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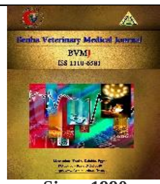

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Original Paper

Natural gums fortified CMC edible coat application to enhance quality of chilled *Oreochromis niloticus* fillets

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ABSTRACT

Consumers desire fresh-like, minimally processed products due to growing worries about the use of preservative in foods, such as fish. Specific requirements and preservation measures are required to reduce the activity of spoilage microorganisms. One of the novel ways for controlling these dangers during storage is to use edible coatings coated with bioactive substances. The purpose of this study is to create a bioactive edible coating from Carboxymethyl cellulose (CMC) reinforced with Arabic gum (AG), Mastic gum (MA), and Olibanum gum (OG) natural extracts, and to assess their effects on the physicochemical, microbiological, and sensory properties of chilled Nile Tilapia fish fillets (*Oreochromis niloticus*). Physico-chemical (TBARS, TVB-N and pH), Bacteriological (total mesophilic count, *Staphylococcus aureus* counts, psychrotrophic count and coliform count) as well as sensory analysis of coated groups showed a significant difference ($P < 0.05$) with control un-coated group. Coated groups fortified with natural gums; CMC/MG (2%), CMC/AG (2%) and CMC/OG (2%) showed the highest reduction in total mesophilic count, *Staphylococcus aureus* count, psychrotrophic count and coliform count. Furthermore, compared to the uncoated control samples, the rate of increase in TBARS, TVB-N and pH for all coated treatments was lower. Additionally, the coated groups showed good quality and acceptability qualities according to the sensory evaluation. In general, application of edible coating enriched with natural gums extracts improved the quality parameters of chilled Tilapia fish fillets.

1. INTRODUCTION

Fish consumption has increased globally in recent years, owing to its low cost and high nutritional content (Afifi et al., 2023). It accounts for around 20% of daily animal protein consumption in developing and under developed nations (FAO, 2022). Consumer preferences for natural preservatives over chemicals lead to the preservation of fish using natural extracts (Ibrahim et al., 2023; Ibrahim et al., 2024). One of the novel approaches in preservation of fish is edible coatings and films, which have the potential to assist sustainable fish production in Asia by reducing packaging waste, increasing shelf life, and improving quality (Tavares et al., 2021; Elsabagh et al., 2023).

Antimicrobial active packaging, which incorporates antimicrobial substances into packaging materials, can preserve fresh fish and extend its shelf life (Eshaghi et al., 2024).

Natural edible coatings operate as an antioxidant and antibacterial barrier, extending the shelf life and quality of fish and related items (Elsabagh et al., 2023). Also suppress microbe development and lower lipid oxidation in fresh fish (Maleki and Mohsenzadeh, 2022).

Polysaccharides, such as carboxy-methyl cellulose (CMC), are ideal natural polymers for packaging film production due to their thermal properties, availability, biodegradable properties and low cost (Panahirad et al., 2021). Furthermore, it is a colorless, non-toxic ionized polysaccharide with superior film-forming properties (Beigomi et al., 2018). Moreover, it resists oxygen, carbon dioxide, and lipid breakdown. The linear structure of cellulose in CMC, which can be fortified with a range of functional additives, results in a flexible, robust, transparent, and stable coating with diverse applications in the food sector (Gregorova et al., 2015).

Gums are a form of water-soluble, hydroxyl-rich, high-molecular- weight carbohydrate polymer found in plants (exudate and non-exudate), algae, microbes, seeds, and other sources. Gums are widely employed in the food sector for thickening, gelling, stabilizing, bulking, and emulsifying qualities, as well as fat alternatives, biodegradable packaging materials, and encapsulating ingredients (Da Silva et al., 2020). Furthermore, gums have innovative techno-functional features that improve emulsification, holding capacity, and film formation (Gao et al., 2024).

Arabic gum (AG) is a hydrophilic and ionic heteropolysaccharide extracted from the discharge of certain acacia trees (Acacia senegal). It is a water-soluble carbohydrate and polypeptide complex (Prasad et al., 2022). It is edible, an effective encapsulate that protects encapsulated goods, and a stabilizer (E414) in the food sector due to its emulsification properties and low viscosity (de Boer et al., 2019). This hetero-polysaccharide is frequently utilized as a coating material due to its properties such as volatile retention, emulsification, affordability (Todorović et al., 2022), negatively charged properties, moderate viscosity, considerable solubility, ease of use, inhibition of oxidation reactions, and colorless solutions (Zabot et al., 2022).

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Its use in food is growing because it has positive advantages such as improved food texture, higher water binding capacity, good emulsification, and longer shelf life (Mugo et al., 2020).

Mastic gum (MG) is an extract from the *Pistacia atlantica* tree with well-known antioxidant and anticancer properties (Rahman, 2018). Furthermore, investigations have demonstrated that MG has remarkable antibacterial capabilities (Paraschos et al., 2007). It is a complex mixture of various phytochemical groups, including mono-, sesqui-, and triterpenoids, as well as phenolic components, many of which have been linked to biological activities such as antibacterial, antioxidant, anti-inflammatory, and anticancer properties (Pachi et al., 2020). Mastic gum is popular in the culinary and pharmaceutical industries due to its pleasant scent and medicinal properties.

Frankincense gum (also known as olibanum) is a fragrant material obtained from Boswellia trees. Frankincense (Boswellia sacra) is a gum resin that has strong antibacterial activity against foodborne pathogens including *S. aureus* and *E. coli* (Almutairi et al., 2022).

Therefore, the current study sought to determine the effectiveness of CMC edible coating supplemented with natural extracts of AG, MG, and OG on the quality of Nile Tilapia filets during chilled storage

2. MATERIAL AND METHODS

Approval Ethics

This study was approved by Scientific Research ethics Faculty of Veterinary Medicine, Benha University, Egypt with ethical approval number (BUFTM 40-11-2023)

2.1. Material

2.1.1 Preparation of extracts:

Ethanolic extracts from mastic gum (MG) *(Pistacia lentiscus)* according to Letsiou et al. (2024), Arabic gum (AG) (*Acacia Senegal)* according to Saleh et al. (2021) and Olibanum gum (OG) (*Boswellia papyrifer*) according to Elhaddad et al. (2023) at National Research Centre, Dokki, Cairo, Egypt.

2.1.2 Fish fillets

Tilapia fillets (*Oreochromis niloticus*) were procured from fish markets in El-Menuofia governorate, sterile packaged then transported directly in an insulated ice container to the food microbiology laboratory at Animal Health Research Institute, Shipin El-Koom lab, for further treatment and analysis.

2.1.3. Edible coat preparation

The edible coat of CMC was prepared according to Mohamed *et al*. (2023) with some modifications to be supplemented with previously prepared plant extracts; AG (2%), MG (2%), and OG (2%) extracts.

2.2. Methods

2.2.1. Experimental design

Tilapia fillets have been classified into five distinct groups., the first one was uncoated (control), the 2nd group was coated with a plain coat of CMC (10%), the 3rd group was the CMC coat fortified by AG (2%), the 4th group was the CMC coat fortified by MG (2%) and 5th group was the CMC coat fortified by OG (2%). Samples were stored at 4 ±1 ºC. Samples were analyzed for physicochemical, bacteriological, and sensory properties during the chilled storage period (zero, 2nd, 4th, 6th, 8th, 10th, 12th, and 14th days) as the experiment was conducted in triplicate and mean values were statistically monitored.

2.2.2. Physico-chemical evaluation

The thiobarbituric acid reactive substances (TBARS) values were examined as stated at AOAC (2009), there values used to determine the samples' oxidative condition. The TVB-N content was assessed using the procedure outlined in AOAC (2009) and reported as mg /100 g of sample. The pH values were calculated using pH meter, the procedure outlined in Zenebon et al. (2008).

2.2.3. Bacteriological quality evaluation

Tilapia fillets samples were examined throughout the storage period to evaluate the total mesophilic count (TMC) using the pour plate method on plate count agar (HIMDIA, M091S) at 35°C (ISO 4833- 1, 2013), *Staph. aureus* count on Baird Parker agar base (HIMEDIA, M043) (incubated at 37°C for 48 hours (FDA, 2001), Psychrotrophic bacterial count (PBC) on plate count agar (HIMEDIA, M091S) at 4°C (FDA, 2001) and total coliform count according to ISO 4832 (2006) on violet red bile agar media (HIMEDIA, M049) then incubated at 37°C for 24 hours.

2.2.4. Sensory Evaluation performed according to ISO 13299 (2003)

Fish quality is mostly determined by sensory qualities, which are the key factors that buyers can consider when buying fresh fish. The color, odor, texture, and overall acceptability are all examples of sensory attributes. The evaluation was based on a ten-point scale (1-10) to determine.

2.3. Statistical analysis

Data was processed using the graph pad prism application. The analysis of variance was performed on all data using one-way ANOVA. Values were expressed as means and SD. p-values were found significant at $p \leq 0.05$ at a confidence level 95%.

3. RESULTS

Data in Fig. (1) Showed the effect of CMC as a natural coat fortified with natural gums (AG, MG, and OM) applied on Nile tilapia fillets to evaluate their impacts on physico-chemical attributes. The results revealed a significant variation ($p < 0.05$) between the physicochemical characters of samples in the control group and CMC treated with natural gums. While, there were no considerable differences between treated groups ($p > 0.05$) as all gum extracts showed higher impacts on enhancing values of TBARS, TVB-N, and pH that reflect the freshness of chilled tilapia fillets samples and prolonged their shelf life. As, control group showed higher values of TBARS, TVB-N and pH which reflects its incipient spoilage from day 4th of chilled storage, while CMC treated samples with AG, MG and OM stayed within accepted range till 14th day of chilled storage.

The results in Fig. (2) represented the bacteriological quality of control, CMC coated group as well as CMC treated groups. The data revealed that the mean counts of total mesophilic count (TMC) ranged from 4.04 to 3.55 (log cfu/g) at zero day of storage. The count of the control group increased to 5.35 log cfu/g at 4th day of cold storage. Also, the count increased in CMC coated group but at a lower rate than control one. TMC decreased from 3.55, 3.57, and 3.55 in AG 2%, MG 2%, and OG 2% to 1.70, 1.57, and 1.55 (log cfu/g), respectively then it increased gradually till the end of the storage period (14th day). Also, all of the used gums extracts have antibacterial effect against *S. aureus* count till 4th day of storage then began to increase till the end of the chilled storage period. Furthermore, the mean coliform and Psychrotrophic bacterial count of the control group increased gradually from 2.72 and 3.33 to 3.48 and 5.44 (log cfu/g), respectively which significantly differed from CMC coated group at 3rd day of storage. While, there was no considerable difference between all treated groups. The treated group have complete antibacterial effect against coliform group at 8th day of storage.

Table 1 shows the sensory aspects of Nile Tilapia fillet samples. Sensory assessment scores showed a significant decrease in the overall acceptability of the control group compared to the CMC and treatment groups during cold storage. It has been discovered that when fish spoils, the flavors become highly fishy, rancid, and rotten.

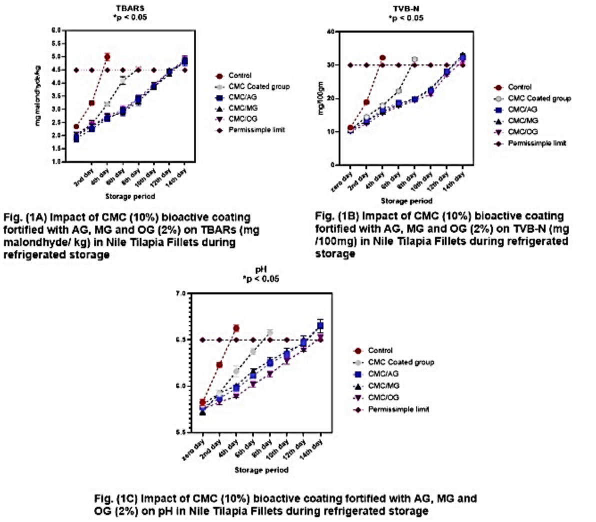

Fig. (1A) Impact of CMC (10%) bioactive coating fortified with AG, MG and OG (2%) on TBARs (mg malondhyde/ kg) in Nile Tilapia Fillets during refrigerated storage

Fig. (1B) Impact of CMC (10%) bioactive coating fortified with AG, MG and OG (2%) on TVB-N (mg /100mg) in Nile Tilapia Fillets during refrigerated storage

Fig. (1C) Impact of CMC (10%) bioactive coating fortified with AG, MG and OG (2%) on pH in Nile Tilapia Fillets during refrigerated storage

Figure (1). Impact of CMC (10%) bioactive coating fortified with AG, MG and OG (2%) on physico-chemical characteristic of Nile Tilapia Fillets during chilledstorage.

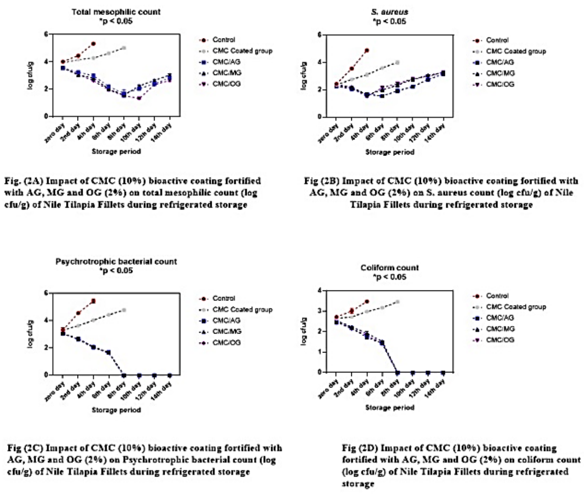

Fig. (2A) Impact of CMC (10%) bioactive coating fortified with AG, MG and OG (2%) on total mesophilic count (log cfu/g) of Nile Tilapia Fillets during refrigerated storage

Fig (2B) Impact of CMC (10%) bioactive coating fortified with AG, MG and OG (2%) on S. aureus count (log cfu/g) of Nile Tilapia Fillets during refrigerated storage

Fig (2C) Impact of CMC (10%) bioactive coating fortified with AG, MG and OG (2%) on Psychrotrophic bacterial count (log cfu/g) of Nile Tilapia Fillets during refrigerated storage

Fig (2D) Impact of CMC (10%) bioactive coating fortified with AG, MG and OG (2%) on coliform count (log cfu/g) of Nile Tilapia Fillets during refrigerated storage

Figure (2). Impact of CMC (10%) bioactive coating fortified with AG, MG, and OG (2%) on the bacteriological quality of Nile Tilapia Fillets during chilled storage.

Table 1 Impact of CMC (10%) bioactive coating fortified with AG, MG and OG (2%) on Sensory attributes of Nile Tilapia Fillets during chilled storage

groups / storage period	Control	CMC coated group	CMC/AG	CMC/ MG	CMC/OG
zero day	9.55 ± 0.07^{a}	9.75 ± 0.06^{a}	9.75 ± 0.10^{a}	9.77 ± 0.03^{a}	9.75 ± 0.07^{a}
2nd day	6.15 ± 0.21^{a}	7.65 ± 0.20^{b}	8.25 ± 0.06^{c}	8.35 ± 0.07^{c}	8.27 ± 0.03^{c}
4th day	3.50 ± 0.35^{a}	6.20 ± 0.28^{b}	7.10 ± 0.10^{c}	7.15 ± 0.07^{c}	7.25 ± 0.05d^{c}
6th day	S	5.25 ± 0.35^{a}	6.30 ± 0.04^{b}	6.40 ± 0.14^{b}	6.35 ± 0.07^{b}
8th day	S	3.90 ± 0.14^{a}	5.75 ± 0.05^{b}	5.77 ± 0.10^{b}	5.80 ± 0.06^{b}
10th day	S	S	5.10 ± 0.03^{a}	5.12 ± 0.10^{a}	5.10 ± 0.07^{a}
12th day	S	S	4.80 ± 0.07^{a}	4.75 ± 0.07^{a}	4.95 ± 0.02^{b}
14th day	S	S	3.82 ± 0.07^{a}	3.85 ± 0.02^{a}	3.80 ± 0.03^{a}

The results are considered significant ($p < 0.05$) when the same row contained different small letters. 10-9: Excellent, 8-7: Very Good, 7-6: Good, 4-5: Acceptable, 2-3: Unacceptable, 1-2: Bad, S: Spoiled.

4- DISCUSSION

Fresh fish has a shorter shelf life (even when refrigerated) than other animal protein meals such as meat and eggs due to its neutral pH, substantial moisture content, and higher levels of nitrogen from non-protein molecules, all of which promote bacterial and chemical deterioration. As a result, customers interest in naturally preserved fish meat products has grown to avoid the health risks accompanied with synthetic preservatives (Ibrahim et al., 2023) as well as the use of edible coatings, which have become a current and future food packaging trend in food sustainability specially fortified bioactive compounds (Mansour et al., 2023).

TBARS is an important biomarker for subsequent lipid oxidation because it measures the amount of malonaldehyde formed during the oxidation of lipid hydroperoxides (Leaes et al., 2022). According to the Egyptian guideline (EOSQC, 2005), 4.5 mg malondhyde/kg in fish flesh is usually regarded as the maximum permissible amount, after which fish meat is considered unsafe for ingestion. After 4 days of storage, the control sample became un safe to be consumed, while the coated group with CMC could not be consumed at 8th day of storage.

Packaging of meats with CMC films affect the lipid oxidation and formation of TBARs (Khezrian and Shahbazi, 2018). All of the CMC-coated treatments were within the permissible range for TBARs levels till the end of the 12th day. TBARs levels in all treatments were higher than the permitted range on the end of chilled storage period (14th day). Several bioactive substances may also be added to CMC coatings to provide antioxidant and antibacterial effects (Mohamed et al., 2020).

Arabic gum, which was employed as a coat for Indian mackerel, delayed the modification of TBARS during the storage period (Binsi et al., 2016). Also, El-Sheikh (2014) employed AG as a coat for chicken boneless breast and discovered that there was no difference in TBARS values over storage time, with the exception of the control sample, which exhibited an increase in TBARs value since the coated samples had lower baseline levels of TBARS. The antioxidant effect of mastic gum occurs due to flavonoid and phenlic compounds (Rahman, 2018).

TVB-N is regarded as a quality parameter for the freshness of fish meat because several volatile compounds, including ammonia and methylamine, formed during fish meat storage in chilled temperatures due to microbial activity, so TVB-N is considered one of the most important biomarkers of fresh fish meat detection (Bekhit et al., 2021). increased with chilled storage duration. The least change in TVB-N values was observed in CMC coated groups treated with natural gums, since these groups were within the authorized range with TVB-N levels of 30 mg N/100 g (maximum limit according to EOSQC (2005), ES: 3494) until the 12th day of storage, at which point they became unacceptable. Arabic gum consists of galactose, rhamnose, arabinose, and glucuronic acid which acts as antioxidant prevent protein deterioration (Piazzon et al., 2012).

The pH of the control group was significantly higher than that of the treatment group, indicating that microbial enzymes released during storage can raise the pH and cause quality loss (Farag, 2012). According to Mansour et al. (2023), CMC edible coating reduced the pH change. The low pH of CMC-treated OG groups after storage could possibly be due to their antioxidant and antibacterial activities (Salavati Hamedani et al., 2022). Roberts et al. (2012) discovered that OG found in Northern Africa, Canada, Western Asia, and India has chemical components such as hydroxyl isoleucine, which have antioxidant properties.

Using CMC edible coating prevents the access of these microorganisms via fish flesh and delays their growth by acting as a gas and moisture barrier, which has various effects on the growth of different types of bacteria (Galus and Kadzińska, 2015; Binsi et al., 2016; Mansour et al. 2023). CMC/AG, CMC/MG and CMC/ OG have antibacterial effect as it decrease the count of TMC, *S. aureus*, PBC, and Coliform count (Binsi et al. 2016; Kocturk et al., 2021; Salavati Hamedani et al., 2022).

According to Rigling et al. (2019), α-Pinene accounts for a majority of MG. α-Pinene's antibacterial activity has been widely reported (de Sousa Eduardo et al, 2018). It is possible that the presence of α-Pinene contributes to the antibacterial properties of MG. MGRE contained α-Linolenic and enoic acids, which have been linked to antibacterial effects in prior research (Tabanca et al., 2020).

The rapid growth of microorganisms in the control group led the sensory score to fall below 4 on the fourth day of storage (Wenjiao et al., 2013). When fish spoils, it becomes exceedingly fishy, rancid, and putrid (Elsabagh et al., 2023). These undesired changes in appearance, texture, flavor, and odor lower the quality. Spoilage induced by bacteria creates volatile amines and biogenic amines, all of which have unpleasant and undesirable off tastes.

5. CONCLUSIONS

The results showed that coating Nile Tilapia filets with CMC incorporated with MG, AG, and OG resulted in decreases in TBARs, pH and TVB, were observed during the chilled storage period. Also, CMC/MG, CMC/AG and CMC/ OG enhance the bacteriological quality and sensory attributes.

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