Biogenic amines content of the Egyptian Domiati cheese in relation to chemical composition and ripening indices

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Abstract: Market Domiati cheese samples representing Baramili and Istamboli types was analyzed for the presence and quantity of biogenic amines (BAs). The total BAs content as an average ±SE was 27.36±9.16 mg/100g of Baramili cheese including tyramine (13.03 mg/100g), cadaverine (8.01 mg/100g), putrescine (3.35 mg/100g) and histamine (2.81 mg/100g) with the percentages of 47.62, 29.28, 12.24 and 10.27 from the total BAs respectively. Tyramine (4.88 mg/100g) was the most prevalent amine in Istamboli cheese representing 57.68% of the total BAs detected (8.46 mg/100 g). This was followed by quantities of 1.69, 1.38 and 0.44 mg/100g for histamine, cadaverine and putrescine in order with the corresponding percentages of 19.98, 16.31 and 5.20 from the total. The BAs Spermidine and spermine were detected in both cheese types in quantities less than 0.10 mg/100g. All cheese samples were analyzed for acidity, pH, moisture, fat, salt, protein, soluble nitrogen (SN), formol ripening index (FRI) and Shilovich ripening index (SRI). The statistical analysis revealed that histamine (Him), tyramine (Tym), putrescine (Put) and cadaverine (Cad) as well as total BAs (TBAs) positively correlated with protein content and negatively correlated with SN/TN content. In this respect Tym had the maximum r-values of +0.776 (P<0.01) and -0.729 (P<0.05) respectively. A highly significant correlations were recorded between both of spermidine (Spd) and spermine (Spm) and acidity and pH. Tym, the most prevalent amine in cheese and the TBAs negatively correlated with SN/TN.

Keywords: Biogenic amines, Domiati cheese.

Introduction

Awareness concerning the importance of food safety in general and their related impacts on human health has recently grown significantly in Egypt. Much emphasis has been given in the research sector to biogenic amines (BAs) in food particularly dairy products, since their toxicity and their potential as food quality marker are the two main reasons for such interest. Presence of BAs is a good indicator also for poor hygienic conditions during food processing (Jayarajahetal 2007) and considered as an index for degree of proteolysis occurred during cheese ripening.

Biogenic amines (BAs) are low molecular weight organic bases with an aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) structure and their names are assigned – in general – depending on the names of amino acid giving rise to it. Moreover, they are designated as biogenic because they are formed by the action of living organisms (Joosten and Stadhouders 1987).

On the other hand, BAs in food are produced mainly by the microbial decarboxylation of the precursor amino acids particularly histidine, tyrosine, lysine, ornithine and arginine (Silla-Santas 1996) or by amination and transamination of aldehydes and Ketones (Karovicova and Kohajdova 2005). Particular bacterial genera mentioned by Vallejos et al. 2012 are capable to decarboxylating amino acids. There are also conditions which favor the decarboxylation process. One of them is the availability of the free amino acids produced by microbial strains with high proteolytic enzyme activity. The conditions, which favor bacterial growth, decarboxylase, and synthesis and decarboxylase activity, are also factors that affect the formation of BAs. The acidic environment (pH 4.5 – 5.5) also favors such decarboxylation (Elsanhoty et al. 2009, Vallejos et al. 2012).

In spite of many earlier studies reviewed by Budal (2013) revealed many important useful functions of BAs if presented in human body in certain quantities, the presence of BAs in fermented foods such as cheese can cause adverse effects if the amounts of BAs are high enough. Such effects, production of carcinogens as Nitrosamine and causing death in very extreme
cases were reviewed in detail by Rawles et al. 1996, Karovicova and Kohajdova, 2005, Spano et al. 2010 and Budal 2013.

In cheese, presence, production and accumulation of BAs depend on the availability of their precursor free amino acids, bacterial load, pH, salt content and storage temperature (Rodriguez et al. 2002), therefore cheese represents an ideal environment for BAs production (Karovicova and Kohajdova, 2005).

Concerning Domiati cheese, it is the most popular soft pickled cheese in Egypt and unique in two aspects: Salt is added to milk before coagulation to counteract the poor bacteriological quality of the raw milk used in the most private dairies and to prevent formation of gas holes and abnormal flavors. The cheese can be consumed fresh more often or after maturation in brine or salted whey for different periods in refrigerator (Baramili Type) or at room temperature (Istamboli type). Elsanhoty et al. (2009) demonstrated that Domiati cheese made from raw milk contained remarkably higher BAs Levels compared with pasteurized milk cheese.

The objective of the present study was to determine BAs content in commercially available Domiati cheese samples representing the two main types of Baramili and Istamboli. Establishing correlations between the BAs and some chemical constituents and parameters of cheese was also taken into consideration.

Materials and Methods

Baramili and Isatmboli cheese samples, which represented the two main types of Domiati cheese in Egypt, were collected in random manner from the local market.

All the ten cheese samples (five for each type) were analyzed for acidity (expressed as % of lactic acid), pH, moisture and fat contents according to the methods given by Association of Official Analytical Chemistry (AOAC 2000). Salt content was also measured (IDF Standard 2001). The micro-kjeldhal method from 1963 was followed for determination of SN and TN, whereas protein content was calculated from the following equation:

\[
\text{Protein} = \text{TN} \times 6.38
\]

Degree of cheese ripening was tested also by measuring formal ripening index (FRI) and Shilovich ripening index (SRI) as described by Tawab and Hofi (1966).

Analysis of cheese samples for the biogenic amines (BAs) content was included six amines: histamine (Him), tyramine (Tym), putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm). In this respect, TCA (10%, wt/vol) was used to extract the BAs from cheese, whereas quantification of them was performed by means of an AAA 400 amino acid analyzer. (Ingos, Praha, Czech Republic) equipped with an Ostion LG ANB ion-exchange column (6 by 3.7cm). The details of the extraction and the operating parameters of determination were as given by Rabieet et al. (2011).

The attained data were statistically analyzed using the SPSS program, version 10-5-0. The Duncan’s multiple test was applied to follow the differences between the attained averages of the data (SPSS.1999).

Results and Discussion

Table (1) reveals the biogenic amines (BAs) content of market Domiati cheese. Histamine is the biogenic amine (BA) from histidine which has many physiological and toxicological effects in human (Ladero et al. 2010, Spano et al. 2010).

Concentration of histamine (Him) varied widely in both Domiati cheese types (Table 1). In Baramili type, the minimum and maximum values were 0.55 and 6.12 mg/100g with an average ±SE of 2.81±0.92. The corresponding values in Istambolicheese were 0.16, 5.16 and 1.69±0.99. Such differences in any cheese type due to individuality of samples were mostly significant (P<0.05). These recorded values are much higher than these given by Mehanna et al. 1989 for Domiati cheese (0.15 mg/100g) and are much lower than values given by El-Zayat (1986) for 60 days old Domiaticheese made from 7% salted milk. He gave values of 35.0 and 31.0 mg/100g for cheese made from reconstituted pasteurized and unpasteurized milk in order.

Salt content and pasteurization seem to have an inhibitory impact on Him content of Domiati cheese. El-Zayat 1986 found that increasing NaCl content of milk from 7 to 14% decreased the Him content in the resultant cheese. El Sanhoty et al. (2009) gave values of 4.42 and 0.98 mg/100g on dry matter basis when cheese was made from raw and pasteurized milk in order.

The pH values seem also to have an effect in this respect. The Baramili cheese of higher pH had also higher Him content than the Istamboli cheese. This agrees with the finding of Joosten 1988 who mentioned that Him incheese with pH 5.39 was twice as much as Him in cheese with pH 5.19. Similar observation was reported by Neamat Allah (1987) who attributed the delay in His formation in Karishcheese to the lower pH and higher concentration of salt.

The data presented in Table (1) revealed that tyramine (tym) content was not significantly different between most of samples of (A) or (B) cheese types. In Baramili cheese, three samples had Tym more than 15.0 mg/100g (P<0.05), whereas two samples had less than 10mg/100g (P>0.05). The recorded average ±SE was 13.03±2.02. The highest Tym content (slightly more than 5mg/100g) was noticed in most of samples belonging to Istamboli cheese with an overall average ±SE of 4.88±0.84 mg/100g.
Table 1: Biogenic amines content (mg/100g) of market Domiati cheese samples of Baramili type (A) and Istamboli type (B)

<table>
<thead>
<tr>
<th>Cheese Type</th>
<th>Him</th>
<th>Tym</th>
<th>Put</th>
<th>Cad</th>
<th>Spd</th>
<th>Spm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.55c</td>
<td>7.80a</td>
<td>2.89c</td>
<td>0.25c</td>
<td>0.24c</td>
<td>0.24c</td>
<td>11.97c</td>
</tr>
<tr>
<td>2</td>
<td>2.90b</td>
<td>16.81a</td>
<td>1.33c</td>
<td>0.20c</td>
<td>0.05b</td>
<td>0.05b</td>
<td>21.34b</td>
</tr>
<tr>
<td>3</td>
<td>2.64b</td>
<td>16.32a</td>
<td>0.44c</td>
<td>0.19c</td>
<td>0.19c</td>
<td>0.02c</td>
<td>19.91b</td>
</tr>
<tr>
<td>4</td>
<td>6.12a</td>
<td>15.82a</td>
<td>9.97c</td>
<td>31.41c</td>
<td>0.03b</td>
<td>0.05b</td>
<td>63.40a</td>
</tr>
<tr>
<td>5</td>
<td>1.83c</td>
<td>8.40b</td>
<td>2.13b</td>
<td>7.75b</td>
<td>0.02b</td>
<td>0.03b</td>
<td>20.16b</td>
</tr>
<tr>
<td>Average</td>
<td>2.81±0.92</td>
<td>13.03±2.02</td>
<td>3.35±1.70</td>
<td>8.01±6.03</td>
<td>0.07±0.042</td>
<td>0.08±0.039</td>
<td>27.36±9.16</td>
</tr>
<tr>
<td>B samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.16d</td>
<td>5.76a</td>
<td>1.13a</td>
<td>0.19c</td>
<td>0.04c</td>
<td>0.05c</td>
<td>7.33b</td>
</tr>
<tr>
<td>2</td>
<td>0.25c</td>
<td>1.53b</td>
<td>0.24c</td>
<td>0.19c</td>
<td>0.02a</td>
<td>0.05a</td>
<td>2.28a</td>
</tr>
<tr>
<td>3</td>
<td>5.16a</td>
<td>5.64a</td>
<td>0.07d</td>
<td>0.24c</td>
<td>0.01a</td>
<td>0.08c</td>
<td>11.20a</td>
</tr>
<tr>
<td>4</td>
<td>0.21c</td>
<td>5.76c</td>
<td>0.48b</td>
<td>4.09b</td>
<td>0.01c</td>
<td>0.05c</td>
<td>10.60a</td>
</tr>
<tr>
<td>5</td>
<td>2.68b</td>
<td>5.70c</td>
<td>0.28b</td>
<td>2.17b</td>
<td>0.01a</td>
<td>0.06c</td>
<td>10.90b</td>
</tr>
<tr>
<td>Average</td>
<td>1.69±0.99</td>
<td>4.88±0.84</td>
<td>0.44±0.18</td>
<td>1.38±0.78</td>
<td>0.02±0.006</td>
<td>0.06±0.006</td>
<td>8.46±1.69</td>
</tr>
</tbody>
</table>


Averages with unlike superscripts are significantly different (P≤0.05).

The values measured in the present study are much higher than Tym content of Domiati cheese given by Mehanna et al. (1989) (1.22 mg/100g), but much lower than those mentioned by El-Zayat 1986 for 60 days old Domiati cheese made from 7% salted milk being 66.0 and 53 mg/100g when cheese was made from raw or pasteurized milk in order.

The most prevalent BA detected in Domiati cheese (120 days old) made by Elsanhoty et al. (2009) was Tym with the values of 36.52 and 1.41 mg/100g on dry matter basis when cheese was manufactured from raw and pasteurized milk respectively.

In general, Tym is formed from tyrosine and has many physiological impacts Berry (2007) and considered as the most abundant BA in cheese as well as it is the principal cause of the so called “cheese reaction”. The toxicological impacts of Tym were recently reviewed by Ladero et al. (2010) and Spano et al. (2010).

It may be of interest to note that richness of all cheese samples with Tym was accompanied with much less quantities of Him content. This agrees with many earlier studies reviewed by Karovicova and Kohajdova (2005). They attributed such finding to sodium chloride which activates tyrosine decarboxylase activity and inhibits histidine decarboxylase activity. The ability of L.buchnerito form Him was partly inhibited at the content of 3.5 % NaCl, but at the content of 5.0%, its formation was stopped. This was also given in the article published by the pre mentioned authors in 2005. However, the maximum permissible limit of Him (10 mg/100g fish) stipulated by FDA (2001).

Table (1) shows that putrescine (Put) content varied widely in cheese due to the individual samples of Baramili cheese (A type) or Istamboli cheese (B type) or due to A and B.

In Baramili cheese, Put was detected in a minimum value of 0.44 mg/100g, whereas the maximum value was 9.97 with an average ±SE of 3.35±1.7 mg/100g. The corresponding values of Istamboli cheese were 0.07, 1.13 and 0.44±0.18 .The value given by Mehanna et al. 1989 was 0.66. In Domiati cheese (120 days old) made from raw or pasteurized milk, the Put contents on dry matter basis were 9.23 and 2.215 mg/100g respectively Elsanhoty et al. (2009).

The present study suggests that cheese with higher pH and lower salt (A) type had more Put than (B) type of lower pH and higher salt content. This agrees with the results of Darwish 1993 for Hungarian cheese.

In general, it is well known that ornithine is precursor of Put production. However in some microorganisms, alternative pathway exists to produce Put from arginine via agmatine (Karovicova and Kohajdova 2005).

Cadaverine (Cad) represented 29.28% from the total BAs in (A) cheese type and came after Tym (47.62%), whereas in Istamboli cheese the Tym, Him and Cad represented 57.68, 19.98 and 16.31% from the total BAs in order (Table 1). However, Cad was detected in a relatively high quantity in sample No. 4 belonging to (A) cheese type (Table 1), whereas the minimum value (0.20 mg/100g) was in sample No.2. The calculated average ±SE was 8.01±6.03. The minimum, maximum and average ±SE
values in case of (B) cheese type were 0.19, 4.09 and 1.38±0.78 mg/100g respectively. Pasteurization of Domiati cheese milk (Elsanhoty et al. 2009) caused a market decrease in Cad content from 22.33 to 3.44 mg/100g (on dry matter).

In general, lysine is precursor of Cad. This is well known, whereas in the literature presence of Cad in cheese could be related to contamination of cheese with species of Enterobacteriaceae and to decarboxylation of lysine into Cad by coliforms (Joosten and Northolt, 1987; Stratton et al. 1991; Marino et al. 2000). This BA was not detected in cheese including white cheese sold in Philippines. Vallejos et al. (2012) attributed that to production of Cad is usually occurred during the early stage of decomposition and the analyzed samples were still in good condition before reaching their expiration.

The present Domiati cheese samples were nearly free from spermidine (Spd) and spermine (Spm). Table (1) shows that less than 0.5 mg/100g was only detected with an average value of 0.07±0.042 in (A) cheese type and 0.02±0.006 in (B) cheese type for Spd and with corresponding averages of 0.08±0.039 and 0.06±0.006 mg/100g for Spm. The available Egyptian studies concerning BAs in Domiati cheese were not included Spd and Spm. A lot of foodstuffs and milk products reviewed by Ladero et al. (2010) contained Spd but different foreign cheeses were free from Spd. However, Rabie et al. 2011 mentioned that Spd was not detected in Blue and Mish cheese (Egyptians cheeses).
but Spm was detected in low concentrations in both Blue cheese (1.6 mg/100g) and Mish cheese (0.7 mg/100g).

Total BAs of Domiati cheese was found to be extremely variable in the two different cheese types (Table 1). The averages \(\pm SE\) were 27\(\pm\)9.16 and 8.46\(\pm\)1.69 mg/100g of (A) and (B) cheese types respectively. Ordanez et al. (1997) attributed such variability to cheese type, the ripening time, the manufacturing process and the microorganisms present in cheese. The two Domiati cheese types vary widely in processing, salt content and storage temperature.

It may be of great importance to note that formation and accumulation of BAs in cheese depend on specific conditions allowing decarboxylase properties of starter (Joosten and Stadouders, 1987) and non-starter (Joosten and Northholt 1987) bacteria as well as many factors related to composition and properties of cheese (Joosten, 1988; Visser, 1993) and of food in general (Karovicova and Kohajdova 2005).

Such variability in BAs content in Domiati cheese types was accompanied with marked differences in composition and properties of cheese presented in Tables (2 and 3).

Averages of acidity and pH were lower and higher in order in Baramili cheese (Table 2) than in Istamboli cheese. The lower the acidity and the higher was the pH, the higher was the total BAs content as in Baramili cheese. El-Zahar (2014) demonstrated that among quality parameters of some other Egyptian cheeses, acidity influenced BAs formation.

Salt content seems to be the most affecting tremendous factor and should be strongly taken into consideration in this respect. Much lower average (11.43\(\pm\)1.37\%) was recorded for salt/moisture in Baramili cheese than in Istamboli cheese (15.46\(\pm\)1.49\%). This was accompanied with higher total BAs in Baramili cheese.

Sensitivity of bacteria to acidity and salt was considered important factors affecting BAs content. Pintado et al. 2008 reported that high pH and low salt content seem to favour BA positive microflora in cheese. As prementioned elsewhere, microorganisms with high decarboxylase activity was reported in the literature as an important factor for production of BAs in cheese. Moreover, some strains of bacteria have proteolytic activity which can also affect the accumulation of BAs in cheese (Galgano et al. 2001).

Concerning proteolysis and ripening indices in cheese, (Table 3) reveals that SN/TN, FRI and SRI were relatively had lower averages in cheese type (A) than in (B) type. This may be due the proteolysis products were used as precursors for BAs production and increased the total BAs content in Baramili cheese than in Istamboli cheese as given in (Table 1). Such higher ripening indices in Istamboli cheese may be due to impact of the high ambient storage temperature of cheese (B type) which may reach more than 35°C during the long summer season in Egypt. No contradiction between the recorded total BAs and the ripening indices, since SN/TN indicates to degree of proteolysis, whereas FRI and SRI (based on changes in buffer capacity) are considered as rapid chemical indices to test cheese ripening (Tawab and Hofi, 1966).

On the other hand, (Table 4) illustrates the correlation coefficient \(r\) between BAs content and some chemical constituents and properties of Domiati cheese samples. Most of the detected BAs (Him, Tym, Put and Cad) and the total BAs (TBAs) positively correlated with protein content, but only Tym was significantly correlated with \(r=0.776 (P\leq0.01)\). The opposite was recorded with the correlations between the prementioned BAs and SN/TN (%), since negative correlations were calculated with only significant one between Tym and SN/TN \((r=-0.729)(P\leq0.05)\). The \(r\) was negative or positive \((P\leq0.05)\) between all the BAs given in (Table 4) and FRI and SRI.

<table>
<thead>
<tr>
<th>Protein, %</th>
<th>Him</th>
<th>Tym</th>
<th>Put</th>
<th>Cad</th>
<th>Spd</th>
<th>Spm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.274</td>
<td>0.776**</td>
<td>0.327</td>
<td>0.400</td>
<td>-0.241</td>
<td>-0.394</td>
<td>0.560</td>
<td></td>
</tr>
<tr>
<td>SN/TN, %</td>
<td>-0.231</td>
<td>-0.729*</td>
<td>-0.261</td>
<td>-0.319</td>
<td>0.143</td>
<td>0.238</td>
<td>-0.484</td>
</tr>
<tr>
<td>FRI</td>
<td>0.254</td>
<td>-0.184</td>
<td>-0.153</td>
<td>-0.084</td>
<td>0.375</td>
<td>-0.277</td>
<td>-0.104</td>
</tr>
<tr>
<td>SRI</td>
<td>-0.081</td>
<td>-0.067</td>
<td>0.019</td>
<td>-0.009</td>
<td>-0.208</td>
<td>-0.288</td>
<td>-0.035</td>
</tr>
<tr>
<td>Acidity, %</td>
<td>0.074</td>
<td>-0.006</td>
<td>-0.198</td>
<td>0.007</td>
<td>0.790**</td>
<td>-0.861**</td>
<td>-0.038</td>
</tr>
<tr>
<td>pH</td>
<td>-0.144</td>
<td>0.275</td>
<td>0.108</td>
<td>-0.185</td>
<td>0.924**</td>
<td>0.892**</td>
<td>-0.010</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>-0.494</td>
<td>-0.659</td>
<td>-0.694</td>
<td>-0.328</td>
<td>0.073</td>
<td>0.294</td>
<td>-0.757</td>
</tr>
<tr>
<td>FDM, %</td>
<td>-0.166</td>
<td>-0.020</td>
<td>-0.508</td>
<td>-0.556</td>
<td>0.130</td>
<td>0.202</td>
<td>-0.430</td>
</tr>
<tr>
<td>Salt/Moisture, %</td>
<td>-0.252</td>
<td>-0.429</td>
<td>0.185</td>
<td>0.294</td>
<td>-0.288</td>
<td>-0.280</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Him: histamine, Tym: tyramine, Put: putrescine, Cad: cadaverine, Spd: spermidine, Spm: spermine
*Significant at 5% level of significance.
**Significant at 1% level of significance.
Activity of decarboxylating enzymes was reported to be more important than precursor availability for production of BAs in cheese (Joosten, 1988), whereas results of free amino acids in Portuguese Terrincho cheese confirmed the interrelationships and BAs production.

The highest negative (-0.861) and positive (+0.790) r values were found between Spm and Spd in order with acidity (P<0.01). Only high significant correlations with r values of 0.924 and 0.892 were recorded between Spd and Spm in order and pH.

Such correlations between BAs and chemical constituents of cheese completed the information given by Pintado et al. (2008) who studied the correlations between BAs and the microbiological quality of Portuguese Terrincho traditional cheese.

Conclusions

Finally, although BAs are present in varied concentrations in foods including cheese and some other dairy products, a shared regulations limiting the amounts of BAs are still lacking (except for histamine in fish). However, the European regulations EC No. 1881/2006 amended by EC No. 629/2008 were completely followed by the Egyptian Standards No. 7136/2010 for contaminants in foods.

References

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