Available online freely at www.isisn.org

Animal Science Journal

Print ISSN: 2220-9549 Online ISSN: 2220-9557

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE ANIMAL SCIENCE JOURNAL, 2020 11(1): 01-14. **OPEN ACCESS**

Evaluation of Biological and Histopathological characteristics of biogenic amines content in soft cheese in vivo

Deabes M.M[1]*, Ali H. M[2] ,Soliman S. A[2] and Elkholy A. I[2]

[1]Food Toxicology and Contamination Department, National Research Center, 33 Bohouth St., 12622 Dokki, Giza, **Egypt**
[2]Department of Dairy, Faculty of Agriculture, Al Azhar University, Cairo, **Egypt**

***Correspondence:** mydeabes@yahoo.com

Biogenic Amines (BAs) are biological nitrogenous organic compounds found in many foods. Polyamines (putrescine, spermine, cadaverine and spermidine) were intake used as a marker for cancer. This study aimed at explores the dietary effects of BAs content in soft cheese samples on the histological, hematological and biochemical variations in *vivo*. To achieve that, eighteen healthy rats were divided into three equal groups. General behavior of animal, hematological and biochemical parameters of blood as well as kidney, liver and testes were evaluated. Six BAs were determined by using high performance liquid chromatography (HPLC). The outcomes of the present study indicated that the levels of histamine in soft cheese samples stored at room temperature were higher than the permissible limits (20mg / 100g) according to the FAO-WHO, the Egyptian standard specifications and the US FDA. On the other hand, BAs concentrations in the stored soft cheese at room temperature were higher than the permissible limits (90mg /100g). While the concentrations of all biogenic amines above (90 mg / 100g) were have a serious negative impact on the consumer's health. Finally, the histological examinations of different tissues of rates such as liver, kidney and tests were performed in contrast with a negative control.

Keywords: Biogenic amines, Soft cheese, HPLC, Histopathology, GOT, GPT.

INTRODUCTION

Soft cheese is one of dairy products and it is a common delicious cheese consumed in Egypt as fresh or after maturation in salt brine. The intake of soft cheese can improve lactose indigestion and it lowers bad cholesterol (Faid, 2017). Cheese has enzymatic and microbial activities cause formation of biogenic amines (BAs) by decarboxylation of amino acid (Linares et al.,2012).

Biogenic amines (BAs) are low molecular weight nitrogenous bases found in many foods, (fish, cheese , meat, vegetables etc.,) and can accumulate at high concentrations in cheese (Mohamed et al., 2013; Ruiz-Capillas and Herrero, 2019). Cheese that has long ripened periods (involving proteolysis) contained high BAs concentrations (Halazs et al. 1994; Fernández, et al. 2007). The microorganism can formation of BAs by decarboxylation enzyme as affected through many factors such as free amino acid, pH, salt, humidity, ripening time, temperature of

storage period and bacterial activity. The consumption of food containing high concentrations of BAs causing toxicological effect (Linares et al. 2011) such as respiratory distress, headache, hyper- or hypo-tension or allergies (Ladero, et al 2010). Polyamines (putrescine, spermine, cadaverine and spermidine) levels were intake together with acetyl conjugates significantly increased in the biological fluids and the affected tissues used as cancer markers in patients of cancer (Khuhawar and Qureshi 2001). The catabolism of polyamine has been implicated in the development of diseases including stroke, other neurological diseases, renal failure, liver disease and cancer (Pegg et al. 2013).

On the other side, tyramine and histamine, more toxic of BAs, are often found in high concentrations in certain foods. Linares et al. (2016) studied a cytotoxicity effect of dietary of histamine and tyramine. They found a tyramine had a stronger and more effect of cytotoxic than histamine. The mode of action of tyramine caused cell necrosis, but histamine induced apoptosis. A positive correlation was found between polyamine consumption and cancer occurrence (Gernerand and Meyskens, 2004).On the other hand, the cytotoxicity of putrescine and cadaverine found that both BA causing cell necrosis; they did not induce apoptosis (Del Rio et al., 2019). High levels of putrescine caused by Helicobacter pylori were found in n gastric carcinoma (Shah and Swiatlo, 2008). Therefore, the present work was performed to study the changes occur of BAs in soft cheese during storage within the validity period. Thereafter, the effect of BAs levels in soft cheese on biochemical and histopathological alterations in the experimental rats based on food safety.

MATERIALS AND METHODS

2.1.Materials

Cheese samples:

Thirty kilogram of soft cheese samples contained milk fat and was packed in plastic cans of 500 gm capacity without whey were collected from plant located in Obour city, Cairo, Egypt.

Chemicals:

Chloroform, n-butanol, n-heptane, acetone, sodium hydroxide, sodium bicarbonate, hydrochloric acid and trichloroacetic acid (T.C.A) were purchased from (Adwic- Co., El-Nasr pharmaceutical chemicals, A.R.E.). Methanol, acetonitrile, diethylether and acetic acid of HPLC grade were purchased from (BDH, England).The pure standard biogenic amines and dansyl chlorides were purchased from (Sigma- Co., Louis, MO 63178 U.S.A). Glutamic oxaloacetic transaminase (GOT) Kit, Glutamic Pyruvic transaminase (GPT) Kit and Urea and Creatinine kits were purchased from Diamond-diagnostic Company, Egypt.

Experimental animals:

Eighteen healthy male albino rats weighting (80-100g) were purchased from the Biological Products and Vaccines Holding Company, Helwan Farm, Cairo, Egypt. Standard synthetic diet, which composed of 12% casein, 8% corn oil, 4% salt mixture, 1% vitamins mixture, 5% fiber (bran) and 70% starch as described by (Ismail, 2013).

2.2. Methods

Soft Cheese samples :

Soft cheese samples were uniformly divided into 2 groups and stored over a period of 120 days at various temperatures (refrigerator temperature (5-7°C) and room temperature (20 ± 5°C). Soft cheese samples were examined of biogenic amines content as fresh and after 30, 60, 90 and 120 days during storage at refrigerator and room temperatures.

Determination of Biogenic amines:

Six biogenic amines included Histamine, Tyramine, Putrescine, Cadaverine, Spermine and Spermidine were extracted and determined according to (Deabes et al. 2013 and 2018).

Condition of HPLC:

Mobile phase solvents consist of; Solvent A: Acetonitrile: 0.02 N acetic acid (1:9); Solvent B: 0.02 N acetic acid: acetonitrile: methanol (1: 9: 9) was applied in linear gradient program at flow rate 1ml /min as follows in table (1).

Table 1: Linear Gradient program of HPLC.

Time (min.)	Flow rate (ml/min)	Solvent		Curve
		A%	B%	
0	1	25	75	-
10	1	10	90	6
20	1	5	95	6
25	1	25	75	6

High performance liquid chromatography (HPLC) was used to dansylamines determination. The system equipped with (Waters 600) delivery system.

HPLC column: Reverse phase, Luna C18 (250x 4.6 mmi.d), 5 um packing (phenomenex,USA). The detection was performed using U.V detector (waters 486) at 254 nm wavelength, using linear program of 25 min period and 1 ml / min constant solvent flow rate. Data were integrated and recorded using a Millennium Chromatography (Waters, Milford MA 01757) using Manger Software 2010.

Housing of experimental animals:

Rats were housed in stainless steel cages under ambient temperature conditions (at 20±5°C and 65±5 relative humidity) in house of animal, Faculty of Agriculture, Al Azhar University, Cairo, Egypt. After an acclimation period for 2 weeks prior to the experimental, all animals were healthy and clinically free from diseases.

Experimental of Animals Design:

Eighteen rats were randomly divided into 3 equal groups. Each group contained 6 rats and fed on one of the following diets every day for 120 days:

Group (1) Control: Rats fed on Control diet (12% casein, 8% corn oil, 4% salt mixture, 1% vitamins mixture, 5% fiber (bran) and 70% starch) and water without any treatment).

Group (2):Rats fed on Control diet + 30% soft cheese was storage at room temperature contained biogenic amines.

Group (3):Rats fed on Control diet + 30% soft cheese was storage at refrigerator temperature contained biogenic amines*.*

Biochemical Investigation:

Colorimetric determination of the activities of this transaminase [Glutamic Oxaloacetic transaminase (GOT), Glutamic Pyruvic transaminase (GPT)],was described by (Reitman and Frankel 1957). Urea and Creatinine concentrations were evaluated by using the enzymatic and kinetic methods respectively according to Patten and Crouch, (1977) and Henry, (1974).

Histopathological Examination:

At the end of the experiment, rats were sacrificed to obtain the livers, kidneys and testes. Immediately after extraction, the livers, kidneys and testes were immersed in formalin concentration 10% until the histopathological examination (Drury and Wallington 1980).

Statistical analysis:

Finally, all data were subjected to analysis of variance and treatment means were compared by Duncan's test at the 5 % level according to (Gomez and Gomez 1984)

RESULTS AND DISCUSSION

3.1.The changes in biogenic amines levels of soft cheese samples during storage:

The consumption of food containing high levels of BAs can cause food poisoning with different symptoms linked to the individual sensitivity and the detoxification activity (Schirone 2016). Marijan et al (2014) who stated that the determination of BAs contents in cheese it is very important for hygienic quality of cheese can be used as a parameter to evaluate the optimum conditions of production and ripening.

The results in table (2) presented that the storage temperature affected in formation of the six BAs (histamine, tyramine, putrescine, cadaverine, spermidine and spermine) were estimated in soft cheese samples during storage.

Histamine levels were increased significantly ($P \leq 0.05$) during storage at room temperature from (6.22 mg / 100g) of soft cheese samples in fresh, to reach (21.78 mg / 100g) at the end of storage time. While the samples increased also at refrigerator temperature until reached to (13.21 mg / 100g) at the end of storage. These increases in histamine levels may be referring to the effect of Glucono delta lactone (GDL) which used in the production of soft cheese. This is in agree with previous studies conducted on the GDL treatment for growth and histamine production of *K. pneumoniae* or *M. morganii* in Trypticase Soy Broth and found the GDL was encouraged two strains of growth and histamine formation. On the other hand, Buncic et al. (1993) and Teodorovic et al., (1994) who reported that GDL was stimulated the histamine production by *M. morganii* in TSBH. In contrast, Maijala et al.(1993) showed that the accelerated pH decrease by GDL addition diminished the formation of biogenic amines.

The levels of tyramine were increased significantly ($P \leq 0.05$) during storage at room temperature from (10.24 mg / 100g) in fresh soft cheese samples to reach (39.27 mg / 100g) at the end of storage. During storage refrigerator temperature of soft cheese tyramine were increased significantly ($P \leq 0.05$) until reached to

(21.13 mg / 100g) at the end of storage. This finding are in agreement with that obtained by (Pinho et al., 2001;Komprda et al., 2007; Komprda et al. 2008;Pachlovà et al., 2012). Polyamines (Putrescine, Cadaverine, Spermidine and Spermine) content differ significantly (P ≤ 0.05) between the two-storage temperature. Thus, the concentrations of Putrescine, Cadaverine, Spermidine and Spermine in fresh soft cheese samples were (15.33, 12.15, 1.17 and 8.65 mg/100g sample) and increased significantly (P ≤ 0.05) to (32.08, 39.30, 19.19 and 32.12 mg/100g samples) at room temperature at the end of storage period. While, at the refrigerator temperature the levels of polyamines increased till reached to (19.32, 17.77, 8.95 and 17.28mg/100g samples) after (120 days) of storage, respectively. The obtained results were simulating to those reported by (Pinho et al.2001; Elsanhoty et al.2009 ;Standarová et al. 2010; Pachlovà et al., 2012; Marijan et al. 2014). On the other hand , Bogdanović el al. 2020 Investigated the presence of biogenic amines in food were obtained from the Croatian retail market, found that histamine and tyramine at concentrations up to 106.4 and 206.6 mg/kg in cheese, respectively.

The levels of Histamine, Tyramine, Putrescine, Cadaverine, Spermidine and Spermine increased significant (P≤0.05) at room and refrigerator temperature with progress the storage period. The increased levels of Histamine, Tyramine, Putrescine, Cadaverine, Spermidine and Spermine of samples stored at room temperature were more significant (P≤0.05) compared to samples stored at the refrigerator temperature. Where the biogenic amines levels of soft cheese samples stored at room temperature were about doubled compared to the samples stored at refrigerator temperature. The levels of histamine in soft cheese samples stored at room temperature were higher than the permissible limits (20 mg / 100g) has a hazard action level 50 mg/100g according to the (FAO-WHO2012;Egyptian Standard Specifications 2005 and the USA FDA 2011) for human consumption at the end of the storage period. This increase of histamine levels may be attributed to the effect of high storage temperature on some factors controlling the histamines formation such as microbial growth, the activity of the decarboxylation enzymes and ripening that gives a chance to proteolysis to occur liberation of histidine throughout the storage period. The obtained results are simulating to those reported by (El-Kosi et al. 2009; Elsanhoty et al. 2009; Standarová et al. and 2010;Marijan et al.2014).

As in table (2), the levels of total BAs in soft cheese samples increased during storage. The concentrations of BAs in soft cheese samples stored at room temperature were significant higher increase more than in refrigerator temperature. Manca, et al. (2020) reported that the ripening duration more than 3.5 months and storage temperature can be characterized as risk factors formation and accumulation of biogenic amines in Italian (Fiore Sardo) cheese. Levels of total biogenic amines in the soft cheese samples stored at room temperature were higher than the permissible limits (90 mg / 100g food), while the concentrations of amines above (90 mg / 100g) have a serious negative impact on consumer's health according to Standarová et al. (2010).

The separation of six biogenic amines by used Linear Gradient program of table (1) it is clear the chromatogram of standard biogenic amines: histamine, tyramine, putrescine, cadaverine, spermidine and spermine as Figure (1) indicated the efficiency of resolution with respect to retention time for the identification of the responded authentic samples.

3.2. Effect of biogenic amine levels in soft cheese on biochemical parameters in rat serum.

The results in table (3) indicated that there were a significant increased (P ≤ 0.05) in the levels of Glutamic Oxaloacetic transaminase (GOT), Glutamic Pyruvic transaminase (GPT), Urea and creatinine in serum of control animals group (1) through the third and fourth months of the experiment with no significant difference between the first and second months and so, the third and fourth months. After the first month of the experiment, the results showed significant differences between the control group (1) and the group (2) but non-significant differences between the group (3) compared to control group. As for the second, third and fourth month of the experiment, the results presented a significant difference between the control group(1) and the group (2).While at the end of the experiment, the data demonstrated a significant difference between the control group and the (2) and group (3). In generally, from the data of liver and kidney functions, the results showed that a significant difference between all groups fed on soft cheese samples containing high levels of biogenic amines compared to the control group(1), which did not feed on soft cheese samples. This alteration due to high content of BAs in soft cheese, those

results were confirmed by (Til et al1997;El-Zahar et al. 2014; Ali et al. 2017).On the other hand, Histamine at 50and 200mg/kg dosage causing hepatic damage. The changes in ALT and AST levels in the groups demonstrate impaired liver functions, and suggestive of liver cell damage (Tripathi et al. 2012).

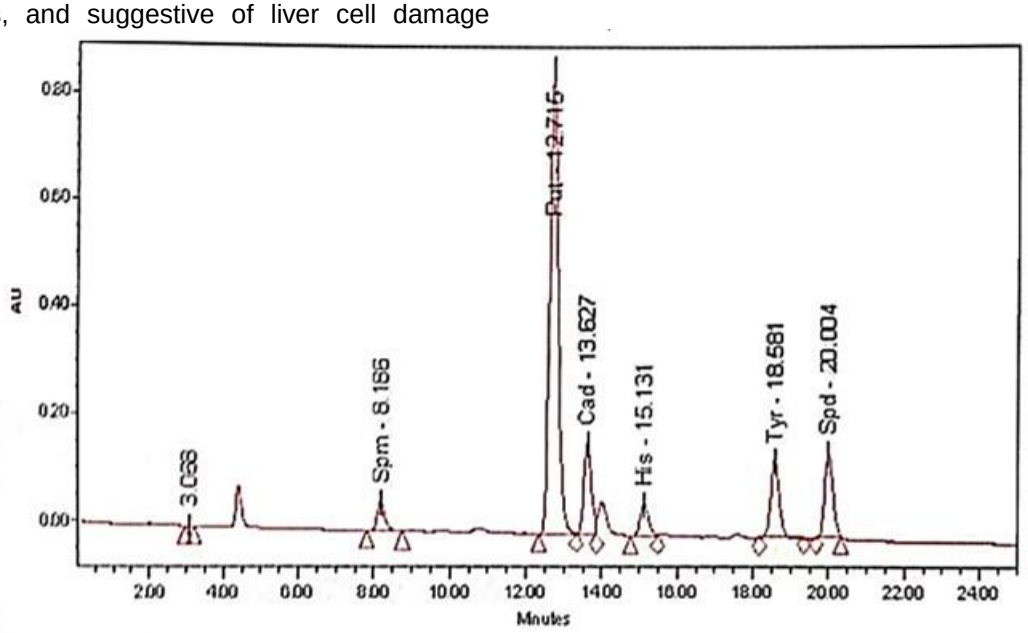

Figure 1: Dansylated of six biogenic amines standard overviewed by high performance liquid Chromatography (HPLC).

His = Histamine, Tyr = Tyramine, Put = Putrescine, Cad = Cadaverine, Spd = Spermidine, Spm= Spermine

Table 2: Biogenic amines levels (mg / 100g) in soft cheese during storage.

Storage temperature	Properties	Storage periods (days)				
		Fresh	**30**	**60**	**90**	**120**
Room temperature	Histamine	$6.22^{Q} \pm 0.061$	$8.08^{M} \pm 0.057$	$11.70^{H} \pm 0.045$	$16.28^{D} \pm 0.070$	$21.78^{A} \pm 0.061$
	Tyramine	$10.24^{P} \pm 0.125$	$20.16^{F} \pm 0.070$	$25.17^{C} \pm 0.050$	$31.37^{B} \pm 0.056$	$39.27^{A} \pm 0.096$
	Putrescine	$15.33^{J} \pm 0.079$	$19.35^{E} \pm 0.056$	$21.92^{D} \pm 0.070$	$27.13^{B} \pm 0.035$	$32.08^{A} \pm 0.031$
	Cadaverine	$12.15^{O} \pm 0.067$	$19.69^{H} \pm 0.047$	$27.32^{E} \pm 0.021$	$31.49^{D} \pm 0.030$	$39.30^{A} \pm 0.179$
	Spermidine	$1.17^{P} \pm 0.095$	$2.43^{L} \pm 0.035$	$6.20^{F} \pm 0.042$	$11.91^{B} \pm 0.070$	$19.19^{A} \pm 0.097$
	Spermine	$8.65^{R} \pm 0.100$	$13.15^{K} \pm 0.040$	$18.45^{F} \pm 0.078$	$24.53^{C} \pm 0.040$	$32.12^{A} \pm 0.057$
	Total	**53.76**	**82.88**	**110.76**	**142.71**	**183.74**
Refrigerator temperature	Histamine	$6.22^{Q} \pm 0.061$	$7.03^{N} \pm 0.040$	$9.27^{K} \pm 0.030$	$11.12^{I} \pm 0.035$	$13.21^{F} \pm 0.045$
	Tyramine	$10.24^{P} \pm 0.125$	$17.07^{K} \pm 0.045$	$17.98^{I} \pm 0.045$	$19.20^{G} \pm 0.050$	$21.13^{E} \pm 0.035$
	Putrescine	$15.33^{J} \pm 0.079$	$16.24^{I} \pm 0.042$	$17.07^{H} \pm 0.046$	$17.95^{G} \pm 0.035$	$19.32^{E} \pm 0.047$
	Cadaverine	$12.15^{O} \pm 0.067$	$12.93^{M} \pm 0.060$	$14.05^{L} \pm 0.035$	$15.01^{K} \pm 0.021$	$17.77^{J} \pm 0.042$
	Spermidine	$1.17^{P} \pm 0.095$	$1.94^{N} \pm 0.056$	$3.17^{IJ} \pm 0.036$	$6.12^{F} \pm 0.035$	$8.95^{D} \pm 0.025$
	Spermine	$8.65^{R} \pm 0.100$	$9.19^{Q} \pm 0.060$	$11.07^{N} \pm 0.055$	$14.00^{J} \pm 0.060$	$17.28^{G} \pm 0.036$
	Total	**53.76**	**64.40**	**72.61**	**83.40**	**97.66**

Means ± standard deviation (SD) with different capital letters within each column and row are significant at 5 % level.

Table 3: Effect of biogenic amines contents in stored soft cheese on Liver and kidney functions in rat serum.

Groups	Properties		Storage periods (days)			
			30	60	90	120
Control Group1	Liver functions	GOT	20.72 ^{N} ± 1.21	25.44 ^{LMN} ± 4.10	32.00 ^{IJKL} ± 1.82	35.96 ^{GHIJ} ± 2.06
		GPT	10.40 ^{L} ± 2.11	13.40 ^{KL} ± 2.78	21.45 ^{FGH} ± 1.57	23.38 ^{FG} ± 2.10
	Kidney functions	Urea	47.33 ^{J} ± 4.15	49.93 ^{IJ} ± 3.16	62.90 ^{FGH} ± 5.20	66.70 ^{EFG} ± 2.71
		Creatinine	0.89 ^{N} ± 0.108	0.99 ^{MN} ± 0.046	1.22 ^{JK} ± 0.044	1.16 ^{JKL} ± 0.045
Group2	Liver functions	GOT	29.92 ^{JKLM} ± 3.85	42.88 ^{EFG} ± 2.64	60.27 ^{B} ± 3.79	75.19 ^{A} ± 0.72
		GPT	15.05 ^{IJKL} ± 1.21	22.65 ^{FGH} ± 3.46	28.13 ^{CDE} ± 1.92	43.09 ^{A} ± 2.13
	Kidney functions	Urea	53.50 ^{HI} ± 4.46	65.33 ^{EFG} ± 6.04	83.83 ^{BCD} ± 14.69	102.80 ^{A} ± 8.25
		Creatinine	1.28 ^{IJ} ± 0.037	1.49 ^{GH} ± 0.110	1.72 ^{BCD} ± 0.064	1.85 ^{AB} ± 0.068
Group3	Liver functions	GOT	24.16 ^{MN} ± 4.09	27.52 ^{LMN} ± 1.11	40.44 ^{FGH} ± 3.49	47.08 ^{DEF} ± 5.15
		GPT	13.30 ^{KL} ± 3.03	14.84 ^{JKL} ± 1.27	23.10 ^{FGH} ± 2.78	28.95 ^{CD} ± 1.83
	Kidney functions	Urea	54.10 ^{HIJ} ± 3.76	58.37 ^{FGHI} ± 6.01	69.03 ^{EF} ± 2.25	86.23 ^{BC} ± 5.40
		Creatinine	1.05 ^{LMN} ± 0.104	1.33 ^{HI} ± 0.099	1.57 ^{DEF} ± 0.091	1.71 ^{BCD} ± 0.169

Means ± standard deviation (SD) with the different capital letters are significantly different at P< 0.05
Group (1) Control without BAs treatment). Group (2):Rats fed on Control diet + 30% soft cheese was storage at room temperature contained BAs. Group (3):Rats fed on Control diet + 30% soft cheese was storage at refrigerator temperature contained Bas.

3.3.Effect of biogenic amines contents in stored soft cheese on histopathological alterations in *Vivo*:

3.3.1.Liver Histopathological Alterations:

The microscopic examination of control rats' liver showed the normal hepatic lobules. The central vein is surrounded by the hepatocytes with eosinophilic cytoplasm and distinct nuclei. The hepatic sinusoids shown between the hepatocytes **(**Fig.2a**)**. Histopathological examination of rats' liver that fed on soft cheese contained milk fat and was storage at room temperature showed macro and micro vacuoles of fatty change (Fig.2b). While, the micrographs of rats' liver that fed on soft cheese contained milk fat was storage at refrigerator temperature showed disturbance of the hepatic lobule associated with huge area of macro and micro vacuoles of fatty change (Fig.2c), but in some rats, examination showed the hepatic lobules appeared more or less like control (Fig.2d). The histological changes that occurred in the rats' liver may be due to an increase in the biogenic amines levels of soft cheese samples during storage. These results are in consistency with (El-Zahar et al. 2014; Ali et al. 2017). Histamine at 50and 200mg/kg dosage causing hepatic damage. The changes in ALT and AST levels in the groups demonstrate have a negative effect on liver functions, and revealing of reasonable liver cell damage .It has been elucidated that departure from the norm in liver function ALT, AST (Tripathi et al. 2012). These elucidte increased speed of hepatocyte apoptosis and necrosis (Masaki et al. 2005; Thapa and Walia, 2007).

3.3.2.Kidney Histopathological Alterations:

From the obtained results in (Fig. 3a) kidney micrograph of rats that fed on basal diet and water without any treatment showed normal structure where the tissue of the kidney is divided into an outer cortex and an inner medulla. The nephron, the functional unit of the kidney, consists of two major components, the renal corpuscle, and the renal tubule. The renal corpuscles lie in the cortex and each is formed of two structures, Bowman's capsule and the glomerulus. As for the microscopic examination of kidney of rats that fed on soft cheese contained milk fat and was storage room temperature showed large areas of hemorrhage in the tubules interstitial areas and degenerative renal tubules. Thickened blood vessel and fibrotic area were seen (Fig. 3b). While the histopathological examination of micrographs of kidney of rat that fed on soft cheese contained milk fat was storage at refrigerator temperature indicated the normal renal corpuscles and tubules that appeared more or less like control (Fig. 3c). In some rats, mild, moderate or severe degeneration of the glomeruli and renal tubules were shown (Fig.3d).The histopathological

changes that occurred in the kidney may be due to an increase in the levels of BAs in soft cheese samples during storage. The observed histopathological changes of the kidney in this current work was agreement with hypothesis by (El-Zahar et al. 2014; Ali et al. 2017)

Figure 2a: Liver micrograph of control rats that fed on basal diet and water without feta cheese showed normal structure of the hepatic lobules (H & E stain, Scale Bar: 20 µm).

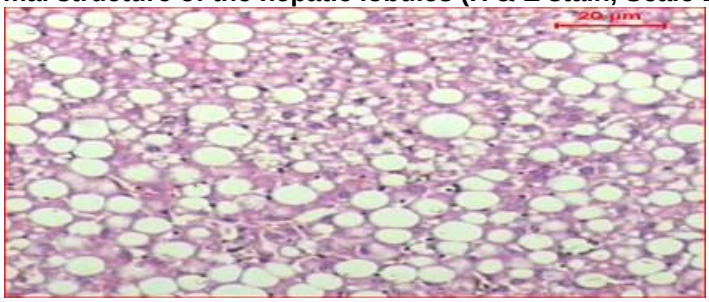

Figure 2b: Micrograph of rats' liver that fed on soft cheese was storage at room temperature shows huge area macro and micro vacuoles of fatty change (H & E stain, Scale Bar: 20 µm).

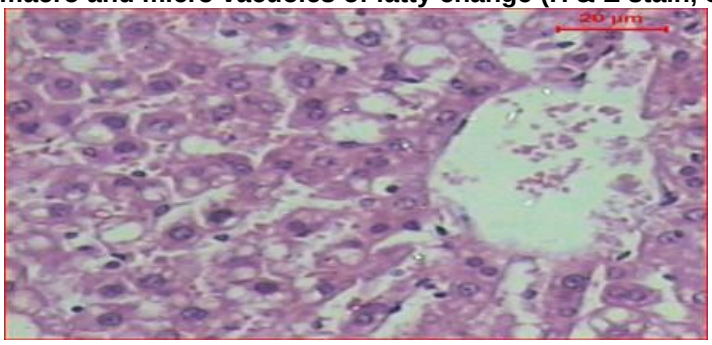

Figure 2c: Micrograph of rats' liver that fed on soft cheese was storage at refrigerator temperature shows disturbance of the hepatic lobule associated with macro and micro vacuoles of fatty change (H & E stain, Scale Bar: 20 µm).

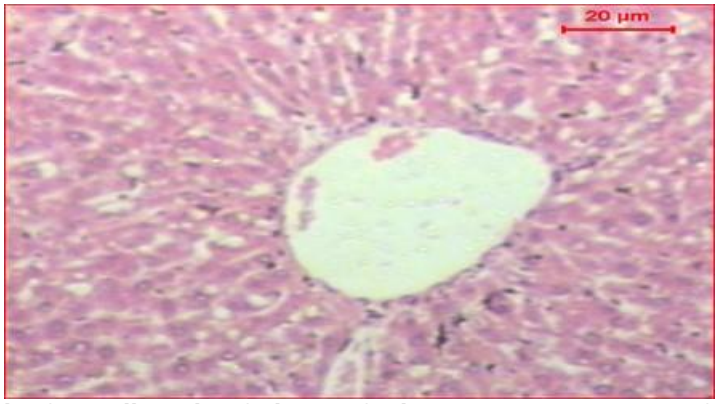

Figure 2d: Micrograph of rats' liver that fed on soft cheese was storage at refrigerator temperature shows the hepatic lobule appeared more or less like control (H & E stain, Scale Bar: 20 µm)

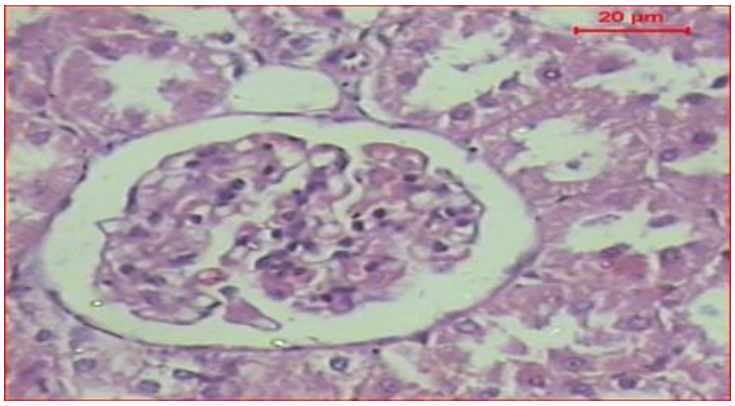

Figure 3a: kidney micrograph of rats that fed on basal diet and water without any treatment showed normal structure of the renal corpuscles and tubules (H & E stain, Scale Bar: 20 µm).

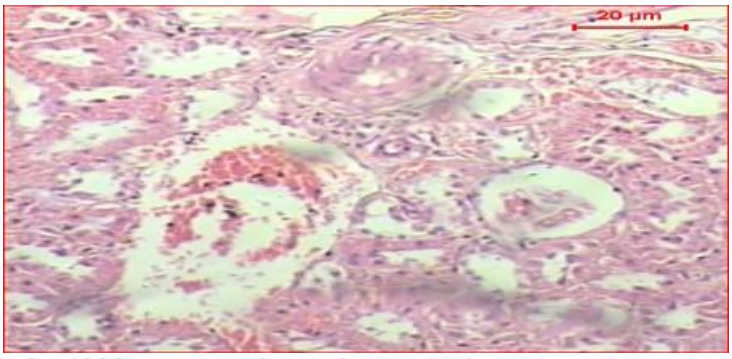

Figure 3b: Micrograph of kidney of rat that fed on soft cheese was storage at room temperature shows large areas of hemorrhage in the tubules interstitial and degenerative renal tubules. Thickened blood vessel and fibrotic area are seen (H & E stain, Scale Bar: 20 µm).

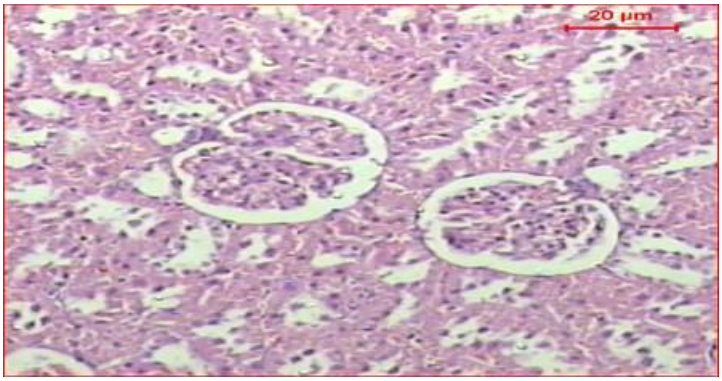

Figure 3c: Micrograph of kidney of rat that fed on soft cheese was storage at refrigerator temperature shows the normal renal corpuscles and tubules appeared more or less like control (H & E stain, Scale Bar: 20 µm).

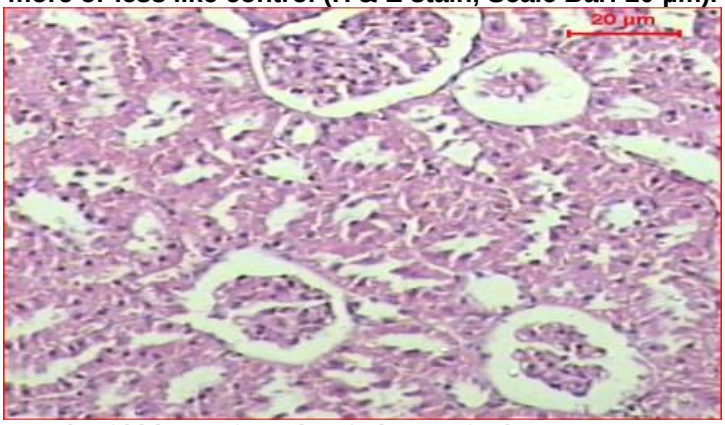

Figure 3d: Micrograph of kidney of rat that fed on soft cheese was storage at refrigerator temperature shows mild, moderate or severe degeneration of the glomeruli and renal tubules (H & E stain, Scale Bar: 20 µm).

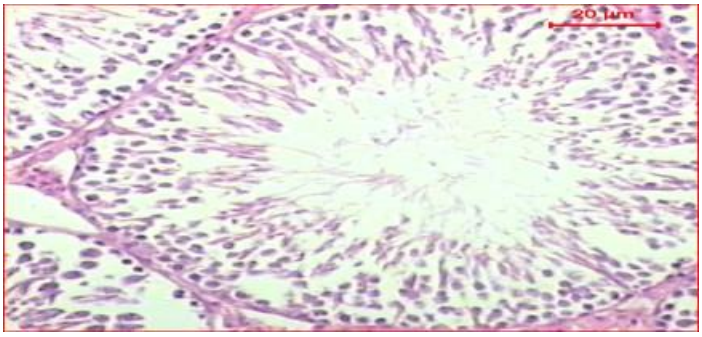

Figure 4a: Micrograph of the testis of control rat show normal structure of seminiferous tubules containing different types of spermatogenic cells. (H& E stain, Scale bar 20 µm).

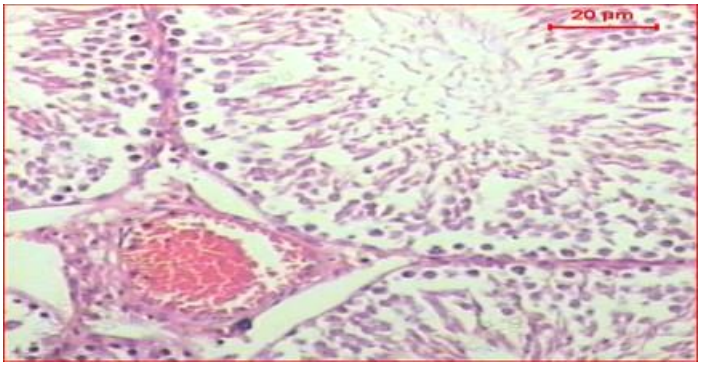

Figure 4b: Micrograph of testis of rat that fed on soft cheese was storage at room temperature shows atrophy of the tissue and disturbance in spermatogenic cell layers of the seminiferous tubule. Congested and thickened wall blood vessel in the interstitial area was seen (H & E stain, Scale Bar: 20 µm).

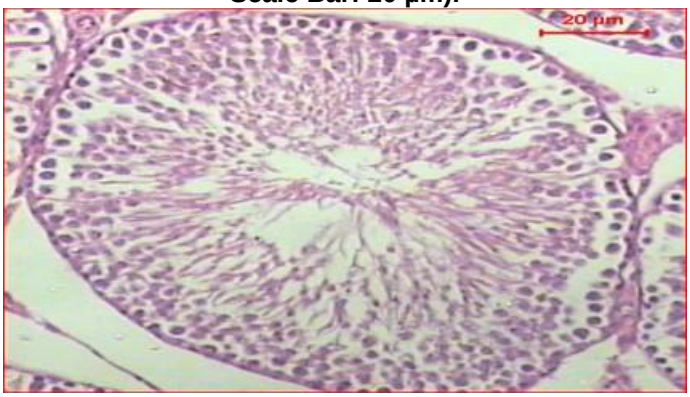

Figure 4c: Micrograph of testis of rat that fed on soft cheese was storage at refrigerator temperature shows the seminiferous tubules indicate that structures are appeared more or less like control. Some spermatogenic cell layers of the seminiferous show disturbance (H & E stain, Scale Bar: 20 µm).

3.3.3.Testes Histopathological Alterations:

The histological observations showed that the testes of control rats had normal arrangement of seminiferous tubules with spermatogenic cells. Evidence of apparently intact seminiferous tubules, as well as active cell division and maturation of the germ cells as evidenced in terminally differentiated cells/spermatozoa, are shown (Figure 4a). In caseof rats that fed on soft cheese contained milk fat and was storage at room temperature, testes showed atrophy of the tissue and disturbance in spermatogenic cell layers of the seminiferous tubule. Congested and thickened wall blood vessels in the interstitial area were seen (Figure 4b).While testes of rats that fed on soft cheese contained milk fat storage at refrigerator temperature showed the seminiferous tubules appeared more or less like control. Some spermatogenic cell layers of the seminiferous showed disturbance (Figure 4c).

These results are in agreement with (Til et al2007; El-Zahar et al. 2014; Ali et al. 2017) who confirmed that the kind and the concentration of biogenic amines accumulation in cheese affected the functions of liver and kidney in albino rats' serum treated with biogenic amines. More ever they explained that histological examination organs were moderately affected by direct injection of biogenic amines as compared with organs from control groups. Liver marked vascular congestion with abnormal double central vein occurrences leading to fibrosis. However, Til et al., (2007) reported that the histopathological examinations also revealed decreased glycogen content in the liver, reduction of spermatogenesis. While the effect of biogenic amines treatment on the histopathological of the kidney marked various degrees of alterations, which vary from mild to marked inflammatory infiltration in the kidney tissues. Also, there were thickened vascular spaces in the kidney (El-Zahar et al. 2014; Ali et al. 2017).The high content of biogenic amines were found in Domiati (Egyptian cheese) storage at 120 days caused negative changes in liver, kidney and testes tissue in rates (Ali et al. 2017).A

negative histological characteristic in the liver caused by the high levels of biogenic amines content in the fish meal Aksnes and Mundheim (1997). On the other hand, Caballero et al.(1999) stated that the biogenic amines content in the diet could affect liver histology. From the obtained results it could be concluded that the high levels of biogenic amines in soft cheese causing the changes in the liver, kidney functions and histopathological of liver and kidney tissue in rats.

CONCLUSION

From the obvious results it can be concluded that the data demonstrated the high levels of biogenic amines in soft cheese caused in alterations of the liver, kidney functions and histopathological of liver, kidney and testes tissue in albino male rats. With the result of our research found that; although BAs are present in many foods and beverages and their levels differ vastly between and within food types, regulation limiting of BAs mount in foods is still lacking except histamine in fish. So, we suggest to put the regulation of BAs in other food to keeping good health for consumers. The low BAs content in food productions depend on the good quality of raw material and fellow up in the steps of processing and storage temperature to discard BAs formations in food.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Thanks to Dr. Abdel Razek Farrag , Pathology Dept., National Research Center, for helping in the histopathological examination in this work.

AUTHOR CONTRIBUTIONS

Deabes MM and Ali H M designed and performleed the experiments and also wrote the manuscript. Deabes MM put the method and analysis of biogenic amines determination by HPLC . Ali H M,Deabes MM,Soliman S.A, and Elkholy AI performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. Ali H M, Deabes MM and Soliman S.A designed experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Aksnes, A. and H.Mundheim, .1997. The impact of raw material freshness and processing temperature for fish meal on growth, feed efficiency and chemical composition of Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture, 149(1-2), 87-106.

Ali, H. M., M.M.Deabes, M.M., S.A.Solimanand A.I.Elkholy, .2017. Effect of feeding the experimental rats with Domiati cheese on their histopathological alterations. J. Food and Dairy Sci. 8(11) 425-430.

Bogdanović, T., S.Petričević, M.Brkljača,I. Listeš and J.Pleadin.2020.Biogenic amines in selected foods of animal origin obtained from the Croatian retail market. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2020 20:1-16. doi: 10.1080/19440049.2020.1726503.

Buncic, S., L.J.Paunovic, V.Teodorovic, D.Radisic, G.Vojinovic, D.Smiljanic and M.Baltic.1993. Effect of glucono delta lactone and *Lactobacillus plantarum*on the production of histamine and tyramine in fermented sausages. International Journal of Food Microbiology. 17:303-309. https://doi.org/10.1016/0168-1605(93)90200-Z

Caballero, M. J., G.López-Calero, J.Socorro, F.J. Roo, M. S. Izquierdo and A. J. Férnandez .1999. Combined effect of lipid level and fish meal quality on liver histology of gilthead sea bream (Sparusaurata). Aquaculture, 179(1), 277-290.

Deabes, M. M., S. A. Ahmed, H. A. Mansour, L.A. Mohamed and D.A. Salah El Din, (2013). Liquid chromatographic determination of histamine in traditionally salted, smoked and frozen fish with relation to microbial load. Toxicology letters 221: S122-S123.

Deabes, M.M., M. Kh. Naguib, A. M .Ayesh, El- E. M. Damaty and G. H. Rowayshed.2018. Comparison of the HPLC and the TLC techniques for the determination of biogenic

amines spiked to sausage and smoked herring samples. Enliven: Toxicology & Allied Clinical Pharmacology.5(1)1-7. https://www.researchgate.net/publication/324278352

Del Rio, B., B.Redruello, D.M. Linares, V.Ladero, P.Ruas-Madiedo, M.Fernandez,M.C Martin, and M.A.Alvarez.2019.The biogenic amines putrescine and cadaverine show in vitro cytotoxicity at concentrations that can be found in foods.Sci Rep;9(1):120. doi: 10.1038/s41598-018-36239-w.
doi: 10.3389/fmicb.2012.00180

Drury, R. A. B. and E. A. Wallington.1980. Carleton's histological technique- 4th Ed - oxford. Oxford University press. https://catalogue.nla.gov.au/Record/2864951 Egyptian Standard Specifications (2005). Salted fish Es: 1725-3/2005.

El-Kosi, O. H.R., E. H .Abdel-Hakiem, A.M.Ayesh and J. I. I. Mohamed.2009. Effect of different storage temperature and period on biogenic amines formation in Ras cheese.SCVMJ,VIX(1)207-219. https://scholar.google.com.eg/scholar?hl=ar&as_sdt=0%2C5&q=Effect+of+different+storage+temperature+and+period+on+biogenic+amines+formation+in+Ras+cheese.+SCVMJ%2C+VIX+%281%29%2C+207-219.&btnG=

Elsanhoty, R. M., H.Mahrous and G. A.Ghanaimy .2009. Chemical, microbial counts and evaluation of biogenic amines during the ripening of Egyptian soft Domiati cheese made from raw and pasteurized buffaloes milk. International Journal of dairy science, 4(2), 80-90. Doi: 10.3923/ijds.2009.80.90

El-Zahar, K. M., A. M. Abd El-Zaher and M. F. Ramadan.2014. Levels of biogenic amines in cheeses and their impact on biochemical and histological parameters in rats. Journal Korean Society for Applied Biological Chemistry, (57), 73-81. Doi:10.1007/s13765-013-4136-z

Faid, S. M. A. 2017. Evaluation of Yogurt and Soft Cheese Fortified with Chia Seeds. World Journal of Dairy and Food Sciences. 12 (1), 01-12. Doi: 10.5829/idosi.wjdfs.2017.01.12

FAO-WHO .2012. Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products. 23-27 July 2012, FAO Headquarters, Rome, Italy.

Fernández, M., D. M.Linares, B.Del-Río, V.Ladero and M.A.Alvarez.2007. HPLC quantification of biogenic amines in cheeses: correlation with PCR-detection of tyramine-producing microorganisms. Journal of Dairy Research, 74(03), 276-282.
Doi: 10.1017/S0022029907002488.

Food and Drug Administration (FDA) .2011. Fish and fishery products hazards and controls guidance. Fourth Edition. Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Food Safety, Washington, DC, USA.

Gerner, E. W. and F. L. Meyskens.2004. Polyamines and cancer: Old molecules, new understanding. Nat Rev Cancer 4: 781-792. Doi:10.1038/nrc1454

Gomez, K. N. and A. A. Gomez.1984. Statistical procedures for agricultural research. John Wiley and Sons, New York 2nd. Ed68p.

Halasz, A.A., L.Barath, L.Simon-Sarkadi and W.Holzapfel.1994. Biogenic amines and their production by microorganisms in food. Trends in Food Science and Technology 5: 42–49. Doi:10.1016/0924-2244(94)90070-1

Henry, R.J. 1974. Clinical Chemistry, Principles and Techniques, 2th ed., Harper and Row, pp. 525. (C.F. Commercial Kit for determination of creatinine. Diamond Diagnostics).
https://doi.org/10.1016/j.fm.2007.11.006

Ismail, E. L. A.2013. Technological and nutritional studies on some supplemented bakery products prepared for school students consumption. M.Sc.; Thesis, Fac. Gric.; AL-Azhar Univ.; Egypt.

Khuhawar, M. Y. and G. A. Qureshi.2001. Polyamines as cancer markers: applicable separation methods. J.Chromatogr B Biomed Sci Appl.;764(1-2):385-407.
https://doi.org/10.1016/S0378-4347(01)00395-4

Komprda, T., R.Burdychová, V.Dohnal, O.Cwiková, P.Sládková and H.Dvořáčková.2008. Tyramine production in Dutch-type semi-hard cheese from two different producers. Food Microbiology,25(2)219-227.

Komprda, T., D.Smělá, K.Novická, L.Kalhotka, K.Šustová and P.Pechová.2007. Content and distribution of biogenic amines in Dutch-type hard cheese. Food chemistry, 102 (1), 129 -

137.https://doi.org/10.1016/j.foodchem.2006.04.041

Ladero. V., M.Calles-Enríquez, M.Fernández and M.A. Alvarez.2010. Toxicological effects of dietary biogenic amines. Current Nutrition & Food Science, 6(2), 145-156. Doi: 10.2174/157340110791233256

Linares, D. M., B.del Rio, B.Redruello, V.Ladero, M. C. Martin, M.Fernandez and M. A. Alvarez.2016. Comparative analysis of the in vitro cytotoxicity of the dietary biogenic amines tyramine and histamine. Food chemistry 197: 658-663. https://doi.org/10.1016/j.foodchem.2015.11.013

Linares, D. M., B.del Rio, B.Redruello, V.Ladero, N.Martinez, , M.Fernandez, M. C.Martin and M. A. Alvarez.2012. Factors influencing biogenic amines accumulation in dairy products. Frontiers in Microbiology , 3, 180, 1-10.

Linares, D. M., M.Martín, V.Ladero, M. A.Alvarez and M. Fernández .2011. Biogenic amines in dairy products. Critical reviews in food science and nutrition, 51(7), 691-703. Doi: org/10.1080/10408398.2011.582813

Maijala, R. L., S.H.Eerola, M. A. Aho, and J. A .Hirn .1993. The effect of GDL – induced pH decrease on the formation of biogenic amines in meat. J. of Food Protection. 56. (2) 125-129. https://doi.org/10.4315/0362-028X-56.2.125

Manca,G., A.Ru, G.Siddi, A.M Maria, G.Murittu and P.L.De Santis.2020.Biogenic amines content in Fiore Sardo cheese in relation to free amino acids and physicochemical characteristics. Italian Journal of Food Safety 9:8457 48-58. DOI: 10.4081/ijfs.2020.8457

Marijan. A., P.Džaja, T.Bogdanović, I.Škoko, Ž.Cvetnić, V.Dobranić and K.Severin. 2014. Influence of ripening time on the amount of certain biogenic amines in rind and core of cow milk Livno cheese. Mljekarstvo/Dairy, 64(3), 159-169. Doi: 10.15567/mljekarstvo.2014.0303

Masaki, T., S.Chiba, H.Tatsukawa, H.Noguchi, T.Kakuma, M.Endo, M.Seike, T.Watanabe and H.Yoshimatsu .2005. The role of histamine H1 receptor and H2 receptor in LPS-induced liver injury. FASEB J 2005;19:1245–52.DOI: 10.1096/fj.04-3195com

Mohamed, A.G., M.M.Deabes, A.M.H Fatma, K.Enab and A.A.K. Abou- Arab. 2013. Biogenic amines and chemical composition of different formulations used for manufacture of processed cheese. J. Appl. Sci. Res. 9 (3): 1477-1483. https://www.researchgate.net/publication/260594505_Biogenic_amines_and_chemical_composition_of_different_formulations_used_for_manufacture_of_processed_cheese

Pachlovà, V., F.Bunka, R.Flasarovà, P.Vàlkovà and L.Bunkovà. 2012. The effect of elevated temperature on ripening of Dutch type cheese. Food Chemistry 132: 1846–1854. https://doi.org/10.1016/j.foodchem.2011.12.017

Patten, C. J. and S.R. Crouch.1977. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem., 49: 464-469. Doi: 10.1021/ac50011a034

Pegg, A. E. 2013. Toxicity of Polyamines and Their Metabolic Products. Chem. Res. Toxicol. 26, 1782−1800. dx.doi.org/10.1021/tx400316s

Pinho, O., I.M.Ferreira, E.Mendes, B.M. Oliveira and M.Ferreira.2001. Effect of temperature on evolution of free amino acid and biogenic amine contents during storage of Azeitão cheese. Food Chemistry,75(3) 287-291.Doi: 10.1016/S0308-8146(01)00109-1

Reitman, S. and S.Frankel .1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology, 28(1), 56-63. Doi: org/10.1093/ajcp/28.1.56

Rowayshed, G.A., A.M. Ayesh and M.M. Deabes. 2002. The influence of some food additives on histamine production by histamine forming bacteria. Egyptian J. Nutrition 17 (1):199-223.

Ruiz-Capillas, C. and A.M. Herrero. 2019.Impact of Biogenic Amines on Food Quality and Safety.Foods.8(2). 1-16. E62. doi: 10.3390/foods8020062.

Santos, M.H.S. 1996. Biogenic Amines: Their Importance in Foods. International Journal of Food Microbiology, 29, 213–231. Doi: org/10.1016/0168-1605 (95)00032-1

Schirone, M., P.Visciano, R.Tofalo and G. Suzzi 2016. Histamine Food Poisoning. Handbook of Experimental Pharmacology .Volume 241 of the series pp 217-235. Doi: 10.1007/978-3-319-58194-1

Shah, P. and E.Swiatlo .2008. A multifaceted role for polyamines in bacterial pathogens. Molecular Microbiol 68 (1) 4 - 16.

https://doi.org/10.1111/j.1365-2958.2008.06126.x

Shalaby, A.R. 1996. Significance of biogenic amines to food safety and human health. Food Research International, 29(7) 675 - 690. Doi:org/10.1016/S0963-9969 (96)00066-X

Standarová, E., L.Vorlová, P.Kordiovská, B. Janštová, M.Dračková and I.Borkovcová. 2010. Biogenic amine production in Olomouc curd cheese (Olomouckétvarůžky) at various storage conditions. Acta Veterinaria Brno,79(1)147-156.Doi: 10.2754/avb201079010147

Teodorovic, V., S.Buncis and D. Smiljanic.1994. Study of some factors influencing the histamine production in meat. Fleischwirtsch. 74,181-183.

Thapa, B.R.and A.Walia.2007. Liver function tests and their interpretation. Ind J Pediatr.2007;74:663–671.https://link.springer.com/content/pdf/10.1007/s12098-007-0118-7.pdf

Til,H.P., H. E.Falke, M.K. Prinsen and M. I.Willems.1997. Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadaverine in rats. Food and Chemical Toxicology, 35(3), 337-348. Doi: 10.1016/S0278-6915(97)00121-X

Tripathi, T., M. Shahid, A.Raza, H.M. Khan, R. A.Khan, A.A.Mahdi, M.Siddiqui, A.Malik and A. A. Khane. 2012.Dose-dependent effect of histamine on liver function markers in immunized rabbits. Experimental and ToxicologicPathology 64 875–881 doi:10.1016/j.etp.2011.03.010.