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Investigation of Biogenic Amine Levels and Microbiological Activity as Quality Markers in Some Dairy and Fish Products in Food Markets in the Kingdom of Saudi Arabia

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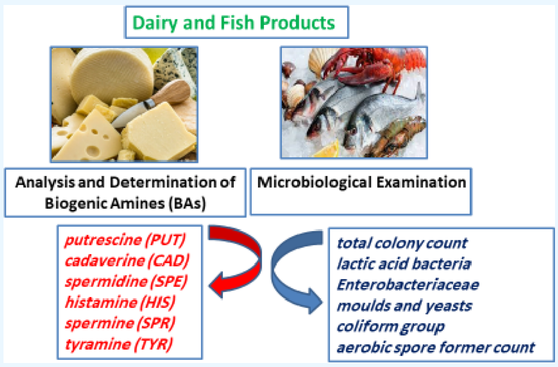

ABSTRACT: This study aimed to verify the presence of biogenic amines (BAs) and evaluate the microbiological activity of some food samples collected from retail stores in the Kingdom of Saudi Arabia. A total of thirty-five dairy and fish products were collected and analyzed for BAs, including putrescine (PUT), cadaverine (CAD), spermidine (SPE), histamine (HIS), spermine (SPR), and tyramine (TYR), as well as for total colony count (TCC), lactic acid bacteria (LAB), Enterobacteriaceae, yeast and mold (Y and M), coliforms, and aerobic sporulation count (ASF). The thin layer chromatography (TLC) method was used in the analytical methodology to identify the BAs. The results showed the presence of BAs in all dairy products, but their concentration did not exceed the maximum permissible limit, which in contrast was established by the Food and Drug Administration (FDA) at 10 mg/100 g. The amounts of BAs in fish products varied significantly. All fish product samples contained levels of BAs below the permissible limit. Results of an independent study also indicated potential toxicity at levels of BAs (>10 mg/100 g) in Egyptian herring. Enterobacteriaceae and the coli group were present in higher concentrations in the Egyptian herring samples, whereas other samples (particularly frozen shrimp) showed increased TCC levels with a higher concentration of histamine-producing bacteria. From a consumer safety perspective, this study also indicated that food samples generally contained acceptable levels of BAs. In conclusion, there is a need to improve and standardize food quality and hygiene practices during production and storage to ensure human safety and prevent HIS formation.

1. INTRODUCTION

One of the main challenges in the food industry is the production of healthy foods free of toxic residues, pesticides, allergens, and microorganisms that can cause diseases. Food toxicity can originate from a variety of sources, including pathogenic microorganisms, viruses, natural toxins, and certain bioactive compounds.[1] Eating foods containing high amounts of amines can have consequences.[2] These effects are significantly exacerbated in individuals afflicted with a range of medical conditions.[3] Biogenic amines (BAs) include putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), spermidine (SPD), and spermine (SPM) and have been linked to a number of disease syndromes with negative effects on human health.[4] BAs originate from the enzymatic activity of decarboxylases, which are found in certain bacterial species, including *Enterobacteriaceae* and *Pseudomonas*.[5] In fermented products, especially cheese, lactobacillus species have been identified as noteworthy contributors to the production of BAs.[6] Furthermore, it has been observed that most BA-producing bacteria are commonly found in raw milk, persist during various stages of the cheese manufacturing process, and may also be present as part of starter cultures or adjunct cultures.[7] Improper storage of food products can lead to the accumulation of BAs. The presence of BAs has been reported in a wide range of food products, including fish and meat products, wine, and beer.[8−11] These BAs are regarded as potential biochemical markers for assessing the quality of raw materials and industrial processes as their accumulation is closely linked to bacterial contamination.[12,13] However, it is worth noting that legally established limits for HIS exist only in fish and fish products, as set by the Food and Drug Administration (FDA) and the European Union. In the human body, BAs can cause headaches, heart palpitations, vomiting, diarrhea, allergies, and hypertensive crises. Detox-

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ification mechanisms for BAs are supported by enzymatic systems, located primarily in the intestine through the action of monoamine oxidase (MAO), diamine oxidase (DAO), and polyamine oxidase (PAO).[14] Elevated consumption of TYR and HIS can overwhelm these enzymatic systems, potentially leading to medical toxicity, including conditions such as hypertension and allergic reactions affecting blood vessels and smooth muscle.[3,15−17] Additionally, impaired DAO activity resulting from factors such as genetic predisposition, gastrointestinal disorders, or the use of DAO inhibitors can result in migraines, skin issues, and gastrointestinal complications. Even minor ingestion of HIS in such cases can lead to a condition known as intolerance, which may not be problematic for individuals in good health.[15,18,19] Although compounds like PUT, CAD, SPD, and SPM themselves do not appear to exert adverse health effects, inhibiting the enzyme systems responsible for their catabolism can reduce the breakdown of TYR and HIS, potentially increasing their toxicity.[11,20] The European Food Safety Authority (EFSA) has recommended the following acceptable values for HIS and TYR intake per meal/person, which are not associated with adverse health effects in healthy people: 50 mg of HIS; 600 mg TYR; 50 mg for third-generation monoamine oxidase inhibitors (MAOIs); and 6 mg for those taking traditional MAOIs.[21]

Foods high in BAs can lead to various forms of food poisoning, including TYR and HIS toxicity. Furthermore, the deleterious effects of HIS and TYR are increased in the presence of other BAs such as CAD and PUT. Remarkably, carcinogenic nitrosamines may also be produced by BAs reacting with nitrate ions (NO_3^-).[1,22−24] Therefore, utilizing BAs as the benchmark for food quality and adhering to quality regulations is a crucial approach to ensuring and guaranteeing food safety.[25,26] Suitability, reliability, and hygiene are all essential facets of food quality.[4]

Many different forms of cheese, including feta, kashkaval, mozzarella, rummy, and mish, are very popular in Saudi Arabia and in many other Middle Eastern countries. Cheese is an important part of many meals, especially those intended for children, as it provides sufficient levels of fat, protein, and nutrients including calcium and magnesium.[27] The most prominent of these countries are Germany, the Netherlands, Norway, Thailand, Cambodia, the Philippines, Japan, China,[28] Turkey,[29] Czech Republic,[30] Spain,[31,32] Italy,[33−35] Croatia,[36] and Egypt.[1] Several food products, such as meat, dairy, and fish products, have been shown to contain significant amounts of BAs in China[37] and Poland.[38]

On the other hand, little information about the presence of BAs in dairy and fishery products from the Kingdom of Saudi Arabia is currently accessible. Therefore, the purpose of the current study is to find out whether dairy and fish products offered for sale in food markets in the Kingdom of Saudi Arabia contain BAs. In addition, research is evaluating the microbial quality of these products.

2. MATERIALS AND METHODS

2.1. Chemicals and Supplies. Crystalline hydrochloride salts of amines (histamine "HIS," putrescine "PUT," cadaverine "CAD," tyramine "TYR," spermidine "SPD," and spermine "SPM") and dansyl chloride (5-dimethylamino naphthalene-1-sulfonyl chloride) were obtained from Merck (Germany). Thin layer chromatography (TLC) plates were coated with silica gel; a 20 × 20 cm aluminum plate (G-60) was purchased from Merck (Germany). All chemicals and solvents were of high analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Food Samples. Thirty-five samples of dairy and fishery products were collected from various retail stores and markets in Saudi Arabia between 2021 and 2022. Food samples were examined within the production company's recommended shelf life. The samples were taken in pairs, with one of them kept at −20 °C until it was used to evaluate BAs. In contrast, the other portion of the samples was sent directly and quickly to the laboratory for microbiological analysis while still being refrigerated.

2.3. Microbiological Examination. Samples of dairy and fish products were prepared for microbiological analysis using the International Commission on Microbiological Specifications for Foods (ICMSF)-recommended techniques.[39] 25 g of each sample was cut using disposable sterile forceps and weighed and homogenized in sterile stomacher bags in 225 mL of 0.1% peptone water Himedia (Bio-Base, mechanic, model: BFH-01). At 450 g in peptone buffer (225 mL); 0.1% Himedia (Bio-Base, mechanic, model: BFH-01). Such a homogenate signifies the preparation of a 10-fold decimal serial dilution after a dilution of 101. The samples of dairy products were tested for total colony count (TCC) and aerobic spore former count (ASF) by the American Public Health Association's APHA.[40] According to the ISO15, 214 handbook,[41] Enterobacteriaceae on violet red bile dextrose agar (Merck, Darmstadt, Germany) were incubated at 30 °C for 24 h aerobically, and lactic acid bacteria (LAB) were grown on pH 5.7 Man−Rogosa−Sharpe (MRS) agar for 72 h. Yeast and mold (Y and M) were determined on potato dextrose agar (Merck, Germany) at 21 °C for 7 days. After this time, the developed colonies were counted, and the results were reported as colony-forming unit cfu/ml or gram of sample according to the American Public Health Association (APHA).[40] Whole histamine-forming bacteria (HFB) were assayed based upon a procedure represented via[40] utilizing whole HFB separation agar.[41] The most probable number (MPN) method is used for the quantitative estimation of coliform.[42] To prepare the fish and dairy product samples for microbiological examinations, the manner according to recommendations of the American Public Health Association (APHA)[43] was used.

2.4. Analysis and Determination of BAs. *2.4.1. Extraction of BAs.* BAs were separated and measured utilizing the method that is outlined in 44. 25 g of each frozen and thawed sample were homogenized, and 125 mL of 5% trichloroacetic acid (TCA) was added. The mixture was then mixed for 3 min in a mixer. Whatman No. 1 was used to filter the homogenized sample. The 10 mL filtrate was put into a glass tube along with 4 g of NaCl and 1 mL of 50% NaOH. It was then sacked and extracted using 3 × 5.0 mL of *n*-butanol: chloroform (1:1 v/v). The upper layers were then moved to a 100 mL separating funnel with 15 mL of *n*-heptane and extracted using 3 × 1.0 mL of HCl (0.2N). The HCl layers were then collected in a glass tube and dried at 95 °C with the help of a gentle air current.

2.4.2. Dansylamine Formation. After carefully mixing 0.5 mL of saturated sodium bicarbonate ($NaHCO_3$) solution into the residue of the samples and working standards, 1.0 mL of dansyl chloride solution (0.5% in acetone) was added and thoroughly mixed using a vortex mixer. For 45 min, the mixture was incubated at 55 °C. 10 ml of distilled water was added and thoroughly mixed with the dansylated mixture.

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Table 1. Levels of BAs and Polyamine in Dairy Products Collected from the Saudi Food Market

			biogenic amine (BA) content(mg/kg)						
sample no.			PUT[a]	CAD[a]	SPE[a]	HIS[a]	SPR[a]	TYR[a]	total BAs
1	Kashkaval cheese	code 1	14.64	00.94	00.06	05.02	ND	02.37	23.03
2	Kashkaval cheese	code 2	03.35	00.15	ND	01.74	ND	00.76	06.00
3	Feta cheese	code 3	00.26	ND	ND	01.62	ND	00.27	02.15
4	Feta cheese	ode 4	00.47	ND	ND	02.11	ND	00.23	02.81
5	Feta cheese	code 5	00.39	ND[b]	ND	03.12	ND	00.41	03.92
6	Istanbul cheese	code 6	01.97	06.33	00.89	04.46	01.65	01.80	17.10
7	Ras cheese	code 7	01.95	06.30	00.69	04.36	01.19	01.97	16.64
8	Edam cheese	code 8	ND[b]	00.68	ND	ND	ND	ND	00.68
9	Edam cheese	code 9	ND	00.72	ND	ND	ND	ND	00.72
10	Istanbul	code 10	01.98	06.52	00.86	05.15	01.69	01.77	17.97
11	Emminil cheese	code 11	ND	00.44	ND	ND	0.43−8	ND	00.87
12	Emminil cheese	code 12	00.29	00.53	ND	ND	0.58−4	ND	01.40
13	Roquefort cheese	code 13	13.59	42.40	ND	12.88	ND	02.65	71.52
14	Labnah cheese	code 14	ND	00.44	ND	ND	0.43−6	ND	00.87
15	Labnah cheese	code 15	ND	00.46	ND	ND	0.54−5	ND	01.00
16	Roquefort cheese	code 16	00.54	01.04	ND	ND	0.22−7	ND	01.80
17	Goat cheese	code 17	00.35	01.35	00.05	ND	ND	01.12	02.81
mean values			03.31	05.02	00.50	04.49	00.79	01.32	15.43

[a]PUT (putrecine), CAD (cadaverine), SPE (spermadine), HIS (histamine), SPR (spermine), and TYR (tyramine). [b]Not detected (ND)

Three volumes of diethyl ether were used to extract the mixture. The ether layers were carefully evaporated to dryness, and the residue was redissolved in methanol.

2.4.3. Determination of Dansylamines by TLC Densitometer. Chromatographic separation of the investigated dansylamines was carried out using one-dimensional silica gel G-60 TLC plates (20 × 20 cm) from Merck, Darmstadt, Germany. 500 μL of methanol was used to dissolve the dry layer of samples and standards. Dissolved samples and standards (10 μL) were spotted. Chloroform, benzene, and triethyl amine (6:4.5:1, *v*/*v*/*v*) were used to develop the plate. All plates were visualized immediately after drying at room temperature, and the fingerprint profile was photodocumented under 365 nm in a UV light source. Using a CS-9000 dual wavelength flying spot scanning densitometer (SHIMADZU) at a wavelength of 254 nm, the resulting spots were then marked, and the marked areas were counted in the "Microanalysis Centre, Faculty of Science, Cairo University, Egypt." BA concentrations in the examined samples were calculated by using the standard curve for each dansylamine.

3. RESULTS AND DISCUSSION

3.1. The Levels of BAs and Polyamine Contents in Some Dairy Products. The BA levels and polyamine contents of 17 samples of commercial dairy products obtained from the food market Saudi Arabia in the period 2021−2022 are summarized in Table 1 and shown in Figure 1, and according to the data, 12 out of the 17 samples had an average PUT concentration of 3.31 mg/kg.

It is worth noting that there was a difference in the PUT levels for specific products. PUT amounts of 14.64, 13.59, 3.35, 1.97, 1.98, 1.95, 0.54, 0.47, 0.39, and 0.35 mg/kg of the following cheese types were displayed: Egyptian feta cheeses with code Nos. 4 and 5, Goat cheese with code No. 17, Kashkaval cheese with code No. 1, Roquefort cheese with code No. 13, and Istanbul cheese with code Nos. 6 and 10. Strikingly, the results also showed that PUT was not detected (ND) in 5 out of the 17 samples or 29.4% of all dairy samples collected. PUT was not specifically found in the samples of Labnah cheese with code Nos. 14 and 15, the Emminil cheese samples with code No. 11, and the Edam cheese samples with code No. 8. These results emphasize how different PUT contents are in the dairy products that were the subject of this investigation.

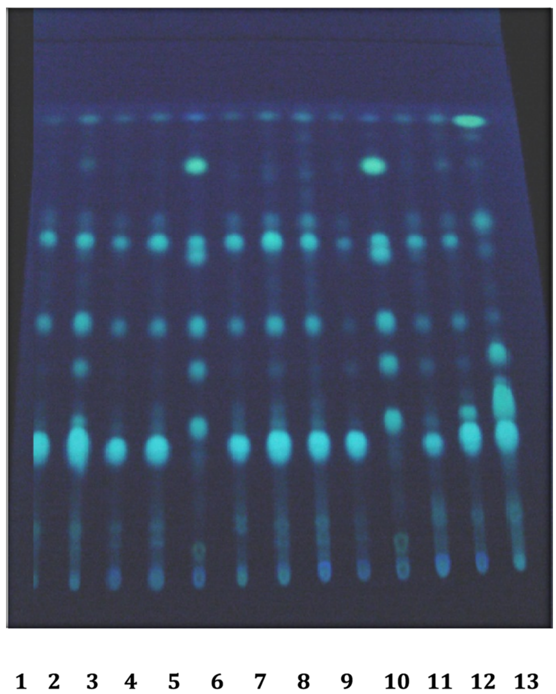

Figure 1. UV illumination at 365 nm of a normal TLC plate developed in a solvent mixture of chloroform, benzene, and triethylamine (6:4.5:1, *v*/*v*/*v*) for BAs in some dairy samples. 1, Kashkaval cheese[1]*; 2, Feta cheese[3]; 3, Istanbul cheese[6];4, Ras cheese[7]; 5, Edam cheese[8]; 6, Istanbul[10];7, Emminil cheese[11];8, Emminil cheese[12]; 9, Roquefort cheese[13];10, Labnah cheese[13]; 11, Labnah cheese[14]; 12, Roquefort cheese[16]; 13, Goat cheese[17] *sample code.

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Table 2. Levels of BAs and Polyamine in Fish Products Collected from the Saudi Food Market

			biogenic amines (BAs) content(mg/kg)						
sample no.	Sample name		PUT[a]	CAD[a]	SPE[a]	HIS[a]	SPR[a]	TYR[a]	total BAs
1	Frozen shrimp	code 1	17.28	10.26	00.93	01.47	ND	00.52	30.46
2	Smoked salmon fillet Scotland	code 2	01.35	00.15	02.62	ND	03.28	ND	07.40
3	Egyptian Fesikh	code 3	02.70	11.76	ND	31.77	ND	02.19	48.42
4	Canned tuna	code4	00.24	01.48	01.78	03.04	02.01	ND	08.55
5	Canned tuna	cde 5	00.28	01.34	01.67	03.11	01.96	01.77	10.13
6	Canned tuna	code 6	00.36	01.12	01.05	04.12	01.89	01.69	10.23
7	Sardines in spicy tomato sauce	code 7	00.62	00.22	01.19	ND	01.16	ND	03.19
8	Milo sardines in vegetable oil	code 8	01.96	06.34	00.88	04.46	01.60	01.80	17.04
9	Sardines Jon in vegetable oils	code 9	01.69	00.90	01.80	ND	01.65	ND	06.04
10	Tuna white meat	code 10	01.44	00.67	01.54	ND	01.34	ND	04.99
11	Tuna white meat	code 11	ND[b]	01.60	02.10	02.56	02.74	ND	09.00
12	Tuna white meat	code 12	ND	01.23	02.13	02.33	02.67	ND	08.36
13	Tuna white meat	code 13	00.76	01.87	02.13	01.93	02.19	ND	08.88
14	Anchovies	code 14	01.23	00.88	02.33	01.09	03.44	ND	08.97
15	White tuna	code 15	00.55	01.41	02.78	02.75	02.37	01.39	11.25
16	Jon west salmon	code 16	01.21	04.89	ND	04.24	ND	ND	10.34
17	Egyptian Herring	code 17	10.33	174.5	10.63	ND	01.83	02.85	200.14
18	Tuna white meat	code 18	ND	00.75	03.87	ND	00.66	ND	05.28
Means			04.41	12.30	02.22	05.24	02.10	01.74	22.71

[a]PUT (putrecine), CAD (cadaverine), SPE (spermadine), HIS (histamine), SPR (spermine), and TYR (tyramine). [b]Not detected (ND)

The concentration of CAD varied greatly, ranging from 0.15 to 42.40 mg/kg with a mean of 5.02 mg/kg. Significantly, samples of Roquefort cheese with code No. 13, Istanbul cheese with code No. 10, Istanbul cheese with code No. 6, Ras cheese with code No. 8, and Goat cheese with code No. 17 had the highest levels of CAD, with values of 42.40, 6.52, 6.33, 6.30, and 1.35 mg/kg, respectively. However, none of the feta cheese samples collected from different markets had any evidence of CAD.

Most samples, ~70.5% (12/17), did not contain SPE, with a mean concentration of 0.50 mg/kg and a range of 0.05 to 0.89 mg/kg. A mean of 4.49 mg/kg for HIS, which ranged from 1.62 to 12.88 mg/kg, was found in 52.94% (9/17) of the dairy product samples.

The Roquefort cheese samples with code No. 13 collected from Al-Qassim had the highest level of HIS and the highest histamine content of any cheese sample (12.88 mg/kg). SPR was measured with a mean of 0.79 mg/kg. The highest value was 1.69 mg/kg in Istanbul cheese (code No. 10), followed by 1.65 mg/kg in Istanbul cheese (code No. 6) and 0.22 mg/kg in Roquefort cheese (code No. 16). Notably, there was no detectable SPR in approximately ~52.9% (9/17) of the samples.

The mean TYR concentration was 1.32 mg/kg and was found in 58.8% (10/17) of all dairy product samples. The common cheese-making bacteria TYR is present. The sample of Roquefort cheese with code No. 13 had the highest TYR concentration (2.65 mg/kg).

It was found that the samples containing the highest contents of BAs were Roquefort cheese (code No. 13), Kashkaval cheese (code No. 1), Istanbul cheese (code No. 10), and Ras cheese (code No. 7), with values of 71.52, 23.34, 17.97, 17.10, and 16.64 mg/kg, respectively. The fact is that these samples stayed below the maximum permissible limits set by the US Food and Drug Adminstration[7] and the Egyptian Organization for Standardization[45,46] for the content of BAs 10 and 20 mg/100 g, respectively. These results are consistent with those of earlier studies[47] that found that the total BA content of Pecorino Del Parco di Migliarino-San Rossore and Formaggio di Fossa cheese was 1578.7 and 2557.7 mg/kg, respectively. Likewise, other research has documented the levels of BAs in a variety of cheese varieties, such as matured Domiati and aged Kareish cheese.[46−53]

3.2. BA Levels and Polyamine in Fishery Products.

Table 2 summarizes and Figure 2 shows BA concentrations in 18 samples of commercial fishery products from the food market in Saudi Arabia in 2021−2022. The information revealed variations in the amounts of BAs in fisheries products. Most fish products had low to moderate concentrations of BAs.

More specifically, the concentrations of all of the BAs (PUT, CAD, SPE, HIS, SPR, and TRP) were less than 10 mg/kg in 58.82% of fish product samples. Furthermore, BA levels below 20 mg/kg were found in 88.24% of all fishery products. These results highlight the BA profile of the fishery products evaluated, which is generally favorable, and most of which fall well within acceptable limits.

PUT and CAD are thought to be important sources of BAs in food products, especially fish production, because they can increase the toxicity of HIS. Table 2 summarizes and Figure 2 illustrates the relatively low concentration of BAs (30.46 mg/kg) in the frozen shrimp sample with code No. 1.

It is worth noting that the Egyptian herring sample, code No. 17, stood out as having the highest BA concentration of 200.14 mg/kg among all of the samples analyzed. The diversity in the composition of these compounds within various fishery products is highlighted by this variation in the BA content. High concentration of BAs (48.42 mg/kg) was administered to the Egyptian Fesikh, code No. 3. In contrast, the Egyptian herring sample, code 17, had the highest level of total BAs (200.14 mg/kg) of any sample examined. The sample of frozen shrimp with code No. 1 had the highest PUT concentration (17.28 mg/kg), followed by the Egyptian herring sample with code No. 17 (10.33 mg/kg). For the remaining samples, PUT concentrations ranged from ND to 0.24 mg/kg, with an average concentration of 4.41 mg/kg for all samples. The CAD

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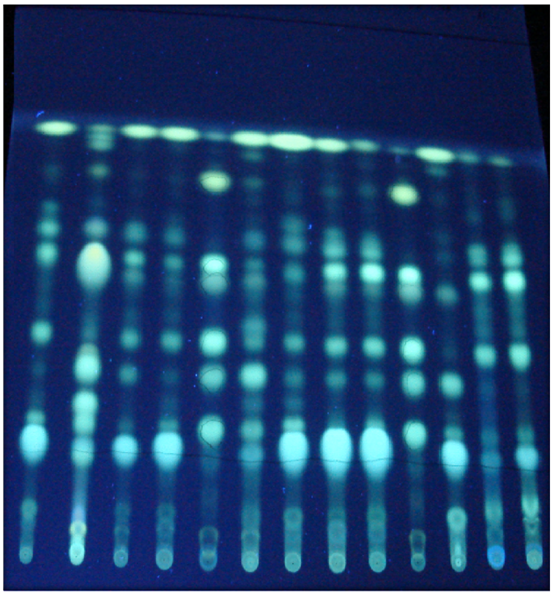

Figure 2. UV illumination at 365 nm of a normal TLC plate developed in a solvent mixture of chloroform, benzene, and triethylamine (6:4.5:1, *v*/*v*/*v*) for BAs in some fish samples. 1, frozen shrimp[1]*;2, smoked salmon fillet Scotland[2]; 3, Egyptian fesikh[3]; 4, canned tuna[4]; 5, sardines in spicy tomato sauce;[7] 6, Milo sardines in vegetable oil;[8] 7, sardines Jon in vegetable oils[9]; 8, tuna white meat[10]; 9, anchovies[14]; 10, white tuna[15]; 11, Jon west salamon[16]; 12, Egyptian herring[17]; 13, tuna white meat. [18] *sample code.

content was the highest in Egyptian herring, reaching 174.50 mg/kg.

The CAD concentration of the frozen shrimp sample (Code No. 1) was 10.26 mg/kg, while the CAD concentration of Milo sardines in the vegetable oil sample (Code No. 8) was 6.34 mg/kg. The mean CAD value for all samples was 12.30 mg/kg, with other sample CAD concentrations ranging from 0.15 to 4.89 mg/kg.

These findings highlight the variations in CAD and PUT concentrations seen in different samples of fishery products.

The mean concentration of SPE in fish products was found to be 2.22 mg/kg. The Egyptian herring sample recorded the highest SPE (10.63 mg/kg). As for the other samples, the concentrations ranged between 0.88 and 3.87 mg/kg. The average SPR in the samples was 2.1 mg/kg, and the highest concentration in the anchovy sample (code 14) was 3.44 mg/kg. SPR values for the remaining samples that tested positive ranged from 1.16 to 3.28 mg/kg.

TYR was found in the examined samples at a mean concentration of 1.74 mg/kg. Among all of the samples, the Egyptian herring samples had the highest TYR concentration (2.85 mg/kg). As for the other samples, the TYR concentrations ranged from 0.52 to 2.19 mg/kg. Crucially, none of the TYR levels in the samples examined exceeded the suggested limit of 100 mg/kg.[54] It is important to note that some samples, such as Egyptian herring, exceeded the 50 ppm histamine limits set by the FDA.

These results show differences in SPD, SPM, and TYR levels among different fish product samples. Most of these samples comply with regulatory limits, with the exception of HIS levels in some samples. The results of this investigation are consistent with the conclusions of preivous studies. According to Santos,[55] salted fish products showed elevated levels of BAs, and this is consistent with what we found in this study.

Furthermore, CAD and PUT were found to be important quality indicators for fish products, especially in vacuum-packed pressurized squid mantle; these results are consistent with previous studies[55,56] that found that canned Brazilian sardines had HIS concentrations ranging from 1.2 to 36 mg/kg. Their findings were similar to ours. HIS values of canned sardines and mackerel imports were found to be higher than 200 mg/kg by Soaress and Gloria,[57] which is consistent with the higher HIS levels found in some study samples.

Zarei et al.[58] found that 57.5% of canned tuna samples had HIS concentrations ranging from 0.12 to 648.20 mg/kg, with a mean of 64.61 mg/kg. These studies collectively emphasize the variability in HIS levels across different fishery products and geographical regions, highlighting the importance of monitoring and quality control in the seafood industry.

When measured the amounts of HIS in sixty-three canned fish samples,[59] it was discovered that seven of the tuna samples had HIS contents higher than 100 mg/kg. Forty-four samples' HIS levels matched the FDA's toxic level of 50 mg/100 g. When compared to other fish products, canned tuna and sardines were found to have significantly higher HIS values.

Fish HIS levels were classified as follows by Shalaby,[1] based on toxicological studies: < 5 mg/100 g (safe for consumption), 5−20 mg/100 g (potentially toxic), 20−100 mg/100 g (likely toxic), and >100 mg/100 g (toxic and unsafe for human consumption).

The adoption of hazard analysis critical control point systems (HACCPs) by different fish processing facilities may be the reason for the comparatively low HIS concentrations found in most fish products in this study.

Antoine et al.[60] suggested that the simultaneous presence of CAD and PUT may enhance HIS toxicity due to the suppression of HIS metabolizing enzymes diamine oxidase and HIS methyltransferase.

Unfavorable levels of BAs can also occur in canned fish because of the handling of low-quality raw fish and improper processing techniques. Additionally, when handling and thawing fish, bacterial populations may grow. Furthermore, as mentioned in earlier research, the environment and equipment used in processing plants may contaminate fish with bacteria that produce HIS.[61−63]

Additionally, HIS decomposition was noted by Sato et al.[64] during storage of common mackerel and by Al-Busaidi et al.[65] during storage of longtail tuna. These reports were linked to the presence of bacteria that break down HIS.

3.3. Microbiological Characteristics of Dairy Products. Table 3 summarizes the microbiological characteristics of the different dairy products that were sold in the food market of Saudi Arabia in 2020 and 2021. The samples' mean total colony count (TCC) was 6.04 ± 1.12 ($\log_{10}$ cfu/g), according to the results. TCC values (8.32 ± 5.02 and 8.02 ± 3.43 $\log_{10}$ cfu/g, respectively) were highest for Labnah sample (code No. 15) and Roquefort cheese sample (code No. 17). Meanwhile, Roquefort cheese (code No. 13) and Istanbul cheese (code No. 6) also displayed high TCC values (7.98 ± 2.12 and 7.84 ± 3.45 $\log_{10}$ cfu/g, respectively). In contrast, Emminil cheese (code No. 11) had the lowest TCC among the samples.

Lactic acid bacteria (LAB) were predominant in all the dairy products, with an average count of 9.17 ± 1.15 ($\log_{10}$ cfu/g). Labnah sample code No. 15, Roquefort cheese (code No. 13), and Istanbul cheese (code No. 10) had the highest LAB counts

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Table 3. Microbial Traits of Dairy and Dairy Products Found in the Saudi Market

				microbial properties of some dairy products					
sample no.	sample name			TCC[a] (10^6 cfu/g)[b]	LAB[a] (10^6 cfu/g)	Enterobac[a] ($\log^a_{10}$ cfu/g)	Y&M[a](10^{a3} cfu/g)	coli group[a] (10^2 cfu/g)	ASF[a] ($\log_{10}$ cfu/g)
1	Kashkaval cheese		code 1	5.71 ± 1.42	10.27 ± 1.09	2.33 ± 0.49	3.67 ± 0.11	3.77 ± 0.22	1.00 x10^4
2	Kashkaval cheese		code 2	6.72 ± 1.34	9.99 ± 2.16	2.11 ± 0.26	4.74 ± $^{0;22}$	3.81 ± 0.21	1.20 x10^4
3	Feta cheese		code 3	7.76 ± 2.39	9.27 ± 3.20	2.98 ± 1.01	4.62 ± 0.21	2.18 ± 0.24	1.30 x10^4
4	Feta cheese		code4	6.74 ± 2.19	8.99 ± 3.26	2.16 ± 0.72	3.82. ± 0.24	2.89 ± 0.23	1.10 × 10^4
5	Feta cheese		code 5	5.93 ± 1.38	9.94 ± 2.87	2.57 ± 0.21	4.13. ± 27	3.0 ± 0.31	1.10 × 10^4
6	Istanbul cheese	from Riyadh	code 6	7.84 ± 3.45	10.32 ± 2.99	3.09 ± 0.29	4.87 ± 0.31	3.24 ± 28	1.40 × 10^4
7	Ras cheese	from Riyadh	code 7	5.74 ± 1.80	9.65 ± 0.13	3.26 ± 38	5.12 ± 0.22	4.0 ± 0.39	1.60 × 10^4
8	Edam cheese	from Riyadh	code 8	4.31 ± 1.98	8.99 ± 1.87	2.41 ± 0.21	4.72 ± 0.22	4.18 ± 0.28	1.30 × 10^4
9	Edam cheese	from Qassum	code 9	5.02 ± 2.03	9.23 ± 3.34	2.00 ± 0.24	4.82 ± 0.21	3.99 ± 0.23	1.20 × 10^4
10	Istanbul	from Qassum	code 10	7.13 ± 1.92	10.36 ± 0.56	2.09 ± 0.31	4.99 ± 0.27	4.02 ± 0.20	1.77 × 10^4
11	Emminil cheese	from Riyadh	code 11	4.53 ± 2.02	8.68 ± 2.78	2.33 ± 0.34	3.98 ± 0.25	4.22 ± 0.25	1.50 × 10^4
12	Emminil cheese	from Riyadh	code 12	3.97 ± 1.76	9.12 ± 2.45	2.26 ± 0.26	4.01 ± 0.18	4.84 ± 0.28	1.40 × 10^4
13	Roquefort cheese	from Qassium	code 13	7.98 ± 2.12	10.33 ± 1.93	3.11 ± 0.23	4.00 ± 0.09	4.21 ± 0.24	1.36 × 10^4
14	Labnah cheese	from Qassum	code 14	7.28 ± 1.67	10.99 ± 3.53	2.34 ± 0.31	2.34 ± 0.23	2.99 ± 0.35	1.00 × 10^4
15	Labnah cheese	from Riyadh	code 15	8.32 ± 5.02	10.82 ± 2.77	2.01 ± 0.26	2.65 ± 0.35	2.27 ± 0.26	1.01 × 10^4
16	Roquefort cheese	from Riyadh	code 16	8.02 ± 3.43	9.36 ± 0.11	3.34 ± 0.29	4.18 ± 0.37	3.22 ± 0.38	1.48 × 10^4
17	Goat cheese	from Qassium	code 17	6.02 ± 2.14	8.90 ± 0.78	3.88 ± 0.42	4.34 ± 0.22	2.19 ± 0.38	1.12 × 10^4
Means				6.04 ± 1.12	9.17 ± 1.15	2.45 ± 0.44	3.94 ± 1.17	3.27 ± 1.06	1.15 ± 1.09

[a]TCC (total colony count), LAB (lactic acid bacteria), Enterobac (Enterobacteriaceae), Y and M (moulds and yeasts), Coli group (coliform group), ASF (aerobic spore former count). [b]cfu (colony-forming unit).

(10.99 ± 3.53, 10.82 ± 2.77, 10.36 ± 5.56, and 10.32 ± 2.99 $\log_{10}$ cfu/g, respectively), while Emminil cheese (code No. 11) had the lowest LAB count.

The data also revealed the presence of Enterobacteriaceae in the samples, with Roquefort cheese sample (code No. 16), Labnah sample (code No. 15), and Ras cheese (code No. 7) having the highest counts (3.88 ± 0.42, 3.34 ± 0.29, and 3.26 ± 0.38 $\log_{10}$ cfu/g, respectively). The mean Enterobacteriaceae count across all samples was 2.45 ± 0.44 $\log_{10}$ cfu/g.

Yeasts and molds (Y and M) were detected in the samples, with Labnah sample (code No. 15) displaying the lowest count (2.34 ± 0.23 × 10^3 cfu/g). The mean count for Y and M in all the dairy products was 3.94 ± 1.17 × 10^3 cfu/g. Istanbul cheese (code No. 10), Istanbul cheese (code No. 6), and Edam cheese (code No. 9) had the highest counts (4.99 ± 0.27, 4.87 ± 0.31, and 4.82 ± 0.21 × 10^3 cfu/g, respectively). Additionally, coli groups were detected in the dairy products, with a mean count of 3.27 ± 1.06 × 10^2 cfu/g. Istanbul

cheese (code No. 10) had the highest coli group count (1.77 × 10^4 cfu/g), while Kashkaval cheese (code No. 1) had the lowest count (1.0 × 10^4 cfu/g).

Aerobic spore former (ASF) count was also observed, with Istanbul cheese (code No. 10) having the highest count (1.77 × 10^4 $\log_{10}$ cfu/g) and Kashkaval cheese (code No. 1) having the lowest count (1.0 × 10^4 $\log_{10}$ cfu/g). The presence of microorganisms such as coliforms, Enterobacteriaceae sp., and *Lactococcus* sp. in dairy products may indicate the quality of the raw materials used and the hygiene conditions during production. Laboratories are considered the most significant microorganisms in dairy manufacturing, and their abundance may vary depending on the type of product. Polluting microorganisms, including Enterobacter, *E. coli*, and Enterococci, can suggest subpar material quality and improper milk sterilization. All of these microorganisms possess significant amino genic potential and may influence the content of BAs, particularly TYR, in cheese.

These findings are in line with previous studies,[66,67] which reported the presence of yeasts, molds, coliforms, and high levels of BAs in certain cheese samples, indicating unsanitary conditions during production and storage.

3.4. Microbiological Properties of Some Fishery Products. Table 4 summarizes the results of the microbiological properties of some commercial fish products in the food market in the Kingdom of Saudi Arabia during the years 2020–2021. The data indicated that anchovy sample (code No. 14) had the highest TCC, followed by the Egyptian herring sample (code No. 17) and frozen shrimp sample (code No. 1) recorded (8.98 ± 1.23, 8.57 ± 3.33 and 8.38 ± 5.1110^6 cfu/g, respectively). The average TCC value was 6.37 ± 1.25 (10^6 cfu/g). The sample with the lowest TCC count was tuna (code No. 11).

Jeya Shaklla et al.[68] found that processed fish products with a "salted, dried, and smoked" flavor had a high microbial capacity, ranging from 102 to 105 cfu/g, additionally; Table 4

Table 4. Microbial traits of fishery products found in the Saudi market

sample no.	sample name		microbial properties of some fish products				
			TCC[a] (10^6 cfu/g)[b]	Coli group[a] (10^2cfu/g)	*Enterobac*[a] (log_{10}cfu/g)	YPC34&M[a] (10^3cfu/g)	HFB[a] (log_{10}cfu/g)
1	frozen shrimp	code 1	8.38 ± 5.11	2.01 ± 0.17	3.34 ± 2.11	3.47 ± 0.15	4.12 ± 1.52
2	smoked salmon fillet Scotland	code 2	7.16 ± 2.33	2.091 ± 0.22	2.62 ± 4.02	1.50 ± 0.210	2.14 ± 1.70
3	Egyptian fesikh	code 3	7.62 ± 2.54	2.03 ± 0.34	2.49 ± 4.00	3.58 ± 0.22	2.19 ± 1.42
4	canned tuna	code4	5.23 ± 2.11	2.80 ± 2.01	2.98 ± 1.78	2.51 ± 0.14	1.22 ± 0.37
5	canned tuna	code 5	5.88 ± 0.28	1.50 ± 2.08	3.01 ± 1.67	2.29 ± 0.19	1.77 ± 1.04
6	canned tuna	code 6	6.01 ± 0.36	1.89 ± 2.28	2.90 ± 1.05	1.67 ± 0.21	1.69 ± 0.78
7	sardines in spicy tomato sauce	code 7	5.70 ± 2.16	2.23 ± 0.34	2.19 ± 4.9	1.22 ± 0.16	1.34 ± 0.55
8	milo sardines in vegetable oil	code 8	6.43 ± 2.76	2.12 ± 0.86	2.39 ± 4.50	2.30 ± 1.20	1.88 ± 1.03
9	sardines Jon in vegetable oils	code 9	7.18 ± 2.56	2.12 ± 1.44	4.99 ± 3.87	3.20 ± 0.89	1.77 ± 0.88
10	tuna white meat	code 10	5.89 ± 1.44	1.34 ± 0.88	2.80 ± 1.54	2.80 ± 0.26	1.20 ± 0.55
11	tuna white meat	code 11	4.90 ± 1.79	1.50 ± 0.44	2.10 ± 1.77	1.20 ± 0.22	1.34 ± 25
12	tuna white meat	code 12	4.81 ± 1.38	2.60 ± 1.12	2.13 ± 1.40	2.33 ± 0.26	1.62 ± 1.03
13	tuna white meat	code 13	5.80 ± 2.44	2.19 ± 0.87	2.13 ± 0.80	1.93 ± 0.27	2.03 ± 1.02
14	anchovies	code 14	8.98 ± 1.23	1.40 ± 0.68	2.99 ± 3.53	1.39 ± 0.19	2.64 ± 0.88
15	white tuna	code 15	6.01 ± 0.55	2.37 ± 1.20	2.78 ± 2.01	2.75 ± 0.34	1.39 ± 0.78
16	Jon west salamon	code 16	5.22 ± 2.66	ND[c]	2.0 ± 0.52	2.17 ± 0.17	2.01 ± 1.04
17	Egyptian herring	code 17	8.57 ± 3.33	2.83 ± 1.45	4.96 ± 4.5	2.83 ± 0.96	2.85 ± 1.02
18	tuna white meat	code 18	4.99 ± 1.80	2.01 ± 0.34	2.0 ± 0.87	2.23 ± 0.28	2.40 ± 1.02
Means			6.37 ± 1.25	1.94 ± 0.89	2.82 ± 1.02	2.12 ± 1.11	1.97 ± 0.23

[a]TCC (total colony count), Enterobac (Enterobacteriaceae), Y and M (moulds and yeasts), Coli group (coliform group), HFB (histamine forming bacteria). [b]cfu (colony-forming unit). [c]Not detected (ND).

summarizes the levels of *E. coli*. The mean value was (1.94 ± 0.89), the lowest value was (1.34 ± 0.88) in the tuna sample (code No. 10), and the highest value was (2.80×10^2cfu/g) in the Egyptian herring (code No.17). The mean value of Enterococci in fish products was 2.82 log_{10} cfu/g; with a range of 2.0−4.96 (log_{10} cfu/g). According to,[69] Enterococci can affect the growth process due to their lipolytic and proteolytic activity as well as their ability to cause acid production when they attach to certain Lactococci. Cheese, fish, and wine are made from phenyl ethyl amine, which is decarboxylated by Enterococci tyrosine decarboxylase.[70] Table 4 summarizes that the mean amount of Y and M found in selected fish products sold in the food market of the Saudi Arabia in 2021−2022 ranged from 1.22 to 3.47 log_{10} cfu/g.

The minimum value of 1.22 log_{10} cfu/g was recorded for sardines in spicy sample (code No. 7), and the maximum value of 3.47 log_{10} cfu/g was recorded for Egyptian Feseikh sample (code No. 3). The average number of Y and M was 2.12 ± 1.11 (log_{10} cfu/g). Similar findings were made by Edris et al.,[70] who discovered that 80% of the 25 imported frozen basal samples that were inspected had a mean value of $3.0 \text{x} 10^3 \pm 2.1 \times 10^2$ cfu/g of mold contamination. Furthermore, it was suggested that 64% of the 25 imported frozen Basa samples that were examined had positive yeast counts, with a mean total yeast count of $5.22 \times 10^4 \pm 1.80 \times 10^4$ cfu/g. Histamine-forming bacteria (HFB) were also counted in fishery products; in tuna white meat (code No. 10), the mean value was 1.97 log_{10} cfu/g, and the lowest and maximum values were 1.20 and 4.12 log_{10} cfu/g, respectively.

Comparable outcomes were found in sardine samples (*Sardinella gibbose*), European sardine (*Sardinella pilchardus*), and Atlantic sardine (*Trachurus trachurus*) studies conducted by Korashy et al.[71] The HPB range for fresh sardine (*Sardinella melanostic*), saury (*Coloabis saira*), and mackerel (*Scomber japonicas*) in Japan was reported by Okuzumi et al.,[72] to be ($1.1 \times 10^4 - 3.0 \times 10^4$, $5.7 \times 10^3 - 2.1 \times 10^5$, and $1.0{-}1.0 \times 10^2$ cfu/g). Our results were lower than theirs.

Additionally, it was observed that the HPBC in mackerel fish in Spain was lower than the levels found in our work (3.1×10^2 cfu/g);[73] the higher HFB found in this work may have been caused by handling practices that were not hygienic during handling and cross-contamination from the surrounding environment. The obtained results were not in agreement with the findings published by Alkuraieef et al.,[74] who reported that improper sanitation practices and inappropriate conditions were present during the fish production and handling processes in the Kingdom of Saudi Arabia and that chemical and microbial contamination in some fish samples exceeded the recommended permissible levels.

Based on the data obtained, inferences concerning the connections among BAs, microbial involvement, bacterial growth, raw material quality, and subpar hygienic quality during production, storage, and handling could be made. Previous studies[75,76] provided evidence in favor of these conclusions. The variances in meat products' BAs and BA contents can be ascribed to the distinct manufacturing processes employed for each product.[77] Overall, the BA content varied significantly between yield types and even within the same type of produce. Numerous variables, such as the type and quantity of microflora, chemo-physical characteristics, the hygienic processing method, the availability of precursors, the amount of meat used, the kinds of ingredients added, and the quality of the raw material, influence these changes.[78,79] Food is the main source of amino-positive bacteria, which are important in the development of BAs.[8]

Raw fresh materials are frequently handled incorrectly, which leads to the formation of BAs. Quality and safety control of food and feed must be implemented preventively from the point of harvest (food) or animal slaughter (feed) to the point of sale to stop the formation of BAs.[20] The main strategy for preventing BA risk should be based on food and feed quality and food safety management, which is based on hazard analysis and critical control points. GMP (good manufacturing practices), GHP (good hygiene practices), and appropriate cleaning and disinfection protocols should all be meticulously followed, starting with primary production in the HACCP process.

4. CONCLUSION

Microbial quality, measurement, identification, and quantification of BAs in food samples are of great importance as BAs are indicators of food quality and safety. Briefly, the BA profiles of thirty-five samples of dairy and fish products were analyzed, and correlations between microbes and BAs were examined. The results showed that all dairy products received results for the presence of BAs but exceeded the maximum permissible limit for BA levels (10 mg/100 g) set by the FDA. BA levels in all of the fish product samples analyzed were below the recommended levels (100 mg/kg). These levels are probably toxic because they exceed international, FDA, and EU regulations as well as an independent study's findings of 100 mg/kg in Egyptian herring. Significant levels of BAs were detected within the acceptable limits (20 mg/kg) recommended by the Egyptian Organization for Standardization and Quality Control (EOS2010) indicated that this was within a safe range. There were differences in the TCC and HPB levels among the samples. Enterobacteriaceae, *E. coli*, and Y and M groups had higher values. This investigation demonstrated that the BA levels in the food samples were appropriate for the consumer environment. These results indicate that the majority of dairy and fish samples have total BA levels within acceptable dosage ranges and toxicity values, as well as that BAs can be assessed and controlled within nutrient yields. However, it also highlights how important it is for safety. The Kingdom of Saudi Arabia oversees the quality of food samples.

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Notes

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