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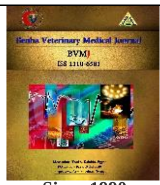

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Original Paper

Evaluating the efficacy of some plant extracts in enhancing the quality of chilled Japanese shrimp (*Marsupenaeus japonicus*) and controlling histamine formation.

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ABSTRACT

Nowadays, the customer's approach towards naturally preserved fish meat products has gained interest in overcoming the health risks of synthetic preservatives. So, our investigation aimed to study the effectiveness of natural preservatives for controlling histamine formation and spoilage in chilled shrimp. The potential of *moringa* extract (MOE), green tea extract (GTE), and olive leaf extract (OLE) (1.5% for each) to act as antioxidants in shrimp was evaluated. The histamine values recorded in the control group significantly differed from MOE, GTE, and OLE ($p < 0.05$) throughout the days of chilled storage. These findings emphasized the effect of the plant extracts to extend the shelf life of chilled shrimp and improve sensory attributes (color, odor, texture, and overall acceptability) and physicochemical characteristics (pH, TBA, and TMA) during a storage period of up to 14 days under refrigerated storage (4°C). These findings suggest that the extract, especially olive leaves, may serve as a food additive, extending the shelf life of shrimp.

1. INTRODUCTION

Shrimp has become popular among consumers as a component of a healthy diet because they are rich in high-quality protein, essential amino acids, and vitamins in addition it is low in fat (Qiao et al., 2019). However, shrimp are highly susceptible to deterioration during transportation, storage, and marketing, which results in a loss of nutrients and a decrease in quality (Ye et al., 2020). shrimp is a highly perishable that spoils rapidly during processing and cold storage (Rossi et al., 2002; Edris et al., 2017), threatening food safety (Biji et al., 2016).

Japanese shrimp (*Marsupenaeus japonicus*) is a high-quality dietary protein source but it's also a critical allergic food. The food allergy (FA) caused by shrimp has become a worldwide public health concern with anaphylaxis risk (de Silva et al., 2022). The extracellular protease of spoilage microorganisms breaks down fish meat into small molecules of amino acids (AA) and oligopeptides, which are used as fuel for the fast growth of the spoilage microorganisms (Yu et al., 2018). Also, Trimethyl-Amine-N-Oxide (TMAO) is reduced to trimethylamine (TMA) (Wright et al., 2019). During these spoilage steps, spoilage microorganisms can use AA as energy for metabolic growth and diamine or decarboxylate FAA to form ammonia and BAs (Zhuang et al., 2021). The accumulation of ammonia and BAs induces rapid deterioration of fish quality, such as increased pH values and total volatile salt nitrogen, off-flavors, and even the outbreak of food-borne diseases (Erikson et al., 2011).

Histamine poisoning is one of the main public health concerns (Yang et al., 2021). Its consumption causes toxicity and food-borne illnesses such as digestive disturbances, hives, and difficulty breathing, and may cause death (FDA, 2001). EC regulations (EC/1441, 2007) set a limit for 100 mg/kg of histamine in fish products. So, accurate and sensitive evaluation of histamine in fish and fish products becomes a demand. HPLC is one of the most sensitive and accurate protocols for histamine detection (Altieri et al., 2016). Moreover, histamine's risk is related to its resistance to detoxification by normal freezing and heat treatment methods such as frying and broiling (Akbari-Adergani et al., 2012). However, limited research on histamine screening using immunoassay has been reported (Xu 2020). Yang et al., (2021) recommend the indirect competitive ELISA as an accurate and sensitive protocol for routine histamine screening in seafood.

Many researchers worldwide seek promising natural preservatives with excellent antioxidant and antimicrobial activity for extending the shelf life of seafood products (Olatunde and Benjakul 2018). *Moringa oleifera* (*M. oleifera*) is a substantial source of natural antioxidants because it has been employed as a good source of rare flavonoids and phenolic acids (Dhakad et al., 2019). Li et al., (2018) stated that green tea primarily comprises polyphenols. Excellent sources of polyphenols can be found in green tea extract, making them a potent substitute for artificial antioxidants. Since they are natural, they are usually less dangerous and seem to have a similar impact on oxidation inhibition (Manea et al., 2014). Extract from olive leaves (OL) has been shown to exhibit antioxidant properties (Kuley et al., 2017). So, the aim of this study was to assess the efficacy of some plant extracts (*Moringa oleifera* (MO),

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green tea (GT), and Olive leaves (OL) extracts) on aspects of shrimp quality, shelf life and toxic histamine biogenic amine formation.

2. MATERIAL AND METHODS

Preparation of extracts

Ethanolic extracts from olives, green tea, *and Moringa oleifera* leaves were prepared according to Abdel-Daim et al., (2020) as following: Leaves from each plant were collected, washed, dried, and crushed to a fine powder using a milling machine. Ethanolic extract of the powder was prepared in absolute 99.8% ethanol solution (for 48 hours) and then filtered (3 times) on 2 mm-sized pores filter paper.

HPLC screening of the phenolic profiles of leaf extracts

The phenolic profile of Moringa *oleifera* (MO), green tea (GT), and olive leaf (OL) extracts was evaluated following Hamad et al., (2023). The phenolic compound of each extract was separated by an Eclipse C18 column and then screened using the Agilent 1260 Infinity HPLC Series, USA.

Shrimp preparation

Fresh medium-sized chilled shrimp (*Marsupenaeus japonicus*) from local supermarkets were collected and washed in a distilled water bath for just one minute. To evaluate the impacts of plant extracts on shrimp, samples were dipped in previously prepared plant extracts as following: control group (without application of any extracts on shrimp), MO group (shrimp + 2% MO extract), GT group (shrimp + 2% GT extract), and OL group (shrimp + 2% OL extract). Samples were packed in polyethylene bags and kept refrigerated at 4 °C for 14 days and examined periodically every two days starting from the zero day of the experiment (Zero, 2nd, 4th, 6th, 8th, 10th, 12th, and 14th). Trials were triplicated, and mean values were statistically monitored and interrupted.

Biochemical quality

Lipid oxidation of the chilled shrimp resembled TBA was evaluated by spectrophotometry to be expressed by the malonaldehyde content of samples. Trimethylamine (TMA) was also assessed by the Conway test. Total volatile base (TVB) content was screened and expressed as mg N/100 g. Moreover, a pH meter was used to evaluate the pH content of chilled shrimp. All previous chemical quality tests were applied following AOAC (2005).

ELISA screening of histamine

Histamine residue in different groups was screened using ELISA following Sadeghi et al., (2019), using RIDASCREEN® Histamine ELISA (R-Biopharm, Darmstadt, Germany). Samples from each group were prepared and homogenized, and a concentration of 1:10 de-ionized water was prepared. Solutions were centrifuged at 2500 RPM for 5 min until 3 phases were formed. After discarding the oily phase, the aquatic phase was diluted in de-ionized water. ELISA kits were used, and binding between acylated samples and acylated histamine with the coated antibody was performed. Enzyme-conjugated antibodies were applied and then washed. The absorbance of samples was read at 450 nm on a microplate reader (BioTeK®, PMT 49984, U.S.A.).

Sensory evaluation

Sensory attributes of chilled shrimp (texture, odor, color, and overall acceptability) were evaluated following Rong et al., (2009).

Statistical analysis

Results obtained were statistically analyzed using two-way analysis of variance (ANOVA) by Graph Pad Prism 8.0.2. Statistical analyses were performed to study the influence of different herbal extracts as well as storage periods on the quality of chilled shrimp. Results were expressed as the mean ± SD of three triplicates (Greenhouse and Geisser 1959).

3. RESULTS

A Detailed Phenolic profile for different leaves extract, obtained by HPLC screening, was illustrated in table (1) and figure (1). It was found that OL extract enriched with Oleuropein with a conc. of 17680.880 ug/ml. concerning GT extract, the higher phenolic compound in it was epigallocatechingallate (3798.266 ug/ml), followed by epicatechingallate (1354.432 ug/ml) and caffeine (1338.341 ug/ml). Rutin (198.511 ug/ml) was the higher phenolic compound in MO.

Results in figure (2) showed the effect of the examined extracts applied on chilled shrimp to evaluate its impacts on physic-chemical, and the significance variation ($P < 0.05$) between physicochemical characters of chilled shrimp in control and those in treated with natural plant extracts. Extracts of OL showed the higher impacts on enhancing values of pH, TBA and TMA that reflect freshness of chilled shrimp samples and shelf life of it. As, control group showed higher values of PH, TBA, TMA reflects its incipient spoilage from day 4th of refrigerated storage, while treated samples with MO, GT, OL stayed within accepted range till 14th day of refrigerated storage.

Concerning sensory attributes of chilled shrimp, results in fig. (3) Showed the impacts of MO, GT, and OL extracts on sensory characters (texture, odor, color, and over all acceptability) and shelf life of chilled shrimp**.**

Results in figure (4) showed that MO, GT, and OL extracts positively reduced formation of histamine levels in chilled shrimp all over storage period (14 days), compared to that of control untreated group.

Table 1 Phenolic profiles of MO, GT, and OL extracts by HPLC-DAD.

Phenolic compound	MO	GT	OL
	concug/ml	concug/ml	concug/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

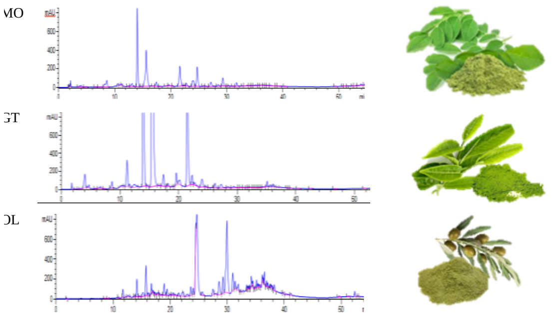

Figure 1 photos and HPLC chromatogram of OL, GT, and MO extracts

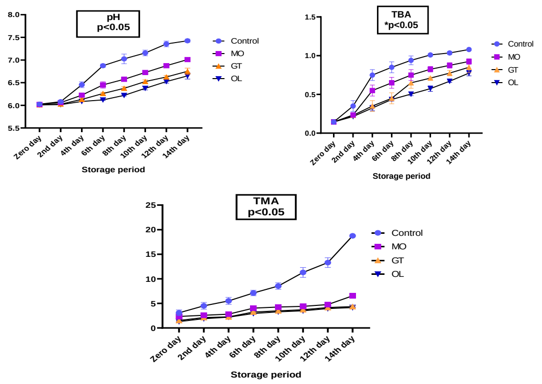

Figure 2 impacts of different plant extracts (MO, GT, and OL) on physicochemical attributes (PH, TBA, and TMA) of chilled shrimp.

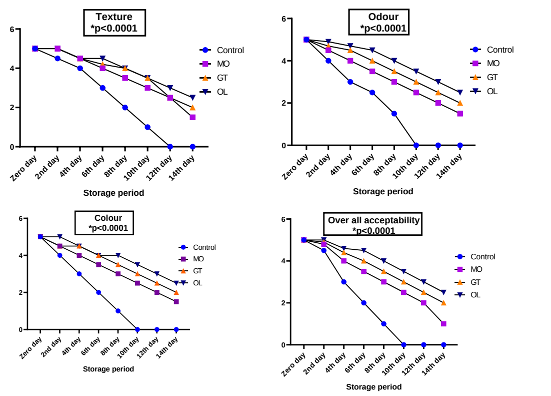

Figure 3 impacts of different plant extracts (MO, GT, OL) on sensory attributes (texture, odor, color, and overall acceptability) of chilled shrimp.

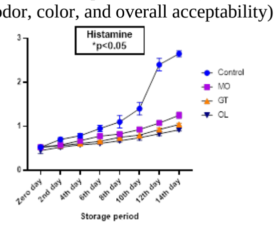

Figure 4 impacts of different plant extracts (MO, GT, OL) on sensory attributes (texture, odor, color, and overall acceptability) of chilled shrimp.

4. DISCUSSION

Nowadays, the customer's approach towards naturally preserved fish meat products has gained interest in overcoming the health risks of synthetic preservatives. In this study, the impact of some plant extracts on shrimp quality was evaluated.

Higher phenolic content MO, GT, and OL extracts highlighted their roles as antioxidants and helped to explain the study's findings, according to a thorough phenolic profile for several leaf extracts that were acquired by HPLC screening. Similar findings were made by Hazra et al. (2012), who recorded that *M. oleifera* is one of the most important sources of natural antioxidants and antimicrobial agents because of the presence of many different types of antioxidant components in its leaves, including flavonoids, ascorbic acid, carotenoids, and phenolics. This, in turn, increases the shelf life of foods containing fat (Al-Juhaimi et al., 2001). Additionally, green tea leaf extract is abundant in flavonoid (Jeyakumari et al., 2021). Furthermore, according to Lee and Lee (2010), oleuropein, luteolin, diosmetin, rutin, verbascoside, apigenin-7-glucoside, and diosmetin-7-glucoside are the main physiologically active compounds of OL. Other notable compounds include hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid, vanillic acid, and vanillin.

In the challenge, results showed the significance of the positive impacts of plant extracts (MO, GT, and OL) on the physico-chemical characteristics (pH, TBA, and TMA) of chilled shrimp. These results agree with those by Elsabagh et al., (2023) in chilled tuna using natural plant extracts. Additionally, Jeyakumari et al. (2021) examined the impact of green tea extract on the biochemical and sensory quality of fish nuggets and found that the treated groups' pH and TMA values slowly changed over time, indicating a significant increase in the shelf life of fish nuggets stored at 2°C. Green tea extract's antioxidant properties are mostly attributable to the presence of polyphenols, particularly catechin (Abdel-Rahman et al. 2011). It has been thoroughly investigated how the presence of phenolic content affects the antioxidant action of green tea extract (Heim et al. 2002). Due to their potential to act as antioxidants, OL has become more and more in demand in recent years for usage in foods, food additives, and functional foods (Lafka et al. 2013).

TMA is considered a marker for fish spoilage that increases in spoiled fish (Feng et al. 2017). During spoilage, trimethylamine oxide (TMAO), choline, and betaine are converted to TMA, which starts to increase gradually and indicates the spoilage of samples (Zhao et al. 2019). The TMA values of the control group significantly increased, while in the treated groups, plant extracts delayed the formation of TMA during the 14^{th} day of storage.

OL extracts showed higher results in enhancing the physico-chemical attributes (PH, TBA, and TMA) of chilled shrimp; this agrees with those by Lahreche et al. (2022), who applied OL to chilled tuna fillets. Elsabagh et al. (2023) also improved the physicochemical properties of fish products by coating them with edible plant extracts. Also, Mansour et al. (2023) revealed that the phenolic compounds present in natural herbal extracts have an antioxidant effect and delay spoilage when incorporated into food.

Concerning sensory attributes of chilled shrimp, results in Table 3 showed the impacts of MO, GT, and OL extracts on sensory characters (texture, odor, color, and overall acceptability) and shelf life of chilled shrimp. A significant variation between the control (untreated) and treated groups revealed enhancement of the overall acceptability of chilled shrimp until the 14^{th} day of refrigerated storage. OL extracts showed higher results in enhancing the sensory attributes and shelf life of shrimp than MO and GT extracts; this agrees with those by Lahreche et al. (2022), who applied OL to chilled tuna fillets. Additionally, Jeyakumari et al. (2021) found that treating fish nuggets with green tea extract improved their sensory qualities and shelf life. They noted that up to the ninth day, there was a significant difference ($p < 0.05$) in texture value between the GTE-treated and control groups. It might be because additional connections or cross-linkages between protein molecules and phenolic compounds, which limit the flexibility of the protein aggregates, cause the gels to become less springy and more rigid (Ranendra et al. 2017).

One of the targets in this study was to control toxic histamine biogenic amine formation in chilled shrimp. The higher impacts on histamine caused by OL extracts may be due to active antioxidants (phenolic and flavonoid compounds), as illustrated by HPLC screening. These results agree with those recorded by previous authors for controlling histamine formation in fish using natural plant extracts, such as Elsabagh et al. (2023) and Nitta et al. (2016).

Nowadays, the customer's approach towards naturally preserved fish meat products gained interest to overcome health risks of synthetic preservatives. In this study impact of some leaves plant extracts on shrimp quality was evaluated.

Higher phenolic content MO, GT, and OL extracts highlighted their roles as antioxidants and also help to explain the study's findings, according to a thorough phenolic profile for several leaf extracts that was acquired by HPLC screening. Similar findings were made by Hazraet al. (2012), who recorded that *M. oleifera* is one of the important sources of natural antioxidants and antimicrobial agents because of the presence of many different types of antioxidant components in its leaves, including flavonoids, ascorbic acid, carotenoids, and phenolics. This, in turn, increases the shelf life of foods containing fat (Al-Juhaimi et al. 2001). Additionally, green tea leaf extract is abundant in a component called a flavonoid (Jeyakumari et al. 2021). Furthermore, according to Lee & Lee (2010), oleuropein, luteolin, diosmetin, rutin, verbascoside, apigenin-7-glucoside, and diosmetin-7-glucoside are the main physiologically active compounds of OL. Other notable compounds include hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid, vanillic acid, and vanillin.

In the challenge, results showed the significance variation positive impacts of plant extracts (MO, GT, OL) on physico-chemical characters (PH, TBA, TMA) of chilled shrimp. These results agree with those by ELsabagh et al (2023) in chilled tuna using natural plant extracts. Additionally, Jeyakumari et al., (2021) examined the impact of green tea extract on the biochemical and sensory quality of fish nuggets and found that the treated groups' pH and TMA values slowly changed over time, indicating a significant increase in the shelf life of fish nuggets stored at 2 °C. Green tea extract's antioxidant properties are mostly attributable to the presence of polyphenols, particularly catechin (Abdel-Rahman et al., 2011). It has been thoroughly investigated how the presence of phenolic content affects the antioxidant action of green tea extract (Heim et al., 2002). Due to their potential to act as antioxidants, OL has become more and more in demand in recent years for usage in foods, food additives, and functional foods (Lafka et al., 2013).

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OL extracts showed the higher results in enhancing physico-chemical attributes (PH, TBA, TMA) of chilled shrimp, this agrees with those by Lahreche et al., (2022) who applied OL on chilled tuna fillets. Also, Elsabagh et al. (2023), enhanced physicochemical of fish products by edible coating incorporated with natural plant extracts. Also, Mansour et al., (2023) revealed that the phenolic compound present in natural herbal extracts have antioxidant effect and delay spoilage when incorporated in food.

Concerning sensory attributes of chilled shrimp, results in table (3) showed the impacts of MO, GT, and OL extracts on sensory characters (texture, odor, color, and over all acceptability) and shelf life of chilled shrimp. A significant variation between control (untreated) and treated groups revealed enhancement of overall acceptability of chilled shrimp till 14th day of refrigerated storage. OL extracts showed higher results in enhancing sensory attributes and shelf life of shrimp than MO, and GT extracts, this agrees with those by Lahreche et al. (2022) who applied OL on chilled tuna fillets. Additionally, Jeyakumari et al. (2021) found that treating fish nuggets with green tea extract improved their sensory qualities and shelf life. They noted that up to the ninth day, there was a significant difference ($P < 0.05$) in texture value between the GTE-treated and control groups. It might be because additional connections or cross linkages between protein molecules and phenolic compounds, which limit the flexibility of the protein aggregates, cause the gels to become less springy and more rigid (Ranendra et al. 2017).

One of the targets in this study was to control toxic histamine biogenic amine formation in chilled shrimp. The higher impacts on histamine caused by OL extracts may be due to active antioxidants (phenolic and flavonoid compounds), illustrated before by HPLC screening. These results agree with results recorded by previous authors for controlling histamine formation in fish using natural plant extracts as Elsabagh et al., (2023) and Nitta et al., (2016).

5. CONCLUSIONS

Results of this study concluded that plant leaf extracts of MO, GT, and OL positively impact all aspects of chilled shrimp quality, including physicochemical, sensory, and control risks of toxic histamine formation. OL extracts proved to be higher in potency than those of MO and GT extracts. Plant extracts are considered promising natural additives that can be safely applied in the fish meat industry.

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