# Biogenic Amines, Microbiological, and Chemical Properties of Market Ras Cheese

Enaam Shokery [1] , Eman Ibrahim[2], Nesma Sallama[2], Susu Alasfoury[3] and Seham Swelam[2]

## ABSTRACT

**Ras cheese is one of the main types of hard cheese that is produced and consumed in Egypt. It is known as "Roumy cheese" throughout Egypt, except in Alexandria, where it is called "Turkish cheese.". It is made from cow's milk or a mixture of buffalo and cow's milk. It requires a longer ripening period, and due to this extended period and its high protein content, which breaks down into a significant amount of biogenic amines. This study aimed to assess the presence and quantity of biogenic amines in Ras cheese and their effects on product quality, spoilage, and safety. The study was based on collecting 22 samples from retail markets in Kafr El-Sheikh to determine the levels of biogenic amines. The study focused on determining the presence of some microorganisms in different samples of cheese and assessing their prevalence.; these samples were analyzed for physicochemical and microbiological properties. The microbiological tests include, total bacterial counts, coliforms, proteolytic bacterial count, molds, and yeasts, while the chemical tests measured moisture, fat, protein, acidity, total volatile fatty acids, formal ripening index, and biogenic amines. In this study, cadaverine was the most abundant amine with a concentration of 203.65 mg/kg, followed by putrescine and histamine, while tyramine was the least concentrated. The study concluded that the presence of a certain amount of biogenic amines in Ras cheese may be attributed to bacterial enzymatic activity and poor storage conditions. To reduce the formation of these amines, the study recommends producing Ras cheese from pasteurized milk, maturing it under hygienic conditions, and ensuring proper transportation and storage for sale under suitable conditions in terms of temperature, humidity, cleanliness, and sanitation. This would help deliver the cheese to consumers in the desired quality**

**Key words**: **Ras Cheese, Biogenic Amines, Tyramine, Histamine, Cadaverine**

## INTRODUCTION

Food quality and safety have garnered increased attention due to their association with potentially hazardous compounds. These substances can be toxic and have been linked to a range of health issues. Among various food items, cheese, a staple in many diets, has become a focal point of this concern. In Egypt, Ras cheese, also referred to as Rumi cheese in several regions and Turkish cheese in Alexandria, is particularly popular. Ras cheese is a hard variety that contains 30–40% moisture, providing a conducive environment for the formation of biogenic amines. These amines can emerge because of microbial activity, especially during the cheese aging. The biogenic amines produced by protein breakdowns can sometimes serve as a quality and safety marker for cheese and other fermented foods (Amer *et al.*, 2023 and Eldenary *et al.*, 2023).

Cheese is well-regarded for its rich protein, vitamins, and minerals, making it an integral component of human nutrition. However, like many fermented foods, cheese can sometimes be associated with biogenic amines (BAs). These nitrogenous compounds are low molecular weight and mostly come from decarboxylation of amino acids like histidine, lysine, ornithine, and arginine by microbes (El-Zahar *et al.*, 2014). Certain cheeses, like Ras Cheese, are noted for higher BAs concentrations, attributable to the activity of decarboxylase-producing microorganisms that transform amino acids. These microorganisms can convert amino acids like tyrosine, histidine, lysine, and tryptophan into biogenic amines, including tyramine, histamine, cadaverine, and tryptamine, respectively. Several factors influence BAs synthesis in foods, including the availability of amino acids, specific microorganisms, and environmental conditions conducive to their growth and activity. Internal and external factors, such as pH, water activity, composition, and microbial population, affect the type and concentration of BAs in foods (Barone *et al.*, 2018 and Farag *et al.*, 2019).

Several common factors affect the formation of biogenic amines in foods, particularly cheese. These include the ripening period, the availability of amino acids, and the proteolysis activity of bacteria to increase biogenic amine content in cheese. The presence of certain microorganisms, such as *Lactobacillus brevis* and *Enterococcus faecalis*, and microbiota like lactic acid bacteria, yeasts, and molds in the fermentation process is also crucial. Decarboxylation conditions, particularly those favoring the formation of cadaverine from ornithine and lysine, are important. A positive correlation exists between the acidity percentage and biogenic amine content (Barone *et al.*, 2018). Tyramine

**DOI: 10.21608/asejaiqjsae.2025.418727**
[1] Faculty of Agriculture, Alexandria university Al Shatby,
2 Faculty of Agriculture.kafr El-Sheikh University ,
3Taif University,P.O. BOX11099, Taif 21944 Saudi Arabia,
Agricultural Research Center – Food Technology Research Institute (FTRI) -Egypt
Received February 10, 2025, Accepted March 23, 2025.

is often found in cheese, leading to dairy-borne intoxications known as "cheese reactions" when present in concentrations exceeding 1000 mg/kg. Contamination with specific bacteria facilitates the formation of tyramine. Histamine, another biogenic amine, is a key factor in food poisoning cases impacting the cardiovascular and central nervous systems. Its presence often indicates poor manufacturing and storage conditions. Polyamines, such as putrescine and cadaverine, not only stop the metabolism of histamine, which makes it more toxic, but they are also linked to the growth of cancer because they create N-nitrosamines that are carcinogenic. The presence of cadaverine in dairy products is an indirect consumer hazard, as it intensifies the effects of tyramine and histamine toxicity by inhibiting detoxification enzymes (Zdolec *et al.,* 2022).

The presence and levels of biogenic amines in cheese have been extensively researched due to their potential health implications. Various factors, including microbial activity and environmental conditions, influence the formation of these amines, with specific attention to amines like cadaverine, tyramine, and histamine. The research underscores the importance of understanding and monitoring these compounds for public health, quality control, and food production improvements, shedding light on their intricate relationship with cheese's chemical and microbiological aspects. Biogenic amines are due to decarboxylation or amination activity by microbial enzymes and different technological processes affecting biogenic amine formation. They are generally low in raw milk, meanwhile, in fermented or ripened cheeses and dairy products, much higher concentrations can be detected (Erdag *et al.,* 2019).

Biogenic amine concentrations are usually low in raw milk and vary due to the different origins and compositions. Several lactic acid bacteria (LAB) belong to different genera, such as *Enterococcus*, *Streptococcus*, *Leuconostoc*, and *Lactococcus*, some non-starter lactic acid bacteria (NSLAB), including pediococci, enterococci, and other thermophilic LAB are BAs producers milk flora introduced during the production procedure. Enterococci are not present in starter cultures but are often found in artisanal cheeses (Benkerroum, 2016). Genera of Enterobacteriaceae and Gram-negative bacteria have represented great amounts of cadaverine and putrescine in Montasio cheese samples, while tyramine was mainly produced by some isolates (Maifreni *et al.,* 2013).

Soft cheeses measured an increase during the storage period (cadaverine, tyramine, and histamine concentrations), and in semi-hard cheeses (Gouda type), tyramine reached maximum levels of 1029 mg $kg^{-1}$ at the expiry date. Contrarily, the higher concentrations of BAs, i.e., histamine and tyramine, were noticed in hard cheeses (Parmesan type) with maximum concentrations of 1025 and 561 mg $kg^{-1}$, respectively. However, tyramine was the most represented amine in blue cheeses, up to values of 1306 mg $kg^{-1}$ at the expiry date (Dabade *et al.,* 2021).

The hygiene level of the raw milk controls the biogenic amine (BAs) content in cheese and depends basically on the occurrence of microbial species producing decarboxylases. Therefore, the selection of decarboxylase-negative starter cultures is the most important factor in regulating BAs activity and their accumulation in cheese. The initial microbial population and the kinds of free amino acids are essential strategies (Benkerroum, 2016). However, the selection of a universal LAB starter according to BAs synesis is an important parameter to be used in fermented dairy products (Zhang *et al.,* 2022). Sensory testing, modified atmospheres such as vacuum, and packaging systems using edible films and coatings have been assessed against BAs formation (Todaro *et al.,* 2018). This study aimed to prevent and control strategies of biogenic amine production and the relationship between the microbiological quality and the chemical properties of biogenic amins formation.

## MATERIAL AND METHODS

### Collection of samples

Twenty-two Ras cheese samples were randomly collected from stores in Kafer El-Sheikh Governorate. The samples were collected in retail packages and transferred in an icebox to the laboratory for analysis within 24 hours.

### Chemical analysis

#### Moisture content:

Cheese's moisture content was determined according to AOAC (2020).

#### Fat content:

A special Gerber butyormeter was used to determine fat% in cheese according to Awad (2006).

#### pH value:

Cheese samples were prepared according to AOAC (2020). 1g of cheese was homogenized with 10 distilled waters, then pH values were measured using a digital pH meter model CRISON-SPAIN.

#### Titratable acidity:

Total acidity of cheese samples as a lactic acid percentage was determined by titration using NaOH 0.1N and ph.ph. as an indicator (Awad, 2006).

**Total volatile fatty acids (TVFA):**

According to Kosikowski (1970), the direct distillation method was used to determine TVFA (ml 0.1 NAOH / 100 gm cheese) in cheese samples.

**Total nitrogen content (TN):**

TN of cheese samples was determined using Kjeldahal as described by AOAC (2020).

**Formol ripening index (FRI):**

FRI was conducted according to Tawab and Hofi (1966) as follows: with gradual addition of 50 ml of distilled water at 40-50 C° from a graduated cylinder, 5g of samples were ground into paste with glass rod in a 100 ml beaker. The supernatant was decanted and filtered under suction on Buchner funnel using two layers of Whatman No.40 filter-paper. A test-tube of about 50 ml capacity was placed inside the suction flask just under the funnel stem to collect the cheese filtrate. After cooling, two 10 ml aliquots of the filtrate were drawn into two 100 ml conical flask. To one of the latter flasks 10 drops of thymolphathalein indicator (0.1%) and 6 drops of phenolphthalein indicator (0.5%) were added to the second. Both flasks were then titrated with 0.1N Sodium hydroxide with a microburette till blue colour was formed in the first flask (A) and a pink colour in the second (B). Both colours should persist for 15 seconds. To the phenolphthalein flask 2ml. of neutral formalin (40%) were added and the contents were retitrated with the same alkali till the pink colour reappeared. The volume of the alkali used was recored (C). The ripening index (RI) of cheese was calculated as follows:-

Shilovich ripening index (SRI) = (A- B) × 100

Formol ripening Index (FRI) = (C) × 100

**Determination of biogenic amines**:

Biogenic amines were determined in cheese samples according to Pinho *et al.* (2001), using the HPLC Ultimate 3000 Thermo Fisher (Germany) equipped with an autosampler, pump, and UV detector set at 254 nm. An Agilent Poroshell, 120 EC-C18, 4 um (4. 6 mm x 450 mm) column was used for biogenic a min separation. Data were integrated and recorded using the Chromeleon software program.

**Microbiological analysis:**

**Preparation of cheese samples:**

Ten grams of each sample were homogenized with 90 ml of sterile 2% sodium citrate solution using stomacher equipment. Serial dilutions were prepared according to ISO 6887-1 (2017): Microbiology ISO 6887-5 (2020).

**Total bacterial count (TBC):**

Total viable aerobic microorganisms' counts were determined on nutrient agar medium. The plates were incubated at 30 for 72 hr, according to Difco (1974).

**Proteolytic bacterial count (PBC):**

The nutrient agar medium was used to count the proteolytic bacterial content of the samples. Sterile skim milk (10%) was added to every plate before pouring the medium (Hammer and Babel, 2002), the plates were incubated for 3 days at 30 °C then added HCl (1%). Colonies are surrounded with clear zone counting proteolytic bacteria.

**Mold and Yeast count:**

It was carried out on potato dextrose Agar (PDA) medium according to Lück and Gavron (1990). The plates were incubated at 24 ± 1°C for five days.

**Coliform bacterial content (CBC):**

Mackoncy agar medium was used to count coliform bacteria following Difco (1974). The plates were incubated at 37 for 72 hr.

**Statistical Analysis:**

The experimental data was analyzed using SPSS29 software, employing one-way ANOVA for statistical evaluation. Duncan's Multiple Range Test was utilized for comparing the means of different treatments, with a significance level set at $p < 0.05$. The entire experiment was replicated twice to ensure the consistency and reliability of the results.

## RESULT AND DISCUSSION

Table (1) shows the chemical composition of the collected Ras cheese samples. The data is presented as the mean ± standard error of the mean (SE) for three replicates. The table includes the following parameters:

**Table 1. Chemical composition of collected Ras cheese samples**

| | Moisture (%) | Fat (%) | Fat/ Dry Matter (%) | Protein (%) | Protein / Dry Matter (%) |
|---|---|---|---|---|---|
| **Maximum** | 44.31±0.74[a] | 35.33±1.45[a] | 49.73±1.04[a] | 38.16±0.0[a] | 56.02±0.14[a] |
| **Minimum** | 25.09±1.12[b] | 16.67±0.88[b] | 25.68±0.98[b] | 22.79±0.0[b] | 33.46±0.38[b] |
| **Average** | 34.7 | 25.67 | 37.70 | 33.71 | 49.49 |

Data means ± SE for 3 replicates.

*a, and b mean that unlike small superscripts within the same column, they are significantly different ($P \leq 0.05$).

In this study, a comprehensive characterization of 22 Ras cheese samples was obtained randomly from Kafer El-Sheikh markets. Chemical analyses found moisture ranged from 25.09–44.31% (average 34.7%), and fat ranged from 16.67–35.33% (average 25.67%), consistent with Ras cheese specifications defined by El-Zahar *et al.* (2014). This indicates acceptable variability in key compositional parameters.

The table shows that the moisture content of the Ras cheese samples ranged from 25.09% to 44.31%, with an average of 34.7%. The fat content ranged from 16.67% to 35.33%, with an average of 25.67%. The fat/dry matter content ranged from 25.68% to 49.73%, with an average of 37.70%. The protein content ranged from 22.79% to 38.16%, with an average of 33.71%. The protein/dry matter content ranged from 33.46% to 65.52%, with an average of 49.49%, these results agree with El-Zahar *et al.* (2014). The data in Table (1) regarding the chemical composition of collected Ras cheese samples presents comprehensive data on Ras cheese samples, revealing a wide range of moisture, fat, and protein contents. Moisture levels varied from 25.09% to 44.31%, while fat content ranged between 16.67% and 35.33%, with protein content spanning from 22.79% to 38.16%. On average, moisture, fat, and protein contents were 34.7%, 25.67%, and 33.71%, respectively. Notably, Ras cheese exhibited relatively high fat and protein levels, surpassing averages for other cheese types like cheddar and mozzarella. Its relatively high moisture content contributed to the cheese's soft and creamy texture. While the fat content varied but tended to be lower than some counterparts, it still enriched Ras cheese's flavor and richness. High protein content, a common trait in cheeses, provides essential amino acids. Moreover, Ras cheese demonstrated elevated fat/dry matter and protein/dry matter ratios, making it a valuable source of both fat and protein on a dry matter basis. This compositional diversity may stem from factors such as milk type, cheesemaking processes, and ripening conditions, yet all samples remained within acceptable quality standards for Ras cheese (El-Zahar, 2014 and Helal & Tagliazucchi, 2023).

Table (2) shows the acidity, pH, formol ripening Index, and TVFA of Ras cheese samples. The acidity of the cheese samples ranged from 1.23% to 2.30%, with an average of 1.77%. Acidity is a measure of the amount of acid in cheese. It plays a direct role in the flavor and texture development of the cheese during ripening. Differences among samples related to pH were described in Table (2) the data were consistent with cheese acidity. The most detectable pH value was 5.89, representing sample 11, but samples 16 and 13 were the least and both recorded 5.42.

The Formol Ripening Index of the cheese samples ranged from 20.00 to 66.67, with an average of 43.33. The Formol Ripening Index is a measure of how ripe cheese is.

The TVFA of the cheese samples ranged from 7.5 to 25.3, with an average of 16.4. TVFA is a measure of the amount of volatile fatty acids in cheese, produced by the lipolysis of cheese fat during cheese ripening, they contribute to the flavor of the cheese. Overall, Table (2) shows that the Ras cheese samples had a wide range of acidity, pH, Formol Ripening Index, and TVFA values. This is likely due to the different factors that affect the ripening process, such as the microflora, the temperature, and the cheese ripening period (El-Zahar *et al.*, 2014 and Amer *et al.*, 2023).

The microbiological assessment revealed total viable microorganism counts of 3.50-3.86 Log CFU/g (average 3.68 Log CFU/g), proteolytic microorganism counts of 0.50-1.98 Log CFU/g (average 1.24 Log CFU/g), and yeast/mold counts of 0.32-2.39 Log CFU/g (average 1.36 Log CFU/g), all within ranges reported by Ibrahim *et al.* (2023) to ensure quality. However, contamination risks depend on intrinsic/extrinsic factors like milk quality and storage conditions (Amer *et al.*, 2023). Six biogenic amines (BAs) - β-phenylethylamine, putrescine, cadaverine, histamine, tyramine, and spermine were quantified using the HPLC method described by Oliveira *et al.* (2001). Levels varied considerably, with histamine exceeding the toxic threshold of 100 mg/kg in some samples (Ladero *et al.*, 2010). High BAs formation may relate to proteolysis by bacteria like *Lactobacillus brevis* and *Enterococcus faecalis* under the acidic conditions favoring decarboxylation reported by Zaccheo *et al.* (2017) and El-Zahar *et al.* (2014) who defined Ras cheese specifications. Ibrahim *et al.* (2023) reported acceptable microbiological ranges. Amer *et al.* (2023) noted contamination risks depend on intrinsic/extrinsic factors. Oliveira *et al.* (2001) described the HPLC quantification method. Ladero *et al.* (2010) reported the toxic histamine threshold. Barone *et al.* (2018) observed conditions favoring BA formation.

Finally, a full analysis of Ras cheese samples from Kafer El-Sheikh markets showed that key compositional parameters like moisture and fat content varied in a way that was acceptable and in line with the requirements. The microbiological assessment indicated that the total bacterial counts, proteolytic counts, and yeast/mold counts were within the reported ranges, ensuring the quality of the cheese. However, it is important to consider intrinsic and extrinsic factors such as milk quality and storage conditions to mitigate contamination

risks. The quantification of biogenic amines highlighted the variation in their levels, with histamine exceeding the toxic threshold in some samples. Specific bacteria's proteolysis under acidic conditions may be responsible for the formation of biogenic amines. These findings emphasize the importance of adhering to defined specifications and implementing proper quality control measures in the production and storage of Ras cheese. Further research and monitoring are necessary to ensure the safety and quality of this popular cheese variety. Table (3) shows the microbiological analysis of collected Ras cheese samples, with the total count, proteolytic count, and yeast and mold count expressed in log colony-forming units per gram of cheese (log CFU/g cheese).

The total bacterial count refers to the total number of viable bacteria present in the cheese sample. The proteolytic count refers to the number of bacteria present in the cheese sample that can break down proteins. The yeast and mold count refer to the number of yeasts and molds present in the cheese sample. The maximum and minimum values in the table indicate the highest and lowest values obtained for each type of count, respectively. The average value in the table indicates the average value for each type of count.

The Table (3) shows that the total count of bacteria in the Ras cheese samples ranged from 3.50 to 3.86 log CFU/g cheese, with an average of 3.68 log CFU/g cheese. The proteolytic count of bacteria ranged from 0.50 to 1.98 log CFU/g cheese, with an average of 1.24 log CFU/g cheese. The yeast and mold count ranged from 0.32 to 2.39 log CFU/g cheese, with an average of 1.36 log CFU/g cheese (Ibrahim *et al.,* 2023). Whereas, Moneeb *et al.* (2024) exhibited the highest fungal count at fresh Ras cheese 2.72 log 10\g on PDA medium. Ras cheese were studied by Mehanna *et al.* (2008) who found that the total count and the proteolytic bacterial count was 7.38 and 3.09 log 10 \g, respectively which consider higher than our average count.

Overall, the microbiological quality of the Ras cheese samples was good. The total count of bacteria was within the acceptable range for Ras cheese. The proteolytic count and yeast and mold count were also within the acceptable range.

However, it is important to note that the microbiological quality of cheese can vary depending on several factors, such as the type of milk used, the cheesemaking process, and the storage conditions. It is therefore important to follow good hygiene practices during cheesemaking and storage to ensure that the cheese remains safe for consumption. Table (4) shows the biogenic amine content in some detected samples in mg/kg. Biogenic amines are naturally occurring compounds found in a variety of foods, including fermented foods, aged meats, and cheeses. They can also be produced by bacteria during food spoilage.

**Table 2. Acidity, pH, Formol Ripening Index and TVFA of collected Ras cheese samples**

| | Acidity (%) | pH | Formol Ripening Index | TVFA* |
|---|---|---|---|---|
| **maximum** | 2.30±0.10[a] | 5.89[a] | 66.67±3.33[a] | 25.3±0.44[a] |
| **minimum** | 1.23±0.07[b] | 5.42[b] | 20.00±0.0[b] | 7.5±0.47[b] |
| **average** | 1.77 | 5.66 | 43.33 | 16.4 |

Data means ± SE for 3 replicates.
[*a, and b] mean that unlike small superscripts within the same column, they are significantly different ($P \leq 0.05$).
TVFA* Total Volatile Fatty Acids (ml 0.1 NaOH/100gm cheese)

**Table 3. Microbiological analysis of collected Ras cheese samples (log CFU/g cheese)**

| | Total Count | Proteolytic bacterial count | Yeast & Mold |
|---|---|---|---|
| **maximum** | 3.86±0.12[a] | 1.98±0.03[a] | 2.39±0.44[a] |
| **minimum** | 3.50±0.21[b] | < 1.0 | < 1 .0 |
| **average** | 3.68 | 1.24 | 1.36 |

Data means ± SE for 3 replicates.
[*a, and b] mean that unlike small superscripts within the same column, they are significantly different ($P \leq 0.05$).

**Table 4. Biogenic amines content in some detected samples (mg/KG)**

| | β-phenyl ethyl amine | Putrescine | Cadaverine | Histamine | Tyramine | Spermine |
|---|---|---|---|---|---|---|
| **maximum** | 112.8[a] | 174.3[a] | 295.5[a] | 187.1[a] | 8.9[a] | 0.2 |
| **minimum** | 8.7[j] | 69.5[j] | 111.8[j] | 26.4[j] | 0.9[i] | 0.1 |
| **average** | 60.75 | 121.9 | 203.65 | 106.75 | 4.9 | 0.15 |

Data means ± SE for 3 replicates.
[*a, and b] mean that unlike small superscripts within the same column, they are significantly different ($P \leq 0.05$).

Table (4) shows the maximum, minimum, and average biogenic amine content for each of the six biogenic amines listed. The maximum values for all six biogenic amines are relatively high, while the minimum values are much lower. This suggests that there is a wide range of biogenic amine content in the detected samples. Table (4) presents valuable information regarding the content of various biogenic amines in a set of detected samples, with measurements expressed in milligrams per kilogram (mg/kg). When consumed in excess, biogenic amines—organic compounds that microorganisms can produce during the breakdown of proteins in food—can have negative health effects. This data is essential for assessing the safety and quality of the analyzed samples, particularly within the context of food safety and potential health risks associated with high biogenic amine levels.

Starting with β-phenyl ethyl amine, it was observed that there was a significant range in content across the samples. The maximum value of 112.8 mg/kg represents the highest concentration found in any of the samples, whereas the minimum value of 8.7 mg/kg represents the lowest concentration detected. The average value of 60.75 mg/kg provides insight into the typical or mean concentration of this amine across the samples. This information can be crucial for determining the variability in β-phenyl ethyl amine content in the analyzed samples.

Moving on to putrescine and cadaverine, similar trends in terms of the range from minimum to maximum values and the average concentrations were also noted. Putrescine has a maximum value of 174.3 mg/kg, while cadaverine has an even higher maximum value of 295.5 mg/kg, indicating a wider variability in these amines across the samples. The average values of 121.9 mg/kg for putrescine and 203.65 mg/kg for cadaverine provide insights into the typical levels found in these samples.

Histamine, another biogenic amine, is of particular concern in food safety due to its potential health implications. The maximum value of 187.1 mg/kg, the minimum value of 26.4 mg/kg, and the average value of 106.75 mg/kg give us a clear picture of the range and typical concentrations of histamine in the detected samples.

In contrast, tyrosine and spermine are present in relatively lower concentrations. Tyramine ranges from a maximum of 8.9 mg/kg to a minimum of 0.9 mg/kg, with an average of 4.9 mg/kg. Spermine, found in very low concentrations, has a maximum value of 0.2 mg/kg, a minimum of 0.1 mg/kg, and an average of 0.15 mg/kg in the samples.

The data in the table suggests that many detected samples contain biogenic amines at a level that could be of concern for some people, especially those with certain medical conditions, such as migraines or hypertension.

The high variability in biogenic amine content among samples suggests that it is important to be mindful of the foods that you eat and to store food properly.

In conclusion, this table serves as a valuable reference for assessing the variability and typical concentrations of these biogenic amines in the samples, which is critical for ensuring food safety and quality. Monitoring and controlling biogenic amine content in food products is essential to mitigating potential health risks for consumers (Gardini *et al.*, 2016; Saad & Tofalo, 2019 and Martuscelli *et al.*, 2021).

The production of BAs in cheeses generally indicates both their quality and safety. Many factors, such as pH, salt concentration, water activity, and redox potential, can influence the growth and metabolic activities of the milk and cheese microbiota involved in BAs production. Besides the initial microbial load of the raw material, the cheese-making process can be responsible of BAs accumulation in the end products, as the proteolysis rate increases with the ripening time, and free amino acids are used by microorganisms as substrate for their decarboxylase activity. The highest probability of detecting bas in cheeses is generally associated with the duration of the aging period. A good storage of the final product is also recommended, considering that high BAs concentrations have also been reported in this stage. The most important hindering factors rely on the selection of decarboxylase-negative or BAs-degrading starter cultures, good hygiene practices during cheesemaking, and appropriate modulation of all parameters influencing the bacterial population responsible for high production of BAs. A global safety assurance program throughout the processing, storage, and retailing of cheeses is essential from the perspective of risk reduction for consumers (Schirone *et al.*, 2022).

This research performed the characterization of 22 Ras cheese samples from Kafer El-Sheikh markets, evaluating key chemical, microbiological, and safety parameters. Chemical analysis revealed moisture and fat contents aligned with defined specifications (El-Zahar *et al.*, 2014), signifying compositional adequacy. Total bacterial counts, proteolytic counts, and yeast/mold counts largely agreed with ranges associated with quality benchmarks from past literature (Ibrahim *et al.*, 2023). However, considerable variability arose in biogenic amine content, with histamine exceeding toxicity thresholds in some samples (Ladero *et al.*, 2010).

While alignment with existing guidelines is reassuring (El-Zahar *et al.,* 2014 and Ibrahim *et al.,* 2023), safety cannot hinge solely on meeting compositional and microbiological requirements. Tailored interventions are necessary to control intrinsic and extrinsic variables influencing decarboxylase activity and biogenic amine levels, according to studies like Amer *et al.* (2023). As this work has illuminated, producing safe Ras cheese is a multifaceted challenge necessitating rigorous process management alongside targeted inhibition of hazards.

## CONCLUSION

Based on the research results, where biogenic amines are present in Ras cheese samples from Kafr El-Sheikh, some innovative strategies as important preventive measures should be recommended to dairy operators regarding Ras cheese production and microbiological evaluation which include: Investigation of optimum storage conditions to reduce mold growth and yeast contamination in Ras cheese during ripening and distribution. Proper temperature, humidity and packaging can mitigate microbial problems. Exploration of generally recognized safe antifungal coatings as alternatives to synthetic preservatives in Ras cheese production. Evaluation of effective and edible inhibitors can enhance safety. Evaluation of anti-mycotoxin probiotic strains as preventive cultures to reduce the risk of mold-derived mycotoxin contamination in Ras cheese. Clarification of the effect of intrinsic factors such as milk quality, coagulation type, ripening duration, etc. on biogenic amine development in Ras cheese through factorial experiments. This can identify critical control points. Expanding sampling studies to validate the guideline biogenic amine concentration ranges for developing safety specifications and risk assessment for Ras cheese varieties. Larger data sets would increase generalizability.

## REFERENCES


Amer, D. A., A. A. M. Albadri, H. A. El-Hamshary, Y. Nehela, A. H. Makhlouf, M. Y. El-Hawary and S. A. Awad. 2023.Changes in Sensory Properties, Physico-Chemical Characteristics, and Aromas of Ras Cheese under Different Coating Techniques. *Foods*, *12*(10). https://doi.org/10.3390/foods12102023

AOAC. 2020. Association of Official Analytical Chemists. In M. Horwitz (Ed.), Official Methods of Analysis, Academic Press.

Awad, S. 2006. Texture and flavour development in Ras cheese made from raw and pasteurised milk. *Food chemistry*, *97*(3):394-400.

Barone, C., M. Barebera, M. Barone, S. Parisi, A. Zaccheo, C. Barone and A. Zaccheo. 2018. Biogenic amines in cheeses: types and typical amounts. *Chemical Evolution of Nitrogen-based*

Benkerroum, N. 2016. Biogenic amines in dairy products: Origin, incidence, and control means. Comprehensive Reviews in Food Science and Food Safety, 15, 801e826.

Dabade, S. D., L. Jacxsens, L. Miclotte, E. Abatih, F. Devlieghere and B. De Meulenaer. 2021. Survey of multiple biogenic amines and correlation to microbiological quality and free amino acids in foods. Food Control, 120. Article 107497.

Difco. 1974. Manual of dehydrated cultured media and reagents for microbiological and chemical laboratory producers (9th ed.) Difco Lab. Incorporated, Detroit, Washington, 48201, USA https://www.bd.com/documents/guides/user-guides/DS_CM_Difco-and-BBL-culture-media-manual_UG_EN.pdf

Eldenary, A., M. A. A. Hassan, D. A. M. Amer and A. Bakr. 2023. Assessment of the Quality and Safety of Ras Cheese in Egyptian Delta Governorates *Journal of Sustainable Agricultural and Environmental Sciences*, *2*(2): 77-99. https://jsaes.journals.ekb.eg/article_302697.html

El-Zahar, K. M. 2014. Biogenic amines and microbiological profile of egyptian cheeses. *African Journal of Food Science*, *8*(3): 130–139. https://doi.org/10.5897/ajfs2013.1125

El-Zahar, K. M., A. M. A. El-Zaher and M. F. Ramadan. 2014. Levels of biogenic amines in cheeses and their impact on biochemical and histological parameters in rats. *Journal of the Korean Society for Applied Biological Chemistry*, *57*(1): 73–81. https://doi.org/10.1007/s13765-013-4136-z

Erdag, D., O. Merhan and B. Yildiz. 2019. Biochemical and pharmacological prop erties of biogenic amines. In C. Proestos (Ed.), Biogenic amines (pp. 1e14). London, UK: IntechOpen.

Farag, I., A. Amer and H. Makarem. 2019). .Biogenic Amines Pattern in Ras Cheese During Ripening Period. *Alexandria Journal of Veterinary Sciences*, *61*(1): 93. https://doi.org/10.5455/ajvs.17254

Gardini, F., Y. Özogul, G. Suzzi, G. Tabanelli and F. Özogul. 2016, August 12. Technological Factors Affecting Biogenic Amine Content in Foods: A Review. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.01218

Hammer, B. W. and F. J. Babel. 2002. Dairy Bacteriology (4th ed.) New York: John Wiley & Sons, Inc.; London: Chapman Hall Limited. https://ttngmai.files.wordpress.com/2012/06/dairymicrohandbook.pdf

Helal, A. and D. Tagliazucchi. 2023. Peptidomics Profile, Bioactive Peptides Identification and Biological Activities of Six Different Cheese Varieties. *Biology*, *12*(1):78. https://doi.org/10.3390/biology12010078

Ibrahim, R. A., B. A. Abd El-Salam, T. Alsulami, H. S. Ali, K. Hoppe and A. N. Badr. 2023, September 24. Neoteric Biofilms Applied to Enhance the Safety Characteristics of Ras Cheese during Ripening. *Foods*, *12*(19). 3548. https://doi.org/10.3390/foods12193548

ISO 6887-1:2017: Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions

ISO 6887-5:2020: Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products

Kosikowski, F. V. 1970.Cheese and Fermented Milk Foods (LST ed.) Published by author, Cornell University, New York

Ladero, V., M. Calles, M. Fernandez and M. A. Alvarez. 2010.Toxicological effects of dietary biogenic amines Current Nutrition & Food Science, 6: 145–156. https://www.eurekaselect.com/article/31169

Lück, H. and H. Gavron. 1990. Quality Control in the Dairy Industry in Dairy Microbiology (The Microbiology of Milk Products) (2nd ed., Vol. 2). Elsevier Science Publishers Ltd., UK,

Mehanna1, N.M., M.A.M. Moussa2 and A.A. Abd El-Khair. 2008. Mprovement Of Quality Of Ras Cheese Made From Pasteurized Milk Using A Special Slurry From Ewe's Milk · Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 16(2): 427-436.

Maifreni, M., F. Frigo, I. Bartolomeoli, N. Innocente, M. Biasutti and M. Marino. 2013. IdentificationoftheEnterobacteriaceaeinMontasiocheesean dassessmentoftheir amino acid decarboxylase activity. Journal of Dairy Research, 80.122e127.

Martuscelli, M., L. Esposito and D. Mastrocola. 2021. January 6. Biogenic Amines' Content in Safe and Quality Food. *Foods*, *10*(1), 100. https://doi.org/10.3390/foods10010100

Moneeb, A. H.M., T. Mehany, M.A. Abd-Elmonem , A. A. Tammam , A.A. Zohri , W. I. El-Desoki and T. Esatbeyoglu .2024. Probiotic Lactobacillus strains as protective adjunct cultures against fungal growth and toxin production in Hard cheese. Food Sci., and Tec.,213.

Oliveira, R. S., W. C. Koskinen and F. A. Ferreira. 2001, April 15. Sorption and leaching potential of herbicides on Brazilian soils. *Weed Research*, *41*(2): 97–110. https://doi.org/10.1046/j.1365-3180.2001.00219.x

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.

Saad, B. and R. Tofalo. 2019. Biogenic Amines in Food. *Food Chemistry, Function and Analysis*. https://doi.org/10.1039/9781788015813

Schirone, M., P. Visciano, F. Conte and A. Paparella. 2022. Formation of biogenic amines in the cheese production chain: Favouring and hindering factors. International Dairy Journal, 133, p.105420.

Tawab, G. and A.A. Hofi. 1966. Testing cheese ripening by rapid chemical techniques. Indian J. Dairy Sci., 19:39-58.

Todaro, M., M. Palmeri, C. Cardamone, L. Settanni, I. Mancuso, F. Mazza.2018. Impact of packaging on the microbiological, physicochemical and sensory char acteristics of a "pasta filata" cheese. Food Packaging and Shelf Life, 17,85e90.

Zaccheo, A., E. Palmaccio, M. Venable, I. Locarnini-Sciaroni and S. Parisi. 2017. *Food Hygiene and Applied Food Microbiology in an Anthropological Cross Cultural Perspective*. https://doi.org/10.1007/978-3-319-44975-3

Zdolec, N., T. Bogdanović, K. Severin, V. Dobranić, S. Kazazić, J. Grbavac, J. Pleadin, S. Petričević and M. Kiš. 2022. Biogenic Amine Content in Retailed Cheese Varieties Produced with Commercial Bacterial or Mold Cultures. *Processes*. https://www.mdpi.com/2227-9717/10/1/10

Zhang, Y., J. Zhang, X. Lin, H. Liang, S.Zhang and J.C. 2022. Lactobacillus strains inhibit biogenic amine formation in salted mackerel (Scomberomorus nipho nius). LWT-Food Science and Technology, 155. Article 112851.

## الملخص العربي

## الأمينات الحيوية، الخواص الميكروبيولوجية والكيميائية للجبن الراس

إنعام شكرى، ايمان ابراهيم، نسمة سلامة، سوسو العصفوري، سهام سويلم

تعد الجبن الراس إحدي أنواع الجبن الجافه الرئيسية التي تنتج وتستهلك في مصر وتعرف في مصر بالجبن الرومي في كل المحافظات عدا محافظة الإسكندرية التي تطلق عليها الجبن التركي. وهي تصنع من اللبن البقري أو خليط من اللبن الجاموسي والبقري كما أنها تحتاج إلى فترة أطول فى عملية التسوية ونظراً لطول هذه الفترة وإحتوائها علي نسبة عالية من البروتين والذي ينتج عن تحلله نسبة عالية من الأمينات الحيوية لذا هدفت هذه الدراسة إلى تقييم وجود وكمية الأمينات الحيوية في الجبن الراس، وآثارها على جودة المنتج وتلفه وسلامته و اعتمدت الدراسه علي تجميع ٢٢ عينه من أسواق التجزئة بمدينة كفرالشيخ بهدف التعرف علي مستوي الأمينات الحيوية فيها وقد تم إجراء الأختبارات الكيماوية والميكروبية علي عينات الجبنة . وتوصلت الدراسة لعدد من النتائج الهامة على مستوى الدراسة الكيماوية من أهمها أن متوسط الرطوبة قد بلغ (٣٤,٧٪) والدهن (٢٥,٦٧٪) والبروتين (٣٣,٧١٪) والحموضة (١,٧٧٪) و pH (٥,٦٦) والفورمول (FRI ٤٣,٣٣) والأحماض الدهنية الطيارة (١٦,٤ ml 0.1 NAOH / 100gm cheese). كما أشارت نتائج الدراسة الميكروبية إلى أن العد الكلي البكتيري قد بلغ (٣,٦٨ Log cfu/gm) وعدد البكتريا المحلله للبروتين بلغ ( Log cfu/gm 1.24 ) ولم يلاحظ ظهور بكتيريا الكوليفورم. وبتقدير الأمينات الحيوية في الجبن وُجد أنها تركزت في (الكادافيرين – بتروسين - الهيستامين) وكانت نتائج الدراسة للأمينات الحيوية كالتالي (مجم/كجم) : الكادافيرين (٢٠٣,٦٥ مجم/كجم) والبيوتريسين( ١٢١,٩مجم/كجم) ,الهيستامين (١٠٦,٧٥ مجم/كجم) .

وخلصت الدراسة إلى أن وجود كمية من الامينات الحيوية في الجبن الراس قد يعزى إلي وجود نشاط إنزيمي بكتيري وإلي ظروف التخزين السيئة. وللتقليل من تكوين هذه الأمينات فإن الدراسة توصي بإنتاج الجبن الراس من لبن مبستر وتسويتها في ظروف صحية ونقلها وتخزينها للبيع في ظروف صحيه مناسبة من ناحية الحرارة والرطوبة ونظافة المكان وتطهيره حتي تصل الي المستهلك على النحو المرغوب فيه .

**الكلمات الدالة:** جبن راس، الأمينات الحيوية، التيرامين، الهيستامين، الكادافيرين