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Potential role of certain herbal extracts in reducing toxic histamine and improving the quality of chilled tuna finger

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ABSTRACT

Around the world, people view fish as one of the key sources of animal protein. Biogenic amines are naturally occurring compounds that can be formed in tuna fish and pose a potential health risk to the consumers and the industry. So, the current study aimed to investigate the effectiveness of different herbal extracts for controlling biogenic amines formation and spoilage in chilled tuna finger. The antioxidant potential of Moringa extract (MOE), Green tea extract (GTE) and Olive leaves extract (OLE) (1.5 % for each) was evaluated by HPLC as natural preservatives. The recorded values of histamine were 1.9±0.05, 1.81± 0.01, 1.1±0.01 (mg/g) with MOE, GTE and OLE, respectively on the 14th day of storage. Moreover, enhancement of chemical quality (PH, TMA, TBA, TVB-N) and sensory profile was detected in treated samples compared to those of control during storage period up to 14 days under refrigerated storage (4°C). These findings suggested that the extract may serve as an alternative choice to use as a food additive extending the shelf-life of chilled tuna fingers.

1. INTRODUCTION

The majority of a person's nutritional needs are generally satisfied by fish products, which are high in protein, vitamins, unsaturated fatty acids, and minerals (Zhuang *et al.*, 2021). The freshness of the fish is important for assessing the quality of fish. One of the main factors for assessing freshness of fish is biogenic amines (BAs) that are classified into three categories according to their chemical structure: aromatic amines as histamine, aliphatic diamines as putrescine and aliphatic amines as Trimethylamine (TMA) (Maintz & Novak, 2007). Due to their effects on food quality and human health, biogenic amine concentrations, particularly histamine, in fish and fish products must be estimated. Histamine levels in fisheries products are restricted to less than 100 mg/kg (Commission Regulation (EC)/1441, 2007). Consuming foods rich in histamine (200 mg/kg) has been linked to hazardous reactions similar to food poisoning in cases of allergy, including vomiting, rashes, stomach aches, difficulties breathing, and even death (FDA, 2001). Instead, meals containing 8–40 mg of histamine can only cause mild intoxication, whereas meals containing 40–100 mg of histamine or more than 100 mg of histamine are connected to intermediate and severe intoxication, respectively (Biji *et al.*, 2016). Trimethylamine is the primary factor responsible for fishy odor (Bedia, 2013). Trimethylamine is used so frequently as a fish degradation indicator.

Natural preservatives have been extensively studied for their antioxidant activities aiming to quality assurance and safety of seafood and fish products (ELsabagh et al., (2023) and Jeyakumari et al. (2021)). These natural antioxidants are reported to be more powerful than the synthetic antioxidants (Yanishlieva *et al.*, 2006).

According to Hazra *et al.* (2012), moringa oleifera (M. oleifera) is regarded as a significant source of natural antioxidant and antibacterial agents. It contains various types of antioxidant components, including flavonoids, ascorbic acid, carotenoids, and phenolics, in its leaves which help extend the shelf life of foods (Al-Juhaimi *et al.*, 2015).

According to Almajano *et al.* (2008), green tea (Camellia sinensis L.) is a source of polyphenolic chemicals with potent antioxidant and antibacterial actions.

Olive extracts are high in hydroxytyrosol which can replace synthetic additions in food items as natural antioxidants and antimicrobials (Martínez*et al.*, 2019).

The current study was applied to evaluate the presence of histamine in fish and chilled tuna finger and evaluate the effect of some herbal extracts (Moringa, green tea and olive leaves extract) (1.5%) on the levels of histamine in the examined fish fingers and its effect on public health.

2. MATERIAL AND METHODS

2.1. Herbal extracts preparation

The moringa, green tea and olive leaves were collected from herbal section in supermarkets then cleaned by washing with distilled water and dried under shade at room temperature then crushed to fine powder. Then, 1 kg from obtained powder was extracted in 1000 ml of ethanol (absolute 99.8%) for 48 h . filtrations process was repeated, then the

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extract was stored in an airtight brown bottle in a refrigerator at 4 ˚C until use (Abdel-Daim *et al.*, (2020) with modification. High performance liquid chromatography (HPLC) profile of phenolic compounds of Moringa,Green tea and Olive leaves extracts are determined according to Hamad *et al.* (2023).

2.2. Sample preparation

Samples of fish tuna fingers were divided into 4 groups, each of 400 gm, control group and 3 treated groups with 1.5% of each of the following herbal extracts: Moringa, green tea and olive leaves and stored in the refrigerator at 4 °. Trial was triplicated (3 times), mean of results was recorded.

2.3. Determination of histamine in fish tuna fingers:

The histamine test kit (RIDASCREEN® Histamine, 96 well, R-Biopharm, Darmstadt, Germany) was prepared using the instructions provided. After that, an acylation and ELISA (Enzyme-linked immune-sorbent assay) was carried out using the reagents in accordance with the protocol included with the kit utilizing an unlimited f50 microplate reader (SN1002002254, Austria), determine the absorbance at 450 nm. The RIDASOFT® Win.NET (Art. Nr. Z9996FF), specialized software, is offered for evaluating the RIDASCREEN enzyme immunoassays.

2.4. Physico-chemical examination:

pH was measured by digital PH Mete (Model 3310 Jenway, sensitivity 0.001) that was previously calibrated. Also, Total volatile basic nitrogen (TVB-N) calculated after distillation and titration of the distillate by using H_2SO_4 solution (0.1N) till the natural red endpoint, then calculated. Thiobarbituric acid (TBA) were performed according to Cai *et al.* (2014) as the sample optical density (OD) was read at a wavelength of 538 nm against the blank on spectrophotometer (Double Beam UV Spectrometer

Model ST-UV-1901PC), then calculated. While Trimethylamine (TMA) assessed according to Ward *et al.* (2009). The optical density was measured photometrically by spectrophotometer at 410 nm wavelength.

2.3. Sensory changes evaluation

All sensory attributes including color, odor, texture, and overall acceptability were considered to evaluate the acceptance of samples. Groups were scored from 5 to 0 (Gao *et* al., 2014). The items were assessed on their sensory qualities, using a 5-point hedonic scale (1 being severely disliked and 5 being greatly accepted).

2.6. Statistical analysis

All data statistically analyzed with Graph Pad Prism 8.0.2 using One-way analysis of variance (ANOVA) (Geisser-Greenhouse's epsilon) with P< 0.001. Post-hoc analysis was performed using Tukey's HSD test. All data were expressed as mean ± SD of three replicates (Greenhouse and Geisser, 1959).

3. RESULTS

Table (1) shows the profile of phenolic compounds of MOE, GTE and OLE. The more prominent acids were Gallic acid, Protocatechuic acid p-hydroxybenzoic acid, Gentisic acid, Catechine, Chlorogenic acid, Caffeic acid, caffeine, Syringic acid, Vanillic acid, Ferulic acid, Sinapic acid, p-coumaric acid, Rutin, apegnin-7-glycoside, Cinnamic acid and quercetin.

Table (2) shows that histamine levels increased significantly (P< 0.0001) throughout the storage period. Histamine level increased rapidly in the control group from 0.135 ± 0.002 to 3.545 ± 0.005 (mg/g). The lowest alteration in histamine levels appeared in the group treated with OLE followed by GTE then MOE. Two-way ANOVA revealed high significance difference (P< 0.0001) between treated and control groups throughout the refrigerated storage (4 °C).

Table 1 HPLC profile of phenolic compounds of MOE, GTE, and OLE

Compound	MOE (ug/ml)	GTE (ug/ml)	OLE (ug/ml)
Gallic acid	6.786	39.815	3.030
Protocatechuic acid	1.380	12.439	89.887
p-hydroxybenzoic acid	0.000	7.122	1.287
Gentisic acid	0.000	0.000	0.000
Catechine	7.412	280.004	62.740
Chlorogenic acid	0.000	4.580	0.000
Caffeic acid	9.165	0.784	0.000
caffiene	0.000	1338.341	0.000
Syringic acid	153.068	2.946	19.764
Vanillic acid	80.434	0.000	2.594
Ferulic acid	2.484	0.000	0.000
Sinapic acid	3.044	0.000	0.000
p-coumaric acid	2.673	15.799	3.442
Rutin	198.511	87.884	31.067
hisperdin	0.000	0.000	0.000
Naringin	0.000	0.000	0.000
Oleuropein	0.000	0.000	17680.880
apegnin-7-glycoside	11.884	9.088	0.000
apegnin	0.000	0.000	0.000
Cinnamic acid	1.909	5.016	1.135
quercetin	1.432	2.778	3.772
Kaempferol	0.000	0.000	1.596
Chrysin	0.000	0.000	0.000
epigallocatechingallate	0.000	3798.266	0.000
epicatechingallate	0.000	1354.432	0.000

Table 2 Changes of histamine in fish tuna fingers fortified with MOE, GTE and OLE during refrigerated storage.

Storage time	Fish tuna fingers			
	Control (mg/g)	MOE (mg/g)	GTE (mg/g)	OLE (mg/g)
0 day	0.14±0.01^{a}	0.14±0.01^{a}	0.13±0.01^{a}	0.13±0.01^{a}
2 day	0.47±0.02^{a}	0.29±0.03^{b}	0.26±0.04^{b}	0.25±0.01^{b}
4 days	0.60±0.02^{a}	0.37±0.01^{b}	0.30±0.01^{c}	0.29±0.03^{c}
6 days	0.69±0.03^{a}	0.43±0.02^{b}	0.36±0.02^{c}	0.31±0.03^{c}
8 days	0.75±0.05^{a}	0.74±0.02^{a}	0.65±0.01^{b}	0.57±0.02^{c}
10 days	0.91±0.03^{a}	0.89±0.05^{a}	0.86±0.04^{b}	0.66±0.01^{c}
12 days	2.95±0.03^{a}	1.55±0.03^{b}	1.34±0.01^{c}	0.91±0.04^{d}
14 days	3.55±0.01^{a}	1.90±0.05^{b}	1.81±0.01^{c}	1.10±0.01^{d}

Values represented as the mean of three determinations ± standard deviation.* the values statistically Significance when p value was<0.001.

Data presented in table (3) revealed that values of pH increased from 5.90 ± 0.09 to 7.9 ± 0.07 for control samples while, it increased from 5.92±0.02, 5.91±0.02 and 5.92±0.03 to 6.91 ± 0.07, 6.72 ± 0.02 and 6.5 ± 0. 1 in samples treated with MOE, GTE and OLE, respectively.

Table 3 Changes of pH in fish tuna fingers fortified with MOE, GTE and OLE during refrigerated storage.

Storage time	Fish tuna fingers			
	Control	MOE	GTE	OLE
0 day	5.90±0.09^{a}	5.92±0.02^{a}	5.91±0.02^{a}	5.92±0.03^{a}
2 day	6.06±0.06^{a}	5.95±0.03^{b}	5.96±0.02^{b}	5.96±0.01^{b}
4 days	6.75±0.04^{a}	6.04±0.01^{b}	6.01±0.01^{b}	5.99±0.01^{c}
6 days	7.00±0.09^{a}	6.16±0.05^{b}	6.11±0.02^{c}	6.02±0.01^{d}
8 days	7.36±0.15^{a}	6.20±0.15^{b}	6.20±0.01^{b}	6.10±0.02^{c}
10 days	7.63±0.15^{a}	6.60±0.07^{b}	6.35±0.05^{c}	6.20±0.01^{d}
12 days	7.80±0.05^{a}	6.86±0.05^{b}	6.55±0.04^{c}	6.30±0.04^{d}
14 days	7.90±0.07^{a}	6.91±0.07^{b}	6.72±0.03^{c}	6.50±0.10^{d}

Values represented as the mean of three determinations ± standard deviation* The values statistically Significance when p value was<0.001.

Table (4) showed that the levels of TVB-N dramatically increased over the course of storage (p< 0.001). In contrast to MOE and GTE, OLE samples showed the lowest TVB-N value variance.

Results shown in table (5) indicated that TBA values of tuna fingers in control group increased from 1.74 ± 0.2 to 6.8 ± 0.5 mg malonaldehyde/Kg at 14thday of storage, while it increased from 1.65 ± 0.1, 1.70 ± 0.03 and 1.70±0.03 (mg malonaldehyde /Kg) to 4.7±0.5, 3.5±0.2 and 3.1±0.2 in examined tuna fingers samples treated with MOE, GTE and OLE, respectively (P < 0.001).

The results presented in table (6) showed that after 14 days of refrigerated storage, the results of control samples for TMA level were 6.82 ± 0.1 increased to 25.5 ± 1.4 mg %

when compared with the other treatments. The lowest alteration in TMA was noticed in the OLE and GTE treatments compared to MOE treatment as it became 16. 4 ± 0.2, 15.3 ± 0.4 and 14.4 ± 0.2 (mg%) for MOE, GTE and OLE, respectively.

Data presented in fig. (3) indicated a spontaneous decrease in the odor and color of tuna fingers as there was a significance difference (P< 0.001) between control group and treated groups. For the control group, the color score decreased from 5 degree to 2.4 degree on the 6th day of cold storage as it became unacceptable. While MOE, GTE and OLE groups become unacceptable at the 10th, 12th, and 14th day of storage, respectively. Texture and overall acceptability constitute the major factors for acceptance of the tuna fingers during cold storage. The score of control group was 1.5 on the 6th day of cold storage while it was 4.1, 4.2, 4.3 (very good) for MOE, GTE and OLE, respectively. The group treated with OLE was the most acceptable group till 12th day of storage (2.75) as it spoiled at the end of storage period (1.25) with (P< 0.001).

Table 4 Changes of TVB-N in fish tuna fingers fortified with MOE, GTE and OLE during refrigerated storage.

Storage time	Fish tuna fingers			
	Control (mg/100)	MOE (mg/100)	GTE (mg/100g)	OLE (mg/100g)
0 day	2.1±0.4^{a}	2.3±0.2^{a}	2.4±0.3^{a}	2.4±0.2^{a}
2 day	12.0±0.5^{a}	10.7±0.6^{b}	10.0±0.2^{c}	9.8±0.3^{c}
4 days	18.0±0.5^{a}	15.1±0.4^{b}	13.5±0.5^{c}	12.6±0.6^{d}
6 days	23.5±0.6^{a}	21.0±0.8^{b}	19.0±0.5^{c}	15.4±0.3^{d}
8 days	28.3±0.3^{a}	22.1±0.6^{b}	20.3±0.3^{c}	19.4±0.2^{d}
10 days	33.3±0.5^{a}	28.2±0.2^{b}	27.4±0.4^{c}	25.7±0.5^{d}
12 days	37.6±0.2^{a}	30.3±0.3^{b}	28.0±0.5^{c}	27.2±0.3^{d}
14 days	42.2±1.8^{a}	36.54±1.1^{b}	30.8±0.4^{c}	28.5±0.41^{d}

Values represented as the mean of three determinations ± standard deviation
The values statistically Significance when p value was<0.001.

Table 5 Changes of TBA in fish tuna fingers fortified with MOE, GTE and OLE during refrigerated storage.

Storage time	Fish tuna fingers			
	Control mg malonaldehyde /Kg	MOE mg malonaldehyde /Kg	GTE mg malonaldehyde /Kg	OLE mg malonaldehyde /Kg
0 day	1.74±0.2^{a}	1.65±0.11^{b}	1.70±0.03^{a}	1.62±0.02^{b}
2 day	2.19±0.02^{a}	1.8±0.11^{b}	1.75±0.10^{b}	1.68±0.03^{c}
4 days	2.7±0.10^{a}	1.9±0.05^{b}	1.85±0.05^{b}	1.75±0.05^{c}
6 days	3.7±0.20^{a}	2.06±0.06^{b}	1.93±0.10^{c}	1.8±0.03^{d}
8 days	4.2±0.11^{a}	2.5±0.11^{b}	2.1±0.21^{c}	1.95±0.05^{d}
10 days	4.8±0.31^{a}	3.1±0.22^{b}	2.5±0.10^{c}	2.1±0.10^{d}
12 days	5.5±0.20^{a}	3.9±0.40^{b}	2.9±0.11^{c}	2.3±0.10^{d}
14 days	6.8±0.50^{a}	4.7±0.51^{b}	3.5±0.20^{c}	3.1±0.20^{d}

Values represented as the mean of three determinations ± standard deviation.
The values statistically Significance when p value was<0.001.

Table 6 Changes of TMA in fish tuna fingers fortified with MOE, GTE and OLE during refrigerated storage.

Storage time	Fish tuna fingers			
	Control	MOE (mg%)	GTE (mg%)	OLE (mg%)
0 day	6.82±0.10^{a}	6.41±0.30^{b}	6.51±0.11^{b}	5.62±0.12c
2 day	8.40±0.21^{a}	6.82±0.40^{b}	6.72±0.10^{b}	5.81±0.20c
4 days	10.50±0.40^{a}	7.80±0.21^{b}	7.40±0.31^{c}	6.90±0.31^{d}
6 days	12.41±0.21^{a}	8.41±0.40^{b}	8.10±0.30^{c}	7.81±0.11^{d}
8 days	15.60±0.40^{a}	10.40±0.21^{b}	9.41±0.10^{c}	8.62±0.40^{d}
10 days	18.50±0.12^{a}	12.81±0.22^{b}	11.3±0.21^{c}	10.61±0.11^{d}
12 days	21.41±0.11^{a}	15.50±0.11^{b}	14.5±0.31^{c}	13.40±0.11^{d}
14 days	25.52±1.40^{a}	16.41±0.20^{b}	15.3±0.40^{c}	14.41±0.20^{d}

Values represented as the mean of three determinations ± standard deviation
The values statistically Significance when p value was<0.001.

4. DISCUSSION

Food spoiling is caused by physico-chemical, microbial, and sensory attributes that render it unsafe to consumers. Natural preservatives could be added to prevent spoiling and lengthen shelf-life (Kuswandi *et al*., 2012). Iso-flavonoids are physiologically active phenolic compounds found in a range of species. These iso-flavonoids have been used as antioxidant and antibacterial agents (Olasupo *et al*., 2018). Moringa, green tea and olive leaves are rich in phytochemical content, which so, they have antioxidant and antibacterial properties (Abdulkadir *et al*., 2018).

There have been claims that GTE possesses antibacterial and antioxidant properties. According to reports, the primary components of green tea include polyphenols (about 90%), amino acids, proanthocyanidins, and caffeine (Li *et al.*, 2018). Also, it is an excellent source of polyphenols, which are potent alternatives to artificial antioxidants. Since they are natural, they are usually less toxic and seem to have a similar impact on oxidation inhibition (Manea *et al.*, 2014).

Biogenic amines (BAs) are used as a sign of food deterioration and, at high levels, can have toxicological effects because they are a crucial tool for determining the sanitary status of foods (Saad *et al.*, 2022). Histamine is an indicator of fish quality (Mendes, 1999). According to the EU, the histamine legal limit in fish is 100 mg/kg (EC, 2005). It's crucial to understand that histamine is a chemical that has neither color nor smell, therefore its presence in a fish has no effect on that fish's appearance (Arnold and Brown, 1978). It is possible for a fish to have a high histamine content while showing no outward signs of deterioration.

The process of proteolysis, which raises the amount of free basic amino acids and causes a buildup of ammonia, amines, and other basic byproducts of bacterial breakdown, may be to blame for the pH value's rise. Also, Mosilhey and Eldeeb (2021) reported that treated group with moringa extract showed delayed alteration in pH more than control group which spoiled rapidly.

Ammonia, methylamine, dimethylamine, trimethylamine, and other compounds produced during the preservation of beef under refrigeration as a result of microbial activity are only a few of the volatile substances that make up TVB-N (Elsabagh *et al.*, 2023). Therefore, TVB-N is one of the most popular techniques for determining how fresh meat and fish are (Mosilhey and Eldeeb, 2021). Furthermore, Karim *et al.* (2018) reported that samples treated with moringa showed more delay in TVB-N formation than control one. Also, Jeyakumari *et al.* (2021) revealed that sample of fish nuggets

treated with green tea extract had a lower TVB-N content and spoiled more quickly than control group. Catechins and flavanols are the major polyphenols in tea leaves responsible for their antioxidant effects.

As a biochemical quality diagnostic for evaluating lipid oxidation in food, the TBA index is frequently utilized. The TBA index measures the amount of malonaldehyde produced in the muscle as a result of lipid peroxide oxidation. Jeyakumari *et al.* (2021) revealed that sample treated with green tea extract had a lower TBA value (0.34±0.01 to 0.44±0.03 mg MDA kg-1) than control (0.82± 0.02 to 2.14±0.01 mg MDA kg-1). According to Khodanazary (2019), the increase of TMA compound might be related to bacterial contamination that degrades proteins. Sensory evaluation is a test or evaluation that reveals how consumers react to food products by employing their senses. It is a way of figuring out if consumers will accept or reject a product (Kenawi and Abdelaal, 2021). The results of the sensory assessment of tuna fingers samples revealed that sensory scores showed a significant reduction in the color, odor, texture, and overall acceptability of control and treated groups during refrigerated storage. It has been established that seafood spoiled recorded on flavors that are very fishy, rotten, and putrid. One of the most crucial factors for consumers is the color of the fish and fish products since it denotes the quality and is linked to the flavor and freshness of seafood (Huynh *et al.* 2022). Kenawi and Abdelaal (2021) revealed that comparison to the control, the color, taste, odor, texture, and general acceptance scores improved with the addition of 1% moringa leaves powder. As a result of enzymatic reactions, it is possible for hazardous substances to be created, such as biogenic amines (Bas), which are basic, non-volatile, low-molecular-weight organic molecules with nitrogen atoms (Mercogliano and Santonicola, 2019).

5. CONCLUSIONS

As a result of the current study, it was determined that moringa extract (MOE), green tea extract (GTE) and olive leaves extract (OLE) increased the shelf-life of tuna fingers under refrigerated storage (4°C) than control group. Changes in biochemical indicators were often highly correlated with the sensory score**.**

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