in rib set, neck and sir loin (Figure 1A-C) but in flank it increased after 5 month of storage (Figure 1D). The reason might be the presence of more blood and less fat content in rib set, neck and sir loin than flank instead. It was known that the increasing of TVBN depended on microbial spoilage and it seemed this reaction can be induced under temperature -18 °C which previously reported (Fan et al., 2009). Increasing of TVBN correlated with increasing of nitrogen compounds like BAs after five months from initial freezing. Threshold of TVBN consumption is about 35 mg/100 g (Connell, 1990). This twelve months survey for monitoring TVBN in frozen beef cuts revealed that there was no serious concern about oxidizing lipids in beef cuts in freezing condition however a noticeable increasing in TVBN especially after five months from initial freezing showed that a sensible deterioration in freshness can be observed in long term storage.

4 Conclusion

Putrescine and cadaverine content of meat cuts as major biogenic amines have major contribute in TVN content. This contribution was fully indicated during long-term storage in freezing condition. The mild increment of purtresine and sharp increment of cadaverine during the first months of storage were recorded and a good correlation between these increments with TVN content was observed. It was also found that levels of BAs like cadaverine increase TVN content especially after fifth months. So this can be as considered as end of period for appropriate freezing time with allowable limit of biogenic amines that revealed significant contribution in increasing TVN content. Thus, due to pathogenicity of biogenic amines that frequently reported as the major cause of neurological disorders, gastrointestinal diseases, abnormal immune responses and carcinogenesis, the characteristic role of these compounds as quality indicators especially at storage in long terms needs to be considered as major parameter in meat quality and hygiene.

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