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Estimation of Formatting Biogenetic Amines Concentration in Fresh and Processed Sardine Fish Products During Different Storage Conditions

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Abstract: FreshSardine fish was preserved with various methods such as; cooling, freezing, salting and canning for estimating of biogenetic amines concentrationlike; histamine, cadaverine and putrescine during differentstorage conditions and its relation with bacterial load exist in samples. Also, determination of pH values and other chemical analysis Total Volatile Base-Nitrogen (TVN), Thiobarbituric Acid (TBA) and Trimethylamine (TMA).Mean values of Biogenetic amines content in cooled sardine fish at zero time, after 4, 7, 10 and 14 days were 1, 2, 53, 79, 130 for histamine; 9, 37, 66, 92, 114 for cadaverine and 8, 13, 24,46,83 for putrescine respectively; frozen sardine fish at zero time, after 2, 4, 6 and 8 weeks were 1,1, 13, 19, 26 for histamine; 9, 14, 29, 56, 85for cadaverine and 8, 9, 12, 11, 14 for putrescine respectively; salted sardine fish at zero time, after 1,2,3 and 4 months were 1, 57, 110, 260, 760 for histamine; 9, 32, 72,143, 360 for cadaverine and 8, 48, 91, 180, 420 for putrescine respectively and canned sardine fish at zero time, after 1,2,3 and 4 months were 1, 5.8, 10, 12, 24 for histamine; 9, 3.2, 5.7, 7.8,13 for cadaverine and 8,3.7, 4.3, 6.1, 8.0 for putrescine respectively. The bacterial load was 5 x 10 in fresh Sardine fish samples and reached to 9.9 x 105; 2 x 102; 2.2 x 107 and 5.5 x 10 in cooling, freezing, salting and canning Sardine fish products, respectively. pH values and other chemical analysis like; T.V.N, T.B.A and T.M.A were 6.58, 11.2, 0.70 and 3.84 in fresh Sardine fish at zero time; 7.73, 42.0, 1.24 and 29.0 in cooled; 6.8, 19.9, 0.53 and 4.78 in frozen; 4.58, 47.2, 1.30 and 32.5 in salted and 6.82, 20.5, 0.74 and 4.11in canned Sardine fish products.

Key words: Biogenic Amines · Bacterial Load · Different Storage Durations · Sardine Fish Products

INTRODUCTION

Fish and fishery products contain high quality protein and other necessary nutrients; they are low in saturated fatty acids and contain high content of unsaturated fatty acids [1]. Fish meal could be a major protein source in food diets. There are essential amino and fatty acids that are present in fish meal but not present in tissue from plants or animals [2]. A well-balanced regime that includes a variety of fish and fish products can contribute to heart health and children's proper growth [3].

Biogenic amines (BA) are organic, basic, nitrogenous compounds of low molecular weight, mainly formed by the decarboxylation of amino acids and with biological activity. According to their chemical structure, they can be classified as heterocyclic (histamine and tryptamine), aliphatic (putrescine and cadaverine) or aromatic (tyramine and phenylethylamine). BAs are naturally occurring in animals and humans. They are involved in natural biological processes such as synaptic transmission, blood pressure control, allergic response and cellular growth control. Nonetheless, BA may be hazardous to human health if their levels in foods or beverages reach a critical threshold [4].

The intestinal tract of humans contains the enzymes diamine oxidase (DAO) and histamine- N-methyl transferase (HMT), which convert histamine to harmless degradation products. However, for large doses of histamine, the capacity of DAO and HMT to detoxify histamine is limited, resulting in toxic effects as histamine enters the bloodstream [5]. On the other hand, putrescine and cadaverine inhibit intestinal enzymes like; diamine oxidase (DMO) and histamine-N-methyltransferase (HMT)

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which metabolize histamine, resulting in an increase of histamine toxicity [6]. Symptoms of toxicity include vomiting, diarrhoea, abdominal cramps, perspiration, flushing, headaches and burning sensations in the mouth [7].

Mean values for histamine content (µg/100g) were 117.6, 110.8, 3.3, 2 &1.74 for feseikh, molouha, smoked herring, frozen fish fillet and frozen fish fingers respectively, meanwhile the mean values for cadaverine content were 803, 921.3, 895, 247&196.4 and 130.3, 208.4, 33, 25 & 22 for putrescine content respectively. Putrescine and cadaverine contents were inversely correlated with pH. Such results can proposed that cadaverine and putrescine are the most objective quality indicators among fish products [8].

The results showed that the free amino acid and biogenic amines content of Feseekh fish high and low salt and other fish products increased significantly during long time of ripening and storage. So it could be concluded that salted-fermented fish (Feseekh, sardine and meloha) can be consumed without any health risks between 20 and 40 days, but it could be hazardous after 60 days, due to the increased free amino acids and biogenic amine content [9].

Commercial fish sauce made from anchovy was found to contain histamine ranging from 10 to 100 mg/100 ml [10]. The maximum allowable histamine in fish sauce imposed by the Canadian Food Inspection Agency is 20 mg/100g [11]. Whereas, high levels of histamine (200.7 mg/100 g), cadaverine (86.3 mg/100 g), tyramine (27.3 mg/100 g) and putrescine (26.0 mg/100 g) were found in the anchovy sample. Putrescine and cadaverine have been reported as potentiates for histamine toxicity[12].Also, histamine at a concentration of 500 mg/kg to be hazardous for human health [13]..

Biogenic amines (BAs), namely putrescine (PUT), cadaverine (CAD), spermidine (SPD), spermine (SPM), histamine (HIM), tyramine (TYM) and tryptamine (TRM) are organic bases that occur not only in fish and fish products, but also in various other foods of proteinaceous origin. Accumulation of BAs in fish flesh is associated with continuing spoilage [14]. The reasons for amine determination in fish are twofold. The first is their potential toxicity; the second is the possibility of using them as food quality indicators [15].

The formation of high levels of histamine in fish products was directlycorrelated with the level of microorganisms present in the product, due tobacterialhistidine decarboxylase action on histidine [16]. High histamine concentrations in cured fish like salted fish could bedue to poor quality of raw material, improper handling or other changesduring storage [17]. Other parameters (pH, water activity, NaCl concentration, additives) may influence the variation of macrobiotic composition and lead to the differences in BAs content [18].

Possibility of regulation of histamine a accumulation in salted and fermented fish products by the addition of halophilic AB, like starter culture, isolated from nukazuke (salted and fermented fish with rice bran) [19]. In a total of 200 isolates from nukazuke fish,13strains produced histamine in histidine containing broth (0.5%) at levels more than 200µg/ml, whereas130 isolates produced no histamine. Many BAs have been found in fish, but only histamine, cadaverine and putrescine have been identified as significant concerns with regard to fish safety and quality [20]. Despite the widely accepted association between histamine and scombroid food poisoning, histamine alone appears to be in sufficient to cause toxicity and putrescine and cadaverine have been suggested to potentiate its toxic activity by inhibiting the intestinal histamine-metabolizing enzymes, diamine oxidase and histamine N-methyl transferees [21].

During processing of fish (butchering, cleaning, brining, salting, smoking, drying, fermenting, pickling, mixing, stuffing, packing, labeling and staging), it is recommended that it is not exposed to ambient temperatures above 4.4°C for more than 12 h cumulatively, if it has been previously frozen or heat processed sufficiently to destroy histamine forming bacteria, or for more than 4 h in the other case [22].

The aim of this study was to investigate the effect of different storage durations on the formation of biogenic amines in fresh, cooled, frozen and processed (salted and canned) Sardine fish products and relationship them with bacterial load exit.

MATERIALS AND METHODS

Samples: Fresh Sardine fish sardines (*Sardinella*spp.) was purchased from market in Egypt and put in ice box for preventing spoilage till divided into to three groups; the first group was fresh for analysis at zero time without storage, second group was preserved with two methods; cooling at 4°C for 14 days and frozen at 0°C for 2 months and the last group processed by two methods; salting and canning at room temperature for 4 months.

Methods:

Determination of Biogenic Amines: Biogenic amine analysis (chemical analysis):

Standard Solutions: Stock standard solution was prepared by dissolving standard accurately 0.1 g of Putrescine, Cadaverine and Histamine (Sigma) in 10 ml of 5% trichloroacetic acid (TCA) solution. The working standard solutions were prepared by diluting of 1 ml of each stock standard solution in 10 ml of distilled water.

Biogenic Amine Analysis:

Extraction: The amines were extracted following the method of [23]. 50 g of fresh fish lateral muscle of anterior-dorsal half of each whole fish were homogenized in 75 ml of 5% trichloroacetic acid solution (TCA) for 2 min and then, centrifuged at 4000 rpm for 10 min. The supernatant solution was recovered and filtered through Whatman No. 41 and into 250 ml flask, the residual material in the test tube, return to the homogenizer and this procedure was repeated for triplicate. in the next stage, 10 ml of the extracted solution with 4g NaCl, 1 ml NaOH (50%) and 5ml chloro form- butanol (1+1) mixed in the test tube and, shaked for 2 min, then, centrifuged at 3000 rpm for 5 min and the supernatant layer (organic layer) was recovered, this stage was duplicate and 15 ml N- heptane and 1 ml of 0.2 N HCl added and was shaken, then the lower layer (water like) recovered. this procedure also was duplicate repeated. finally, 1 ml distilled water added and the solution dried under the N2 or evaporation bathroom.

Drivatization: The amines were drivatizated following the method of [24]. To the extracted dry matter 1 ml of a 2 M NaOH solution and 5 µl of benzoyl chloride (derivatizator) were added. The mixture was shaken vigorously in a vortex mixer and allowed to stand for 20 min; 2 ml of a saturated NaCl solution were added to stop the benzoylation. finally, 2 ml of Diethyl ether is added, then, centrifuged at 2500 rpm for 5 min; the supernatant layer was recovered and dried under the N2 or evaporation bathroom.

Injection: This stage was achieved following the method of [24]. The extracted dry mater according to the procedures reported in section drivatization, dissolved in 200 µl of HPLC-grade methanol, filtered through Millipore (0.45 µm) and 20 µl of the filtrate were injected in the HPLC using a Hamilton syringe. Amines were detected under the UV light at 254 nm and the separation were performed under inverse phase with isocratic conditions using a mobile phase composed of methanol/water (70/30) and the flow rate 1.1 ml/min.

Bacteriological Examination: Ten grams from each fish sample were aseptically homogenized in a sterile homogenizer flask with 90 ml of sterile 0.1% peptone (Oxoid CM9) for 2 minutes to provide a homogenate of 1/10 dilution. From the resulting dilution, ten-fold serial dilutions were prepared using the same diluents. A100 µl from the original and the subsequent decimal dilutions were inoculated into duplicate plates of plate count agar (Oxoid CM463) and incubated at 30°C for 2 days [25].

Chemical Composition Analysis: Proximate composition analysis of Sardine fish samples were determined as moisture, crude protein, total lipids and crude ash content by the method described in [26].

Physicochemical Analysis:

Measurement of pH: The pH value of examined fish samples was measured directly on the muscles at room temperature using digital pH meter with a probe type combined electrode (Suntex TS-1), where three readings were recorded and the average was calculated [27].

Tri Methylamine: Trimethylamine (TMA) of raw materials was determined by the Modified Dyer Picrate method [28].Whole fish (20 g) was homogenized in 80 ml cold 7.5% (w/v) trichloroacetic acid. The homogenate was centrifuged at 8000 rpm (PK 121R, ALC Intl. Srl) at 4 °C for 10 min. The supernatant was further extracted in toluene and reacted with 1% picric acid. Absorbance was measured at 410 nm using trimethylamine as a standard. TMA was expressed as mg TMA/100 g sample.

Total Volatile Base-nitrogen: Total volatile base-nitrogen (TVB-N) of raw materials was determined by the steam distillation [29]. Ten grams of homogenized anchovy was added to 2 g MgO and 40 ml distilled water. Steam distillation was performed using a Kjeldahl distillation unit (Vapordest 30, Gerhardt, Königswinter, Germany). for 5 min. The distillate was titrated with 0.1 *N* HCl and TVB-N was calculated and expressed as mg N/100 g sample.

Thiobarbituric Acid Value: (TBA, mg malonaldehyde/kg sample) was determined by a distillation method, according to this method 5ml TBA reagent (0.2883g/100ml

of 90% glacial acetic acid) were added to 5ml distillate that is collected from distillation 10g of fish burger with 4M hydrochloric acid. After that, it was shaken and heated in boiling water for 35 min. A blank was prepared using 5ml water with 5ml reagent. Then were cooled in water for 10 min and measured the absorbance against the blank at 538nm using1cm cell [30]. TBA number (mg malonaldehyde per kgsample) = 7.8 D

RESULTHS AND DISCUSSION

Proximate composition analysis of Sardine fish samples were determined as moisture, crude protein, total lipids and crude ash content are shown in Table 1.

The moisture content of Sardine fish samples were 70.1% at zero time of fresh Sardine fish samples, 72.8, 69, 34.8 and 59.2% at the end of the storage duration of preserved Sardine fish samples by cooling, freezing, salting and canning, respectively.

The crude protein and ash ranged from 17.3 and 1.3% of cooled to 24.5 and 5.9% of salted Sardine fish samples, respectively whereas, canned Sardine fish samples recorded the highest content of total lipids was 15.2% followed it the salted Sardine fish samples was 13.7% while the fresh Sardine fish samples recorded the lowest content was 8.5%.

The changes in different biogenic amines concentration (mg/kg) and Bacterial load (cfu/g) in cooled sardine fish during storage at 4°C±1 for 14 days are shown in Table 2.

The results revealed that the concentration of cadaverine and putrescine were higher than histamine in fresh sardine fish samples (1, 9 and 8mg/kg) respectively, at zero time. Also, it observed that histamine content start in increasing after 4 days reached to 130 mg/kg compared with cadaverine and putrescine were 114 and 83mg/kg respectively, at the end of storage duration.

These data were in the allowance limits of FDA [22] which reported that it ranged from 100-200 mg/kg of fresh fish. Also, the maximum allowable histamine in fish sauce imposed by the Canadian Food Inspection Agency is 20 mg/100g [31]andEC[32]ruled that the level of histamine in food (200mg /Kg) was established in Germany. Only 100mg /Kg of food was accepted in Canada, Finland and Switzerland.

These results agreement with Křížek *et al*. [33] who observed that the shelf life of fillets and mince at 3°C was 11-16 days and 7-10 days, respectively. Content of putrescine seems to be a good quality indicator for all examined fish species. The fish species and the method of flesh processing did not have a significant influence on the putrescine formation. Samples of good and acceptable quality did not contain toxicologically significant concentrations of histamine or tyramine. Also, Kerr *et al*. [34] discovered that the storage of fish under refrigerated conditions from the time it is caught until when it is consumed has been found to be very important in reducing outbreaks of histamine poisoning.

These study revealed that the total mean of bacterial load (cfu/gm) among examined samples ranged from 5 x 10 at zero time to 9.9 x 105 at the end of storage duration (after 14 days) at 4°C. Data are shown in Table (3) results that the histamine concentrate increase slowly during frozen storage until reached to 26 mg/kg whereas, cadaverine was the highest (85mg/kg) andputrescine was the lowest (14mg/kg) at the end of storage duration.

These results agreement with ESS[35]who reported that the histamine content must not exceed200 mg/kg for caught fish are generally low, usually below 0.1mg/100g [36]. Rossano *et al*. [37] studied the influence of storage temperature and time of freezing on histamine formation in anchovies, showing the ability of freezing to inhibit or slow down its formation. Also, low temperatures control bacterial histamine formation during fish processing. So, tuna fish are usually covered with ice or simply placed on ice to prevent spoilage. It is mainly caused by consumption of scombroid fish including tuna, mackerel, saury and non- scombroid fish including bluefish, mahi-mahi, sardine, anchovy, herring and marlin [38].

Bacterial growth in frozen sardine fish products was slowly until reached to 2 x 102 after 8 weeks at 0°C.

The changes in different biogenic amines concentration (mg/kg) and Bacterial load (cfu/g) in salted sardine fish during storage at 4°C±1 for 14 days are shown in Table (4).

The results obtained that the salted sardine fish product samples have the highest concentrate of biogenic amines among all sardine fish samples reached to 760, 360 and 420mg/kg for histamine, cadaverine and putrescine respectively, from the previous data detected that histamine was the highest followed putrescine and then cadaverine after 4 months at room temperature.

These data were out of allowance limits of FDA [22] which reported do not more than 200-400 mg/kg of processed fish products.But in Egypt, histamine content must not exceed 200 mg/kg for salted, smoked and frozen fish product [35].

Table 1: Chemical composition of fresh, cooled, frozen, salted and canned sardine fish after the storage durations (gm/100gm).

Examined Samples	Moisture	Crude protein	Total lipid	Crude ash
Fresh	70.1	19.6	8.5	1.6
Cooled	77.4	18.9	8.4	1.4
Frozen	67.0	20.2	8.6	1.9
Salted	34.8	24.5	13.7	5.9
Canned	59.2	22.3	15.2	3.9

Table 2: Changes in different biogenic amines concentration (mg/kg) and Bacterial load (CFU/gm) in cooled sardine fish during storage at 4±1°C for 14 days

	Storage time (day)				
Biogenic amines (mg/kg)	At zero time	After 4 days	After 7 days	After 10 days	After 14 days
Histamine	1	2	53	79	130
Cadaverine	9	37	66	92	114
Putresine	8	13	24	46	83
Bacterial load (CFU/g)	5 x 10	2 x 102	2.6 x 103	6.3 x 104	9.9 x 105

Table 3: Changes in different biogenic amines concentration (mg/kg) and Bacterial load (CFU/g) in frozen sardine fish during storage at 0°C for 8 weeks (mg/kg)

	Storage time (week)				
Biogenic amines (mg/kg)	At zero time	After 2 weeks	After 4 weeks	After 6 weeks	After 8 weeks
Histamine	1	1	13	19	26
Cadaverine	9	14	29	56	85
Putresine	8	9	12	11	14
Bacterial load (CFU/g)	5 x 10	4.6 x 10	8.7 x 10	11 x 10	2 x 102

Table 4: Changes in different biogenic amines concentration (mg/kg) and Bacterial load (CFU/g) in salted sardine fish during storage at 30±5°C for 4 months (mg/kg)

Biogenic amines (mg/kg)	Storage time (Month) At zero time	After month	After 2months	After 3months	After 4months
Histamine	1	57	110	260	760
Cadaverine	9	32	72	143	360
Putresine	8	48	91	180	420
Bacterial load (CFU/g)	5 x 10	3.2 x 102	1.1 x 104	9.3 x 105	2.2 x 107

The results revealed that the salted sardine fish products are safe until 3 months after that it might not be safe and it become healthy risk after 4 months, due to the increment of biogenic amines concentration and bacterial load. These results agreement with Ahmed *et al.* [8] who could be concluded that salted fish products werethe worst examined fish products concerning its highbiogenic amines content and the mean values for frozen fish fillet and fish finger were 6 log cfu/g.Also, Histamine forming bacteria was also found to be high in salted sardines, salted lethrinids, salted Indian ilisha and salted Tiger perch, besides smoked tuna, as [5]. Whereas, Shakila *et al.* [39] who reported that the putrescine forming bacteria were isolated in high numbers in salted fish than the other amine forming bacteria. The reason of previous data High histamine concentrations in cured fish like salted fish could be due to poor quality of raw material, improper handling or other changes during storage [17].

Total mean of the bacterial count of salted samples recorded the highest content reached to 2.2 x 107 cfu/g after the end of storage duration at room temperature where bacterial load increase with increasing time and temperature of storage duration. These data agreement withShakila *et al.* [39] who reported that the total bacterial load in the salted fish ranged from 104 to 105 cfu/g. Also, Jeyasekaran and Shakila [40] who studied the total bacterial load in the samples of cured fishery products (salted, dried and smoked) ranged from 102 to 105 cfu/g. Hence, Sodium chloride enhanced activity of histidine decarboxylase of *Staphylococcus* spp. isolated from salted anchovies. Hernández-Herrero *et al.* [41]. The high salt concentration can prevent the growth of spoilage microorganisms such as *Escherichia* sp. *Serratia*sp. *Pseudomonas* sp. and *Clostridium* sp. in fish sauce [42].

The results of the canned sardine fish product samples are shown in Table (5) have the lowest concentrate of biogenic amines among all sardine fish samples reached to 24, 13 and 8mg/kg for histamine, cadaverine and putrescine respectively, from the previous data detected that histamine was the highest followed cadaverine and then putrescine after 4 months at room temperature.

These results agreement with the study revealed that the highest concentration of biogenic amines was found in feseekh (low salt) (1799 mg/kg) followed by feseekh (high salt) (1078 mg/kg), followed by smoked (282 mg/kg) on the other hand the lowest concentration of biogenic amines was found in canned fish (18 mg/kg). The highest concentration was for histamine [9].

The maximum total BAs content of lightly cured horse mackerel was 484.42 mg/kg compared to 167.86 mg/kg or less for the other salted and fermented fish products. In the Spanish mackerel sample, histamine was detected within the range of 15.74-28.70 mg/kg, whereas The bacterial growth was the lowest counts in canned sardine fish products that agreement withKerr *et al.* [34] who obtained that the canning die the bacteria which

Table 5: Changes in different biogenic amines concentration(mg/kg) and Bacterial load (CFU/g) in canned sardine fish during storage at 30±5°Cfor 4 months (mg/kg)

Biogenic amines (mg/kg)	Storage time (Month) At zero time	After month	After 2months	After 3months	After 4months
Histamine	1	5.8	10	12	24
Cadaverine	9	3.2	5.7	7.8	13
Putresine	8	3.7	4.3	6.1	8.0
Bacterial load (CFU/g)	5 x 10	ND	0.7 X 10	2.2 X 10	5.5 X 10

ND: Not detected

Table 6: Physicochemical analysis parameters in cooled sardine fish during storage at 4°C±1 for 14 days

Parameters	At zero time	After 4 days	After 7 days	After 10 days	After 14 days
PH	6.58	6.72	7.00	7.41	7.73
T.V.N (mg/100g)	11.2	26.5	28.9	34.1	42.0
T.B.A (mg/kg)	0.70	0.79	0.86	0.91	1.24
T.M.A (mg/100g)	3.84	8.8	12.4	19.9	29.0

Table 7: Physicochemical analysis parameters in frozen sardine fish during storage at 0°C for 8 weeks.

Parameters	At zero time	After 2 weeks	After 4 weeks	After 6 weeks	After 8 weeks
PH	6.58	6.78	7.10	6.90	6.80
T.V.N (mg/100g)	11.2	18.9	19.8	18.5	19.9
T.B.A (mg/kg)	0.70	0.68	0.63	0.59	0.53
T.M.A (mg/100g)	3.84	3.69	4.10	4.3 8	4.78

produce biogenic amines during the cooking and sterile under pleasure. In addition, modified atmosphere packaging and vacuum packaging represent popular preservation methods which may inhibit the growth and increase the lag phase of microorganisms with amino acid decarboxylase activity [43].

It could be concluded that the frozen and canned sardine fish samples are safe and acceptable for human consumption under storage condition at 0°C and room temperature for 8 weeks and 4 months, respectively. Whereas, the salted sardine fish product samples could be hazardous after 4 months due to the increment of biogenic amines and bacterial load followed it the cooled sardine fish which stored at 4°C for 14 days.

pH values and other chemical analysis parameters of cooled Sardine fish products at 4°C for 14days are shown in Table (6).

pH value, TVN, TBA and TMA of fresh Sardine fish samples were 6.58, 11.2 mg/100g, 0.70 mg/kg and 3.84 mg/100g at zero time, respectively. The results revealed that the previous parameters increase with increasing of storage duration at 4°C. Where the data reached to 7.73, 42.0mg/100g, 1.24mg/kg and 29mg/100g after the end of storage duration, respectively. From the previous results, it is observed that all data were out the allowance limits after 14 days storage at 4°C. It could be concluded that the cooled fish samples become unsafe for human consumption because of healthy risks. The previous data agreement with Pearson[29]who suggests that for white-fleshed fish, TVN levels below 200 mg N/Kg indicate that the fish is fresh, whereas the fish would be rejected for human consumption when the TVN level exceeds approximately 500 mg N/Kg [44] for the ambient trial this level is reached after 2 days and for the trials at 4°C and at 0°C this level is reached after 10 days.

pH values and other chemical analysis parameters of frozen Sardine fish products at 0°C for 8 weeks are shown in Table (7). pHvalues were inversely correlatedwith T.M.A of Sardine fish products during frozen storage at 0°C for 8 weeks where the pH value increased from 6.58 at zero time to 7.10 after 4 weeks but, it decreased to 6.80 after 8 weeks while TMA values decreased from 3.84 at zero time to 3.69 after 2 weeks but, increased to 4.78 mg/100g after 8 weeks. Also, TVN values were inversely correlatedwith T.B.A of Sardine fish products during frozen storage at 0°C for 8 weeks where the TVN value increased from 11.2 at zero time to 19.9 after 4 weeks while TBA decreased from 0.70 at zero time to 0.53 after 8 weeks.

These results agreement withMahmoudzadeh *et al.* [45] who found that the TVN values increased from 11.66 to 20.97 and 10.68 to 20.97 at the end of second and first months, respectively, but then significantly decreased in the deep flounder fish burger (group A)at the end of storage whereas in Brushtooth lizard fish burger (group B) no changes were observed between end of the first month and end of the frozen storage. The TBA values of both groups significantly decreased from 1.01 to 0.22 and 0.70 to 0.26 at the end of storage, respectively and there were no significant differences between values of two groups at the end of 5th month. Also, Ahmed *et al.* [8] who proved that the frozen fish products were the lowest products inhistamine, cadaverine and putrescine contents as somebacterial populations responsible for biogenic amines production cannot with stand the low temperature ofstorage and the high pH value were 7.5 and 6.15 of fish fillet and fish finger.

pH values and other chemical analysis parameters of salted Sardine fish products at room temperature for 4 months are shown in Table (8).

Table 8: Physicochemical analysis parameters in salted sardine fish during storage at 30°C ±5 for 4 months

Parameters	At zero time	After month	After 2 months	After 3 months	After 4 months
PH	6.58	6.1 2	5.79	5.10	4.58
T.V.N (mg/100g)	11.2	22.5	26.1	38.9	47.2
T.B.A (mg/kg)	0.70	0.79	0.92	1.1	1.3
T.M.A (mg/100g)	3.84	6.23	15.9	23.7	32.5

Table 9: Physicochemical analysis parameters in salted sardine fish during storage at 30°C ±5 for 4 months

Parameters	At zero time	After month	After 2 months	After 3 months	After 4 months
PH	6.58	6.30	5.97	6.47	6.82
T.V.N (mg/100g)	11.2	14.9	17.5	19.2	20.5
T.B.A (mg/kg)	0.70	0.66	0.65	0.69	0.74
T.M.A	3.83	3.72	3.91	4.32	4.11

The results obtained from the previous data revealed that the PH values decrease with increasing the storage period ranged from 6.58 at zero time to 4.58 after 4 months. Whereas, both T.V.N, T.B.A and T.M.A increase with increasing the storage period ranged from 11.2, 0.70 and 3.84 at zero time to 47.2 mg/100g, 1.30 mg/kg and 32.5 mg/100g, respectively after 4 months. These results agreement with Zaman *et al.*[46]who obtained that the level of pH and salt content in all samples ranged from4.8% to 5.7%. Also, Ahmed *et al.* [8] who proved that thePutrescine and cadaverine contents were inversely correlatedwithpHsince biogenic amines formation is enhanced in acidic pH.Also, some technological processes such as salting, ripening, fermentation or marinating can increase the possibility of formation of BAs, a low pH (4.0-5.5), which can be achieved in salted anchovies, for instance, is favorable for enhanced amino acid decarboxylase activity [47]. The affects the production and activity of the enzyme because in low pH environment, bacteria are more stimulated to produce decarboxylase as a part of their defense mechanisms against the acidity [48].

pH values and other chemical analysis parameters of canned Sardine fish products at room temperature for 4 months are shown in Table (9).

The results obtained from the previous data revealed that the PH values decrease with increasing the storage period ranged from 6.58 at zero time to 5.97 after 2 months but it increased to 6.82 after 4 months. Whereas, both TVN, TBA and TMA increase with increasing the storage period ranged from 11.2, 0.70 and 3.84 at zero time to 47.20.5 mg/100g, 0.74 mg/kg and 4.11mg/100g, respectively after 4 months.

There is no relationship between TVN and histamine formation at each temperature, TVN increased continuously with time. The biogenic amines likehistamine, typically show a period of time where histamine is not formed, production then commences and then declines with time. At 0°C, problematic levels of histamine are not encountered, however, the corresponding TVN values indicate that the fish would not be fit for consumption [34].

It could be concludedthat the biogenic amines trend to increase in the concentration as the result of increase the temperature and time storage duration except histamine that increase slowly at between 0 - 4°C after few days. Most sardine fish product samples contained trace amounts in the allowance limits of FDA (below 200mg/kg) except of salted sardine fish can be consumed without any health risks until 3 months after that, it could be become hazardous because of outing biogenic amines concentration and bacterial load about allowance limits.

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