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COMBINED EFFECTS OF VACUUM PACKAGING AND MORINGA LEAF POWDER ON THE QUALITY AND STORABILITY OF NILE TILAPIA (*Oreochromis niloticus*) PICKLE DURING STORAGE

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ABSTRACT

The phytochemical profile of moringa leaf and the combined effects of moringa leaf powder (MLP) with vacuum packaging on the biochemical, bacteriological and sensory attributes of Nile tilapia (*Oreochromis niloticus*) pickle during storage at room temperature (25 ± 5 °C) for a period of 42 days were investigated. There were four treatments, such as control 1 (untreated; air pack), control 2 (untreated; vacuum pack), treatment 1 (1% MLP with vacuum packaging) and treatment 2 (2% MLP with vacuum packaging). The HPLC-ESI-Q-TOF-MS analysis showed the presence of several phenolic acids such as chlorogenic acids and quercetins in moringa leaf. During the storage period, MLP treated pickles exhibited significantly ($P < 0.05$) lower pH, total volatile basic nitrogen, free fatty acids, total viable count, and psychrotrophic bacterial count than the untreated pickles. Moreover, both MLP treated pickles had acceptable sensory characteristics up to 35 days of storage followed by control 2 (28 days) and control 1 (21 days) pickles. Findings of this research demonstrated that 1% MLP treated pickle with vacuum packaging delayed the quality deterioration and extended the shelf life for 14 and 7 days longer than control 1 and control 2, respectively during storage at room temperature. In conclusion, moringa leaf powder could be used as a natural additive of Nile tilapia pickles under vacuum packaging conditions to obtain better quality and storability during storage.

Keywords: Nile tilapia pickles, moringa leaf powder, phytochemicals, storability, storage

INTRODUCTION

Nile tilapia is an important and demanding fish in Bangladesh. Bangladesh has secured the 4th position in Nile tilapia production internationally and the 3rd position in Asia with an annual production of 407,359 metric tons (MT) in 2021-2022 fiscal year (**DoF, 2023**). Nile tilapia farming has expanded globally due to its high productivity, adaptability, and consumer preferences (**de Lima *et al.*, 2017**). But fish and aquatic products frequently have a short shelf life due to microbiological, enzymatic, and chemical reactions, resulting in a decrease in quality and safety (**Vijayan *et al.*, 2021**). These changes lead to the formation of objectionable metabolites such as TVBN, biogenic amines and ammonia (**Monterio *et al.*, 2018**). Again, lipid oxidation poses a risk to the quality of various foods, particularly like fishes, which are rich in unsaturated fats. It can result in alterations in color, flavor, nutritional content, and the storage life of the food product (**Secci & Parisi, 2016**). Pickling is an ancient preservation method, which has been successful with various fish species, indicating its potential for Nile tilapia. However, the storability of ready-to-eat foods is a critical factor in ensuring satisfaction to consumer and efficient product delivery. Due to highly perishable nature of fish, fish pickles have comparatively short shelf life (**Shikha *et al.*, 2018**) than livestock products during storage at room temperature. This knowledge permits better quality control, optimum management, and meeting consumer expectations regarding product freshness and safety (**Isra *et al.*, 2022**). Additionally, customers want to purchase high-quality, functional food items for both consumption and storage. However, pickling by itself is not sufficient to stop fishery products from losing quality and to increase the storage ability of pickled products (**Isra *et al.*, 2022**). Traditionally, various synthetic preservatives have been used, but excessive use of these preservatives cause public health hazards including allergic reactions, asthma, and even cancer etc. that food safety concerns state the necessity for natural alternatives (**Saini & Keum, 2018**). Plant extracts are rich in antimicrobial and antioxidant properties, which offer safer and environment friendly options (**Rathod *et al.*, 2021**). Additionally, vacuum packaging improves the storability of processed fish and fish products more effectively (**Rasul *et al.*, 2022**). Moreover, the incorporation of thyme and rosemary extracts having vacuum packaging was reported as a highly efficient method for prolonging the storage life of fish ball (**Balikçi *et al.*, 2011**).

Recent research demonstrates the effectiveness of natural preservatives like as ginger (**Islam *et al.*, 2022**), water lily (**Dulal *et al.*, 2023**), seaweeds (**Afrin *et al.*, 2023**; **Shahrier *et al.*, 2023**) in extending Nile tilapia fillet's shelf life. Moringa leaf and seed extracts displayed promise as natural antifungal agents, inhibiting spoilage fungi and offering essential nutrients (**Ayirezang *et al.*, 2020**). The presence of phytochemical compounds like steroids, triterpenoids, flavonoids, tannins, alkaloids, and saponins in moringa leaf, can effectively hinder the deterioration process in fish (**Farooq *et al.*, 2021**). Including moringa leaves powder in the diet improves the food's functional characteristics and makes the products more storable over an extended period of time (**Dhibi *et al.*, 2022**; **Isra *et al.*, 2024**). Moringa leaf showed positive results and extended shelf life in case of products like minced *Pangasius hypophthalmus* (**Chakraborty *et al.*, 2017**), mutton patties (**Mashau *et al.*, 2021**), dried *Puntius sophore* (**Rasul *et al.*, 2022**), Nile tilapia fillet (**Putri *et al.*, 2023**), and *Pangasius hypophthalmus* ball (**Isra *et al.*, 2024**). Despite these advancements, there is no existing literature on the use of moringa leaves powder with vacuum packaging on the storability of Nile tilapia pickle. Therefore, the current research was carried out to identify the phytochemical constituents of moringa leaf and to investigate the combined effects of moringa leaf powder and vacuum packaging on the biochemical, bacteriological and sensory attributes of Nile tilapia (*O. niloticus*) pickle during room temperature storage.

MATERIALS AND METHODS

Collection of moringa leaves

Fresh moringa leaves were harvested from the local area of Gazipur Sadar and transported to the Fish Processing Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. The leaves were carefully washed under running water and sun-dried in open air for two consecutive days. Then the leaves were finely ground and sifted using a 40 µm sieve. Following the standard protocol outlined in the Association of Official Analytical Chemists (AOAC, 2010), the proximate composition of powdered moringa leaves was examined in triplicate. The resultant powder was stored at frozen temperature (−18 ±1°C) in airtight plastic bags for further application.

Determination of phytochemical constituents' profile of moringa leaves by HPLC-ESI-Q-TOF-MS

The composition of phytochemicals in aqueous ethanolic (80%) extract of moringa leaves were analyzed by shaking technique following the method of **Chakma *et al*. (2023)**. The phytochemical constituent profile analysis was conducted using an 80% aqueous ethanolic extract of moringa leaves that was obtained using the shaking technique. An acquit high-performance liquid chromatography (HPLC) system was used to analyze the extract. (Waters Corporation, MA, USA) using an ACQUITY UPLC HSS T3 column (150 mm × 2.1 mm, 1.8 µm particle size; Waters Corporation, MA, USA). Following a protocol established by Nakagawa et al. (2020), chromatographic separation was carried out. Chemical formulas were predicted using MassLynx software (Waters Corporation, MA, USA).

Collection of fish sample

Live Nile tilapia (*O. niloticus*) (average weight 900 ± 45 g) was collected from a local fish farm of Gazipur Sadar. Harvested fish was iced immediately maintaining fish: ice ratio 1:1, in an insulated box and transported to the Fish Processing Laboratory of BSMRAU, Gazipur. After being gutted with sharp knives and cleaned with sanitized water, the fish was filleted with sterile knives. Two fillets were taken from each fish, and the fillets were then cut into slices that were 1 cm by 1 cm by 1 cm (length by width by thickness).

Ingredients and Nile tilapia pickle preparation procedure

The fish pickle was made in the product Laboratory of the Department of Fisheries Technology, BSMRAU using **Shikha *et al.* (2018)**'s approach with slight modifications. The ingredients needed to make Nile tilapia pickle are listed in Table 1.

Table 1 Ingredients used for Nile tilapia pickle preparation

Ingredient name	Amount	Ingredient name	Amount
Nile tilapia muscle	500 g	Vinegar	50 ml
Chili powder	20 g	Black pepper	2 g
Turmeric powder	2 g	*"Pachforon"*	10 g
Cumin powder	10 g	Sugar	50 g
Onion	20 g	Salt	40 g
Garlic	80 g	Tomato sauce	33 g
Ginger	10 g	Tamarind	20 g
Cloves	2 g	Sodium benzoate	1 g
Mustard oil	150 ml	MLP	10 g (for 1% MLP) 20 g (for 2% MLP)

The boneless fish pieces were marinated for at least an hour at room temperature using the necessary amounts of salt, chili powder, and turmeric powder. Following that, the marinated fish pieces were fried in mustard oil until they turned brown and kept aside. According to the experimental protocols, to prepare 4 types of pickles including two controls (Control 1 with air packaging; Control 2 with vacuum packaging) and two treatments (Treatment 1 with 1% MLP and vacuum packaging; Treatment 2 with 2% MLP and vacuum packaging), pickles were made separately in individual frying pans. In brief, in 3 different frying pans, onion, garlic and ginger paste were fried till light brown in color. The definite quantities of cumin powder, "*pachforon*", cloves, salt, vinegar, sugar, and MLP were mixed into the spices during the frying process. 1% and 2% MLP were added in 2 pans of fish pickles; however, no MLP was used in the pan containing control pickles. After that, the pre-fried fish pieces were added with the fried spices. Until the vinegar was absorbed, the mixture was cooked. Finally, tamarind water and tomato sauce were added to each pickle. After thoroughly mixing the ingredients, the pickle was cooked for ten more minutes. Lastly, the pickles were allowed to cool at room temperature (Figure 1).

Packaging and storage of pickle

Pickle samples were packed in multilayered polyethene bags (Oriented polypropylene/OPP, Polyvinyl acetate/PVA, Linear low density polyethene pouch/LLDPE). After that, the pickles were kept for 42 days at room temperature (25 ± 5 °C). Every seven days, the pickles' quality parameters were examined until it was determined that they were unfit for human consumption and chemical parameters were subsequently examined as well.

Chemical analyses

Proximate composition—moisture, crude protein (using Kjeldahl Apparatus; model: Digestor, DK 6; Distillation unit, UDK 129, Italy), crude fat (using Soxhlet apparatus; model: SER148 solvent extraction unit, Velp Scientifica Srl, Usmate, Italy), and ash content of pickle samples were analyzed following the standard protocol of AOAC (2010). A pH meter (model: Jenway, Model no. 3510, UK) was used to measure pH directly according to the method of **Afrin *et al.* (2021)**. Using the **AOAC (2010)** method, the total volatile basic nitrogen (TVBN) was calculated and expressed as mg N/100 g fish pickle. Using the **Kirk and Sawyer (1991)** method, the free fatty acid (FFA) of pickle was calculated and expressed as oleic acid equivalent (g/100 g sample).

a. Control 1 and 2 pickle b. 1% MLP treated pickle c. 2% MLP treated pickle

Figure 1 Nile tilapia pickle at zero day

Bacteriological analyses

The fish pickles' total viable count (TVC) and psychrotropic bacterial count (PBC) were estimated using **Shahrier *et al.*'s (2023)** method and reported as log colony forming units (CFU)/g of sample.

Sensory evaluation

The sensory assessment of fish pickles was carried out using a slightly modified version of the Isra et al. (2022) methodology. On the basis of flavor, color, general appearance, taste, and chewiness, a 5-point rating system was used. The sensory evaluation was conducted in various sensory booths. The panel contained ten highly experienced assessors from BSMRAU's Department of Fisheries Technology. Excellent quality is indicated by a sensory score of 1, and poor quality is indicated by a score of 5.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The statistical analysis was performed using the Statistical Analysis System (SAS, 2023, Version 9.4, SAS Institute, Cary, NC, USA). All experiments were conducted three times. A two-way analysis of variance (ANOVA) and a post-hoc test using Duncan's multiple range test were applied to the data. A difference was considered significant at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemical constituents' profile of moringa leaf extracts

Table 2 represents the composition of phytochemicals in aqueous ethanolic (80%) extract of moringa leaf along with the mass spectral data and tentative identification of the compounds detected. According to previous reports, the extracts of moringa leaves contained a total of eleven different compounds (Figure 2) (**Coppin *et al.*, 2013; Duranti *et al.*, 2021**). Two unknown compounds were also found in the extracts. Table 2 presents an identification of the peaks. The moringa leave extracts contained mainly chlorogenic acid and several type of quercetin. Furthermore, it also contained glucosinolates, which is characteristic phytochemical of moringa.

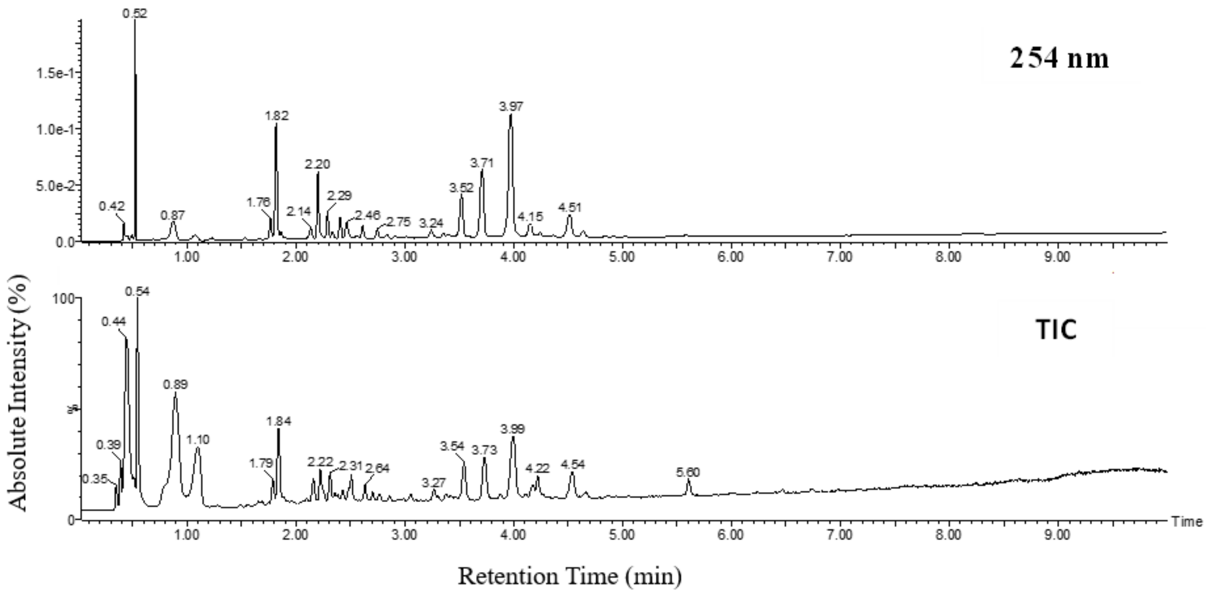

Figure 2 HPLC-ESI-Q-TOF-MS chromatograms of the extracts prepared from moringa leaf powder.

Singh *et al.* (2009) reported that the existence of polyphenolic compounds might play a significant role in their comprehensive antioxidant capabilities and anti-quorum sensing characteristics. Thus, moringa has the potential to serve as an excellent option for the nutraceutical, functional food, and biopharmaceutical sectors. Moreover, the major phytochemicals found in moringa leaf, including cryptochlorogenic acid, isoquercitrin, and astragalin, are responsible for the antioxidant, anti-hypertensive, and anti-inflammatory properties (**Verma *et al.*, 2009; Vongsak *et al.*, 2012**).

Table 2 Mass spectral data and tentative identification of compounds detected in moringa leaf powder

Peak	Proposed Identification	Retention Time (min)	Formula	m/z experimental [M-H]-	m/z calculated [M-H]-	Mass Error (ppm)
1	Quinic acid	0.52	$C_7H_{12}O_6$	191.0563	191.056	0.7
2	Glucosoonjnain	0.87	$C_{20}H_{29}NO_{15}S_2$	586.0948	586.090	4.7
3	Glucomoringin	1.10	$C_{20}H_{29}NO_{14}S_2$	570.0971	570.095	2.0
4	Neochlorogenic acid	1.76	$C_{16}H_{18}O_9$	353.0878	353.087	0.5
5	Chlorogenic acid	1.82	$C_{16}H_{18}O_9$	353.0878	353.087	0.5
6	Quercetin-di-O-glucoside	2.20	$C_{27}H_{30}O_{17}$	625.1385	625.140	-2.0
7	Crypto-chlorogenic acid	2.29	$C_{16}H_{18}O_9$	353.0878	353.087	0.5
8	Rutin	3.52	$C_{27}H_{30}O_{16}$	609.1486	609.146	3.0
9	Isoquercetin/ Isoquercitrin	3.71	$C_{21}H_{20}O_{12}$	463.0898	463.088	2.1
10	Quercetin-O-b-D-glucose-acetate isomer	3.97	$C_{23}H_{22}O_{13}$	505.0975	505.098	-0.7
11	Astragalin/ Luteoloside	4.15	$C_{21}H_{20}O_{11}$	447.0916	447.093	-1.2
12	Unknown	4.51	$C_{23}H_{22}O_{12}$	489.1032	489.1033	-0.1
13	Unknown	5.61	-	326.0684	-	-

Dried moringa leaf contain quercetin at a concentration of 100 mg/100 g in the form of quercetin-3-O-β-D-glucoside, also known as isoquercitrin. Quercetin is known for its numerous therapeutic properties (**Lako *et al.*, 2007; Atawodi *et al.*, 2010**). Additionally, chlorogenic acid is a dihydrocinnamic acid ester and a primary phenolic acid found in moringa (**Amaglo *et al.*, 2010**). **Coppin *et al.* (2013)** also reported that 70% methanolic extracts of moringa leaf contained various flavonoid compounds, such as quercetin and kaempferol glycosides, acetyl glycosides, malonyl glycosides, and succinoyl glycosides, found in substantial quantities. Peak 12 was predicted to have the chemical formula C23H22O12 based on its m/z. The substances may be the glycosides such as Kaempferol 3-(6"-acetylglucoside) or Luteolin 7-(6"-acetylglucoside).

Proximate composition

Proximate composition analysis showed that the moringa leaf contained 8.11% moisture, 25.72% crude protein, 5.87% crude lipid and 9.39% ash content (Table 3). Raw tilapia muscle had moisture, crude lipid, crude protein, and ash contents of 73.56, 6.17, 18.27, and 1.16%, respectively which shows that the Nile tilapia was of the premium quality and fresh. Proximate composition analysis showed that the moringa leaves contained 8.11% moisture, 25.72% crude protein, 5.87% lipid and 9.39% ash content. The moisture, crude protein, crude lipid, and ash content of control (1 and 2), treatment 1 and treatment 2 were ranged from 40.79 to 42.88%, 23.48 to 27.21%, 17.52 to 19.31%, and 2.99 to 5.63%, respectively just after preparation of pickles (Table 3).

Table 3 Proximate composition of fresh muscle and pickles of Nile tilapia[1]

Proximate Composition	Fresh Nile tilapia muscle	Nile tilapia Pickles		
		Control 1 & 2	Treatment 1	Treatment 2
Moisture (%)	73.56 ± 2.06	42.88 ± 1.81[a]	41.28 ± 1.09[a]	40.79 ± 1.51[a]
Crude protein (%)	18.27 ± 0.41	23.48 ± 1.77[b]	25.22 ± 1.23[ab]	27.21 ± 1.42[a]
Crude lipid (%)	6.17 ± 1.02	17.52 ± 0.94[a]	18.77 ± 0.72[a]	19.31 ± 0.88[a]
Ash (%)	1.16 ± 0.20	5.63 ± 0.26[a]	2.99 ± 0.17[c]	3.52 ± 0.21[b]

[1]Values expressed as mean ± standard deviation (SD) (n=3). Different letters indicate significant differences ($P < 0.05$) among the means and same letters indicate no significant differences.

The protein content of fresh Nile tilapia muscle measured at 18.27%, which increased to 23.48, 25.22 and 27.21 in control, treatment 1 and treatment 2 pickles, respectively. An increase in the protein content is also reported in case of pomegranate and rosemary treated fish patty (**Martínez-Zamora *et al.*, 2020**), moringa treated sun-dried *P. sophore* (**Rasul *et al.*, 2022**) and bael pulp powder, guava powder, and dragon fruit peel powder treated *Wallago attu* nuggets (**Vidyarthi *et al.*, 2022**). These findings indicate that the increase of protein content in MLP treated pickles might be attributed to the protein content present in moringa leaf and a decrease in the water content.

pH value

All of the samples had pH values between 4.64 and 4.74 on day zero. A progressive rise in pH levels were observed during the storage period (Fig. 3). pH values were reached to 6.63 ± 0.17, 6.78 ± 0.19, 6.90 ± 0.21 and 6.61 ± 0.31 at the 28th, 35th, 42th and 42th day of room temperature storage for control 1, control 2, treatment 1 and treatment 2 pickles respectively (Figure 3). However, increment of pH was comparatively lower in moringa treated pickles compared to control pickles. The pH values of all the treatments remained within the maximum permissible limit of 6.8 to 7.0 for fishery products recommended by **Erkan *et al.* (2011)**. Tilapia fillets soaked into a moringa leaf extract solution exhibited lower pH values compared to the untreated group due to the influence of antimicrobial substances presents in moringa leaf extract, specifically tannins and flavonoids (**Putri *et al.*, 2023**). More or less similar trend of pH values were reported using moringa leaf extracts in *Pangasianodon hypophthalmus* fillets (**Greeshma *et al.*, 2019**) and pomegranate peel powder in breaded cod stick (**Panza *et al.*, 2021**). It was found that plant extracts/powder have the ability to stabilize and maintain a pH level below 7 (**Guan *et al.*, 2019**). The accumulation and formation of volatile chemicals and alkaline molecules such as trimethylamine and ammonia throughout the storage period could be the cause of the pH increase (**Martínez-Zamora *et al.*, 2020**).

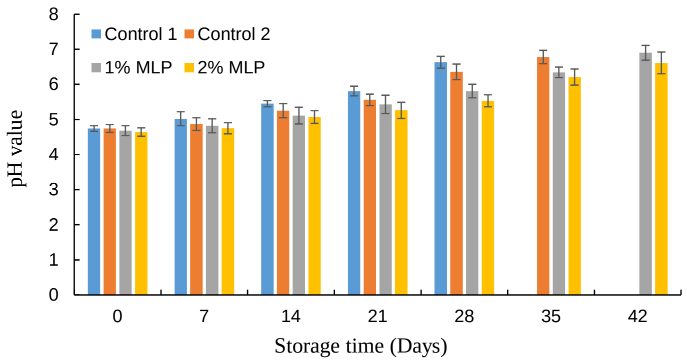

Figure 3 Variations in pH values of Nile tilapia pickle during storage at room temperature. The error bars represent means ± SD of triplicates.

Total volatile basic nitrogen

With the increment of storage period, the amount of total volatile basic nitrogen (TVBN) in all control and moringa-treated pickles enhanced significantly ($P < 0.05$). The TVBN values for pickles in control 1, control 2, treatment 1 and treatment 2 were 38.64, 39.11, 37.17 and 35.54 mg N/100 g, respectively at 28th, 35th, 35th and 35th day of storage time (Figure 4), which was within the maximum acceptable level of 35-40 mg N/100g (**Connell, 1995**). This delay in TVBN formation in MLP treated pickles can be attributed to a more rapid reduction in bacterial population, a decreased bacterial capacity for oxidative deamination of non-protein nitrogenous compounds, or a combination of both factors (**Frangos *et al.*, 2010**). Gelatin-enriched moringa extract was used in *Mustelus mustelus* fillet, and positive results or reduced TVBN values were reported (**Mezhoudi *et al.*, 2022.** Accordingly, vacuum-packed dried *P. sophore* treated with 2.5% moringa water extract showed reduced TVBN values. (**Rasul *et al.*, 2022**) *and in* vacuum-packed mackerel fish balls (**Balikçi *et al.*, 2022**) with natural extracts (thyme, rosemary and basil) during storage.

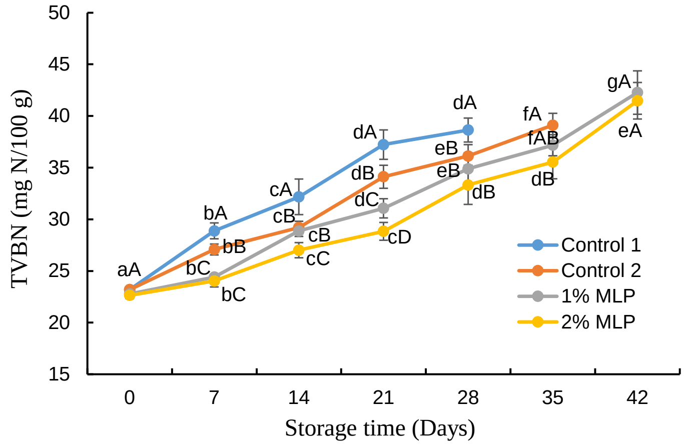

Figure 4 Variations in total volatile basic nitrogen (TVBN) values of Nile tilapia pickle during storage at room temperature. The error bars represent means ± SD of triplicates. [a-g] Small letters in each line indicate significant ($P < 0.05$) differences of means within the storage time. [A-D] Capital letters indicate significant ($P < 0.05$) differences of means within the treatments.

Free fatty acid

The initial free fatty acids (FFA) value of tilapia pickles ranged from 3.29 to 3.54% oleic acid (Figure 5). As less than 7% oleic acid is the acceptable limit (**Bimbo, 1998**), control 1 and control 2 sample was found unacceptable at 28th and 35th day with a value of 7.68 and 7.57% oleic acid, respectively. But, at day 42th, FFA values were 6.58% and 6.42% in treatment 1 and treatment 2 pickle samples, respectively. The rate of rise in FFA value was lower in MLP treated pickles. **Ninan *et al.* (2011)** reported that the rise in FFA could be attributed to the cooking process, as the cooking of minced meat may have deactivated the lipolytic enzymes. Different natural extracts also showed positive results in lowering the FFA value such as whole radish extract treated *Tor khudree* steaks (**Maqbool *et al.*, 2020**), rosemary extract treated Atlantic mackerel (**Uçak *et al.*, 2011**) and vacuum-packed mackerel fish balls with thyme, rosemary and basil extracts (**Balikçi *et al.*, 2022**). However, at 42th days of storage, there were no significant ($P > 0.05$) variations in the FFA values of the pickles treated with MLP at 1% and 2%.

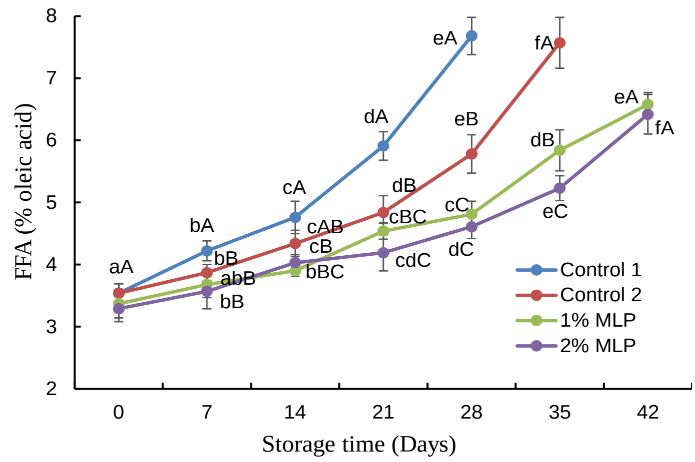

Figure 5 Variations in free fatty acid (FFA) values of Nile tilapia pickle during storage at room temperature. The error bars represent means ± SD of triplicates. [a-f] Small letters in each line indicate significant ($P < 0.05$) differences of means within the storage time. [A-C] Capital letters indicate significant ($P < 0.05$) differences of means within the treatment.

Total viable count

The initial total viable count (TVC) value indicates that top quality fish was utilized for pickle preparation as the recommended allowed limit is ≤7 log CFU/g fish muscle (**ICMSF, 1986**). Pickles' TVC rose significantly ($P < 0.05$) as storage duration increased., and reached to 6.48, 6.74, 6.46 and 6.17 log CFU/g for control 1, control 2, treatment 1 and treatment 2 pickles at 21th, 28th, 35th and 35th days of storage period, respectively (Figure 6). According to the results, addition of MLP with vacuum packaging significantly ($P < 0.05$) decreased the TVC in the fish pickles and this is because MLP holds numerous biologically active substances such as saponins, phenolics, flavonoids, tannins and alkaloid compounds that have antibacterial action (**Khalid *et al.*, 2023**). However, at 42th days of storage, the 2%

MLP-treated pickles had a lower TVC than the 1% MLP-treated pickles; however, there were no significant ($P > 0.05$) differences between the values. MLP was also found to be effective in reducing the TVC values of herbal chicken sausages (**Jayawardana *et al.*, 2015**). Similarly, application of rosemary essential oil showed lower bacterial count in products like refrigerated carp fish fingers (**Abdeldaiem *et al.*, 2017**) and refrigerated bonito fish patties (**Guran *et al.*, 2015**). The presence of antimicrobial phenolic compounds in MLP could be a contributing factor to the reduced value of TVC in the treated pickles.

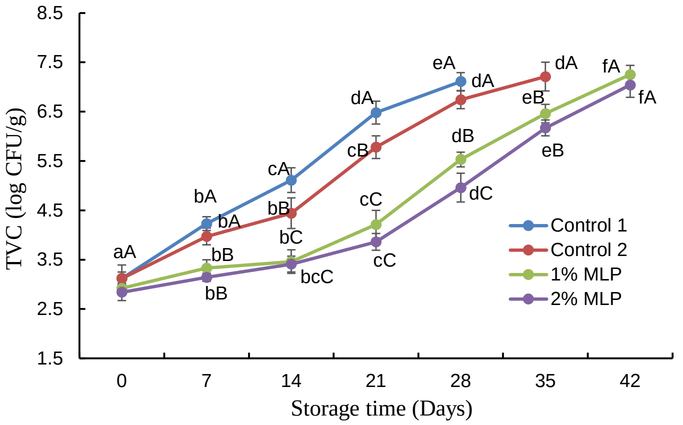

Figure 6 Variations in total viable count (TVC) values of Nile tilapia pickle during storage at room temperature. The error bars represent means ± SD of triplicates. $^{a-f}$ Small letters in each line indicate significant ($P < 0.05$) differences of means within the storage time. $^{A-C}$ Capital letters indicate significant ($P < 0.05$) differences of means within the treatment.

Psychrotrophic bacterial count

The initial psychrotrophic bacterial count (PBC) of the tilapia pickles at zero day were ranged from 2.63 to 2.91 log CFU/g. As storage time increased, there was a significant ($P < 0.05$) increase of PBC in of both of the treated and control pickles (Figure 7) and PBC reached to 6.78, 6.91, 6.17 and 5.78 log CFU/g for control 1, control 2, treatment 1 and treatment 2 pickles at 28th, 35th, 35th and 35th days of storage period, respectively (Figure 7). Pickles treated with moringa had lower PBC values than control which might be due to the presence of flavonoids and tannin that can inhibit the growth of fish spoilage bacteria (**Abdallah *et al.*, 2023**). More or less similar result was also reported by **Anastasio *et al.* (2014)**, who reported that use of rosemary effectively reduced the bacterial load in vacuum-packed swordfish steaks. **Panza *et al.* (2021)** also reported lower psychrotrophic bacterial count when pomegranate peel powder was applied in breaded cod sticks. The lower value of PBC in moringa treated pickles might be for the synergistic impacts of antimicrobial compounds present in moringa and vacuum packaging.

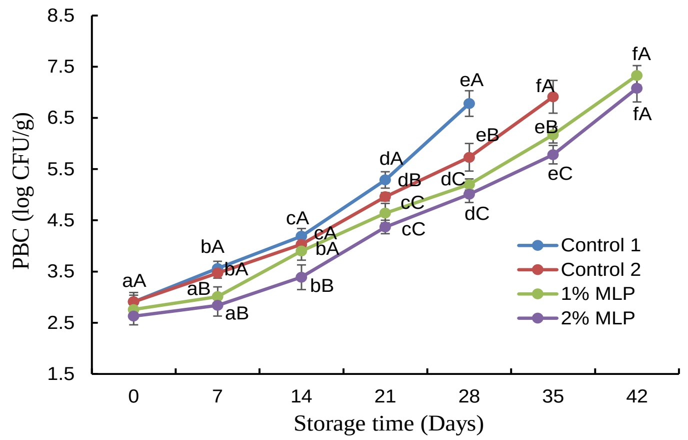

Figure 7 Variations in psychrotrophic bacterial count (PBC) values of Nile tilapia pickle during storage at room temperature. The error bars represent means ± SD of triplicates. $^{a-f}$ Small letters in each line indicate significant ($P < 0.05$) differences of means within the storage time. $^{A-C}$ Capital letters indicate significant ($P < 0.05$) differences of means within the treatment.

Sensory evaluation

A sensory score of 5 was considered as unacceptable for human consumption (**Isra *et al.*, 2022**). Sensory attributes such as taste, flavor and chewiness were deteriorated at a lower rate in MLP treated pickles than control pickles. Sensory evaluation results revealed that the control 1, control 2, treatment 1 and treatment 2 pickles were found unacceptable at 28th, 35th, 42th, and 42th days, respectively during storage at room temperature (Table 4). Treatment 1 showed comparatively better scores than treatment 2 pickles. Initially, the color and general appearance of pickles was better in control pickles than MLP treated pickles. At the day of rejection, all pickle samples possess a moderate to strong odor with a point of 5 approximately. Although the general appearance was within the acceptable limit throughout the entire storage, both type of pickles (MLP treated and control) felt bad in taste as well as felt rubbery and elastic when chewed at the day of rejection. Considering all the sensory characteristics, the pickles treated with MLP exhibited superior sensory quality than control one which may be because of MLP's functional properties. (**Hodas *et al*., 2020**). The results of the sensory examination also match the bacteriological and chemical data those were discussed earlier. Improved and better sensory attributes were also reported using pomegranate peel powder in breaded cod stick (**Panza *et al.*, 2021**), thyme and rosemary extract in vacuum-packed mackerel fish balls (**Balikçi *et al.*, 2022**), rosemary in swordfish steaks (**Anastasio *et al.*, 2014**) and pomegranate peel powder in fish burgers (**Abou-Taleb, 2022**) and moringa leaves powder in pangasius fish ball (**Isra *et al.*, 2024**). All of these not only made the product last longer but also made the product highly appreciated to consumers.

Table 4 Variation in sensory properties of Nile tilapia pickle during storage at room temperature[2]

Storage time (Days)	Control 1	Control 2	Treatment 1	Treatment 2
Color				
0	1.00 ± 0.00^{aC}	1.00 ± 0.00^{aC}	1.22 ± 0.12^{aB}	1.50 ± 0.13^{aA}
7	1.41 ± 0.11b^{AB}	1.32 ± 0.05^{bB}	1.55 ± 0.10^{bA}	1.63 ± 0.18^{aA}
14	2.10 ± 0.22^{cA}	1.76 ± 0.36^{cA}	2.17 ± 0.27^{cA}	2.30 ± 0.25^{bA}
21	3.14 ± 0.23^{dA}	2.38 ± 0.34^{dB}	2.68 ± 0.33^{dAB}	2.77 ± 0.18^{cAB}
28	4.02 ± 0.25^{eA}	3.52 ± 0.16^{eB}	2.95 ± 0.22^{dC}	3.18 ± 0.15^{dC}
35		4.16 ± 0.25^{fA}	3.54 ± 0.21^{eB}	3.78 ± 0.23^{eA}
42			4.83 ± 0.21^{fA}	5.00 ± 0.00^{fA}
Flavor				
0	1.00 ±0.00^{aA}	1.00 ± 0.00^{aA}	1.12 ± 0.20^{aA}	1.17 ± 0.17^{aA}
7	1.38 ± 0.21^{aA}	1.33 ± 0.09^{aA}	1.48 ± 0.12^{bA}	1.54 ± 0.12^{bA}
14	1.77 ± 0.30^{aAB}	1.58 ± 0.21^{aB}	1.81 ± 0.08^{bAB}	2.03 ± 0.16^{bA}
21	3.70 ± 0.23^{bA}	2.74 ± 0.25^{bB}	2.26 ± 0.11^{bC}	2.39 ± 0.21^{bBC}
28	5.00 ± 0.00^{cA}	4.11 ± 0.29^{cB}	2.67 ± 0.27^{cC}	2.97 ± 0.22^{cC}
35		5.00 ± 0.00^{dA}	3.90 ± 0.47^{dB}	4.11 ± 0.13^{dB}
42			4.87 ± 0.19^{eA}	5.00 ± 0.00^{eA}
Taste				
0	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}	1.18 ± 0.24^{aA}	1.08 ± 0.09^{aA}
7	1.40 ± 0.33^{bA}	1.18 ± 0.29^{aA}	1.60 ± 0.36^{abA}	1.16 ± 0.13^{aA}
14	2.62 ± 0.22^{bA}	1.29 ± 0.33^{aC}	2.10 ± 0.24^{bB}	1.51 ± 0.14^{bC}
21	3.21 ± 0.25^{cA}	2.73 ± 0.29^{bAB}	2.55 ± 0.30^{bAB}	2.48 ± 0.10^{cB}
28	5.00 ± 0.00^{dA}	3.13 ± 0.22^{bB}	2.65 ± 0.31^{bB}	2.76 ± 0.34^{cB}
35		5.00 ± 0.00^{cA}	3.19 ± 0.18^{cC}	3.73 ± 0.22^{dB}
42			5.00 ± 0.00^{dA}	5.00 ± 0.00^{eA}

General appearance				
0	1.00 ± 0.00^{aB}	1.00 ± 0.00^{aB}	1.13 ± 0.12^{aA}	1.23 ± 0.18^{aA}
7	1.29 ± 0.05^{bB}	1.26 ± 0.10^{aB}	1.36 ± 0.05^{aA}	1.45 ± 0.09^{aA}
14	1.59 ± 0.16^{bA}	1.42 ± 0.22^{aB}	1.48 ± 0.30^{aA}	1.90 ± 0.12^{bA}
21	2.71 ± 0.21^{cA}	2.25 ± 0.15^{bB}	2.01 ± 0.17^{bB}	2.26 ± 0.38^{bAB}
28	3.95 ± 0.29^{dA}	2.79 ± 0.16^{cB}	2.13 ± 0.27^{bC}	2.33 ± 0.15^{bC}
35		4.21 ± 0.26^{dA}	2.84 ± 0.16^{cC}	3.44 ± 0.22^{cB}
42			4.78 ± 0.19^{dA}	4.95 ± 0.21^{dA}
Chewiness				
0	1.14 ± 0.08^{aA}	1.14 ± 0.08^{aA}	1.26 ± 0.12^{aA}	1.28 ± 0.13^{aA}
7	1.61 ± 0.14^{bA}	1.45 ± 0.16^{bA}	1.66 ± 0.08^{bA}	1.71 ± 0.12^{bA}
14	2.46 ± 0.18^{cA}	1.94 ± 0.12^{cB}	2.18 ± 0.16^{cA}	2.30 ± 0.11^{cA}
21	3.52 ± 0.26^{dA}	2.50 ± 0.14^{dC}	2.75 ± 0.13^{dBC}	2.87 ± 0.09^{dB}
28	5.00 ± 0.00^{eA}	3.59 ± 0.19^{eB}	3.10 ± 0.14^{eC}	3.43 ± 0.16^{eBC}
35		5.00 ± 0.00^{fA}	4.04 ± 0.21^{fB}	4.36 ± 0.16^{fB}
42			5.00 ± 0.00^{gA}	5.00 ± 0.00^{gA}

[2]Values expressed as mean ± SD (n=10). $^{a-g}$ small letters indicated significant (*P* < 0.05) differences of means during various storage time for same treatment. $^{A-D}$ Capital letters indicate significant (*P* < 0.05) differences of means among the treatments on the same day.

CONCLUSION

The investigation of the HPLC-ESI-Q-TOF-MS showed MLP contains several phenolic acids and polyphenols such as chlorogenic acids and quercetins. According to the present research findings, pickles treated with MLP show lower pH, TVBN, FFA, TVC, and PBC values than control pickles. The study's conclusions display that adding MLP to fish pickles had antibacterial and antioxidant qualities that slowed down bacterial growth, ammonia production, and other lipid oxidation products which improved the pickles' overall quality and shelf life. The findings of the bacteriological, biochemical, and sensory analyses demonstrated that when vacuum-packed with 1% MLP, Nile tilapia pickles maintained their quality and had a 35-day shelf life when stored at room temperature. In order to preserve different fish and value-added fisheries products, MLP can be used as a functional food ingredient and natural preservative in the food industry.

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