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Innovative Chitosan-Based Coating for Enhance Preservation and Sensory Quality of Rainbow Trout (*Oncorhynchus mykiss*) Fillets Using Microencapsulated Milk Thistle (*Silybum marianum*) Extract

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ABSTRACT: This study investigated the effects of chitosan-coated milk thistle (MT) extract on the quality of rainbow trout (*Oncorhynchus mykiss*) fillets for 21 days at 4°C. The fillets were divided into four groups: uncoated (control group), coated with chitosan only (Ch), free milk thistle extract (Ch + MT), and microencapsulated MT extract (Ch + Micro). The coatings were applied at different concentrations (1.5, 2, 2.5, and 3% w/v) and analysed for chemical (pH, TBA, TVB-N), microbiological (psychrotrophic bacteria and total viable count), and sensory properties. The study found that chitosan coating with encapsulated MT effectively inhibited microbial growth and chemical spoilage and extended the shelf life of rainbow trout fillets. Microencapsulation also slowed down MT release, enhancing sensory properties. The encapsulated samples showed no significant loss in color, odor, or overall acceptability, making it a promising alternative for maintaining the quality and freshness of rainbow trout fillets.

Keywords: Active Biopolymer, Active Packaging, Biodegradable Films, Chitosan, Milk Thistle, Shelf-life.

Introduction

The rainbow trout (*Oncorhynchus mykiss*), belonging to the salmonidae family, holds a prominent position among the globally farmed aquaculture fish species. According to the FAO report in 2019, Iran was acknowledged as the primary producer of freshwater rainbow trout, contributing to 28% of the global production, which amounted to 708,489 tons (FAO/ FishStat, 2020). Rainbow trout provides superior quality protein alongside all the essential amino acids. Furthermore, it is abundant in unsaturated fatty acids, predominantly omega-3 fatty acids which make the product highly **prone to** both microbiological and chemical deterioration (D'Agaro *et al*., 2022). In addition to low oxidative stability and enzymatic autolysis, the metabolic activities of microorganisms contribute to a limited shelf-life in seafood products (Greenlee *et al*., 2007). Nowadays, the biggest challenge for fresh fish is the prolongation of shelf-life, particularly for refrigeration

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(approximately 5–7 days) (Zouharová *et al*., 2023). Therefore, the short shelf-life of the raw fish reveals the requirement of developing novel preservation methods. Over the past decade or two, active packaging has been recommended to fulfill customer requirements and enhance the protective properties of food packaging by incorporating of antimicrobial agents into the packaging system (Yucel, 2016). Edible films and coatings have emerged as highly promising active food packaging technologies, particularly for perishable food products like fish fillets, to effectively extend their shelf-life (Gennadios *et al*., 1997). To date, among the film-forming biopolymers, polysaccharides have been reported as a good base ingredient (not allergenic, tasteless and odorless) to offer a shelf-life extender for fish (G.Volpe *et al*., 2010; Gutierrez *et al*., 2009; Kilincceker *et al*., 2009a; Ojagh *et al*., 2010; Salgado *et al*., 2015). Chitosan, as a cationic biopolymer, has been widely applied in forming edible films and coatings due to its solubility in acetic and hydrochloric acid, relatively stable chemical structure, biodegradability, biocompatibility, high antimicrobial activity and non-toxicity (Alves *et al*., 2018; Ke et al., 2021; Volpe et al., 2015). Active ingredients such as antimicrobial agents and antioxidant substances can be incorporated into film-forming to increase the durability of foods and consumer confidence (Alves *et al*., 2018; Beigzadeh Ghelejlu *et al*., 2016; Chandra Mohan *et al*., 2016; Gutierrez *et al*., 2009). Moreover, the demand for natural antioxidant and antimicrobial compounds is growing together with consumer awareness towards the use of harmful synthetic preservatives (Ježek & Buchtová, 2007). In this respect, plant-derived bioactive compounds have received far more attention because of their strong antioxidant, antimicrobial activities and safety (Beigzadeh Ghelejlu *et al*., 2016; Gutierrez *et al*., 2009; Madene *et al*., 2006).

Silybum marianum or **milk thistle** (MT) is an annual/biennia herb belonging to the Asteraceae family that commonly grows wild worldwide, including the northern, western, and southern areas of Iran. Flavonolignans is the main active constituent of *S. marianum* seeds referred to as silymarin (approximately 4-6%) (Evren & Yurtcu, 2015), which possesses antioxidant, anti-inflammatory, anti-arthritic, and antimicrobial properties (Fanoudi *et al*., 2020; Rehman *et al*., 2020). MT can be exploited as a promising alternative to synthetic preservatives. However, the direct incorporation of plant extracts into food as complex system, raises significant concerns like undesirable impacts on the integrity of the food chemical composition or physical stability and degradation of the bioactive **compounds′ biological activity** (Ghaly *et al*., 2010; Sawale *et al*., 2017). To overwhelm these challenges, an encapsulation approach can be employed to prevent unwanted reactions, protect unstable bioactive compounds, improve their oxidative stability during storage and processing and dictate the controlled delivery (Bouarab Chibane *et al*., 2019; Gombotz & Wee, 2012; Mozafari *et al*., 2006; Saberi-Riseh *et al*., 2021). In this regard, calcium alginate gel generally identified as a wall material used to trap active agents. Several studies have been conducted on the encapsulation of functional compounds from plant extracts within the food matrix and also the use of such compounds in the preparation of coatings and edible films (Chongsrimsirisakhol & Pirak, 2023; Lee *et al*., 2018; Muñoz-Shugulí *et al*., 2021; Stojanovic *et al*., 2012).

In the present study, the effect of incorporating MT (active coating) in direct (free) and microencapsulated forms into a chitosan-based edible film on extending the shelf life of trout fillets was investigated. In order to evaluate the efficacy of the active coating, various parameters such as pH, total volatile basic nitrogen (TVB-N), Thiobarbituric acid (TBA) value, bacterial growth and sensory characteristics were studied in rainbow trout fillets stored at 4 ℃ for 21 days.

Materials and Methods

- *Materials*

All chemicals used in the study were of analytical grade and were obtained from Merck, Darmstadt, Germany. Milk thistle (MT) seeds were kindly provided by the Ministry of Agriculture, Jahad, Isfahan, Iran, and the Medicinal Plants Research Center, Iran.

- *Preparation of milk thistle aqueous extract*

The MT extract was prepared by conventional water extraction according to the following procedure: 100 g of the MT sample was milled and then extracted with distilled water (500 mL) at 60 ℃ with stirring (model HP-840, Alfa Company, Iran) for about 1 h. The extract was then extracted with distilled water (500 mL). The solution was then centrifuged at 7000 rpm for 10 min using a Sigma 2-16P centrifuge (Germany). Finally, the supernatant was filtered through a 0.45-µm cellulose filter (Alltech Associates, Deerfield, IL, USA) and stored in dark glass containers at 4 ℃ until ready for use (Sajadi et al., 2016).

- *Preparation of Alginate hydrogel beads for encapsulation of MT extract*

The MT extract was micro-encapsulated using the emulsion extrusion technique, following the procedure of Chan (2011). The alginate solution was prepared by adding 3g of sodium alginate powder to 100ml of distilled water, stirring until fully dissolved. The solution was left to stand at room temperature for a night to remove bubbles. 100 ml sodium alginate solution was mixed with 2 ml of MT extract, then agitated and sprayed using an INOTECH IE-50 Encapsulator (Switzerland) into a 0.05 M calcium chloride solution. This created sol-gel microcapsules, containing the alginate and MT extract. The microcapsules were left to harden in the $CaCl_2$ solution for a duration of 30 minutes under continuous agitation, resulting in the formation of sol-gel microcapsules. The extract-loaded alginate beads were sieved and rinsed twice with sterile deionized water to remove calcium chloride solution. Tissue paper was used to absorb excess water and dry the wet microcapsules to the appropriate moisture level for further analysis.

- *Preparation of coating solution*

An aqueous chitosan solution was prepared as described previously by Ojagh *et al*. (2010) with minor modifications. Briefly, 2 g of chitosan powder dissolved in 100 mL of 0.1 M acetic acid and stirred for 2 h at 95 °C. Then, solution was filtered through Whatman No. 3 paper to remove any undissolved solids. Glycerol was used as a plasticizer in a chitosan solution (0.75 ml/gr) and agitated using a stirrer/hot plate at 60 °C for 30 min. The film-forming solution was then combined with encapsulated and free MT extract in concentrations ranging from 1.5% to 3%, stirred for 1 hour, and used to prepare an edible coating.

- *Preparation of trout fillets*

Live rainbow trout was purchased from

a local fish market (Tehran) and immediately transported to the laboratory for further processing. Then they were filleted in the size of (6×6 cm and 2 cm thickness) after decapitated, gutted and skinned by hands and washed using drinkable water in the lab. Fillet samples coated using the immersion method divided into four groups: (1) uncoated samples (control), (2) fillets coated solely with chitosan (Ch), (3) fillets coated with free milk thistle extract (Ch + MT), and (4) fillets coated with microencapsulated MT extract (Ch + Micro).

Fillet samples were coated individually for 1 minute, dried at 25 °C for 15 minutes, and stored in sealed sterile plastic petri dishes in a refrigerator at 4 °C. All treatments were randomly analyzed within 21 days of storage (1, 7, 14 and 21) at room temperature.

- Antioxidant activity assessments

- DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity of MT extract was determined following the procedure Wang *et al*. (2008). Different concentrations of MT extract (100 µL) were added to 3.9 mL of a methanolic solution containing DPPH (0.1 mM). The mixture was then vortexed for 5 min and left to stand in the dark for 30 min at the ambient temperature. The absorbance was measured at 517 nm against a blank using a UV–vis spectrophotometer (T70, Japan). The scavenging ability was determined using the following equation [1]:

$$\text{Scavenging activity (\%)} = (A_{517\ control} - A_{517\ sample})/A_{517\ control} \times 100 \qquad (Eq.1)$$

Where A_{control} and A_{sample} are the absorbance value of DPPH with pure ethanol and MT extract at 517 nm respectively. The IC_{50} is defined as the effective concentration of the antioxidant extract necessary to inhibit 50% of the DPPH free radicals which was attained by interpolation from the following linear regression equation [2]:

$$y = 15.386x + 9.53 \qquad (Eq.2)$$

Where y is the antioxidant activity of the extract and x is the concentration of the MT extract. MT extract could inhibit DPPH free radicals, with an IC_{50} of 2.63 µg/mL. According to the obtained results from the IC_{50} test, the concentrations of 1.5, 2, 2.5 and 3% were chosen for the experiments.

- Chemical analysis

- pH measurements

For pH determination, approximately 5 g of fillet sample was mixed with 50 mL of distilled water and agitated for 5 minutes. After the samples were homogenized, the pH was measured using a digital pH meter (Metrohm, Switzerland) (Sallam & Samejima, 2004).

- TBA

TBA (Thiobarbituric Acid) was quantified using a colorimetric method (Kirk & Sawyer, 1991). Briefly, 200 mg of fillet sample was placed into a 25 ml volumetric flask, and 1-butanol was added to reach the volume. The mixture was vortexed for homogenization. After filtration, 5 ml of the resulting solution was transferred to a stoppered test tube and mixed with 5 mL of TBA reagent. Subsequently, the test tube was kept in a water bath at 95 ºC for 120 min to allow the reaction occurred. After cooling the solution to room temperature, the absorbance of the sample was measured at 530 nm using a UV/Vis spectrophotometer. Distilled water was

utilized as a blank in this measurement. The TBA value was expressed as mg malondialdehyde (MDA) per kilogram (kg) and calculated using the following equation [3]:

$$TBA = \frac{(Abs_{sample} - Abs_{blank}) \times 50}{200} \qquad (Eq\ .3)$$

- TVB-N

TVB-N (Total Volatile Basic Nitrogen) was determined following the method described by AOAC (2002). For this, 10 g of the sample was weighed and transferred to the distillation bottle along with 2 g of MgO (magnesium oxide). The mixture was then mixed with 300 ml of distilled water. Steam distillation was carried out using a Kjeldahl apparatus. The distillation process lasted for 30 minutes, and the distillate was trapped in a flask containing 10 mL of 2% boric acid and an indicator solution of methyl red in ethanol (0.1:100 w/v). The contents of the collection flask were then titrated from yellow to red with 0.1 N sulfuric acid. TVB-N value was expressed as mg of nitrogen per 100 g of fish sample and calculated from the following equation [4]:

$$TVB - N = V \times 14 \qquad (Eq\ .4)$$

Where v is a volume (mL) of H_2SO_4 used.

- Microbiological analysis

In a sterile environment, 5 g of samples were weighed and placed in a stomacher bag along with 45 ml of Ringer's solution. The mixture was then homogenized for 1 min using a stomacher (Lab Blender 400). Serial dilutions were subsequently prepared from the homogenized mixture for microbiological analyses. Total viable counts (TVC) were enumerated using plate count agar (PCA, Merck, Germany) which incubated at 28 ℃ for 3 days. In addition, total psychrotrophic count (TPC) was determined according to Ariyapitipun et al. (1999). In this regard, **psychrotrophic bacteria** were enumerated using PCA and incubated at 10 ℃ for 7 days and expressed as colony-forming units (CFU).

- Sensory evaluation

A panel of 11 semi-trained evaluators aged 20-27 (six females and five males), evaluated the sensory properties of raw fish samples, including odor, color, and overall acceptability. Samples were randomly coded and presented in visible light using a 5-point Hedonic scale ranging from 5 (extremely good) to 1 (extremely bad) was employed. Scores below 4.0 were rejected, and the sensory attributes were considered unacceptable. The panelists were divided into six genders. (Fan *et al*., 2008; Ojagh *et al*., 2010).

- Statistical analysis

The experimental data was analyzed using one-way ANOVA, and significant differences were determined using Duncan's multiple range test (DMRT) with a 95% confidence level. Results were presented as means ± standard deviation, and statistical analysis was performed using SPSS software (version 26).

Results and discussion

- Chemical analysis

- pH

Figure1 shows the pH values of the filleted trout with and without coating during storage at 4 ℃ for 21 days. The Initial pH was 5.72, that is in agreement with the report by Tooryan & Azizkhani (2020) and slightly lower as reported by Kilincceker *et al*. (2009b). Postmortem period increases pH due to the decomposition of nitrogenous compounds which negatively influences various qualities of the product during storage

including odor, color, and texture (Abbas *et al*., 2009; Simeonidou *et al*., 1997). Ludorf & Meyer (1973) report suggests fish's acceptable pH ranges from 6.8-7.0. In this study, the pH of the control sample enhanced gradually from 5.63 to 7.25 during 21 days of storage which was slightly higher than the acceptable limit, whereas in Ch treatment, the final pH reached a value of 6.97. There were no significant ($P < 0.05$) changes in pH values for uncoated trout fillet and coated samples with chitosan, and no significant differences in pH values for free and microencapsulated MT, likely due to the preservative impact of the MT extract. The Ch + Micro treatment with 3% MT effectively protects a product during storage by maintaining a pH below 6.19. This is due to the MT extract's preservative effect, which reduces bacterial growth and protects against substrate decomposition. This finding aligns with previous studies (Berizi *et al*., 2018; Pabast *et al*., 2018; Volpe *et al*., 2015).

- TBA changes

TBA measurement is an indicator of secondary lipid oxidation and provides evidence for the production of malondialdehyde, a compound responsible for oxidative rancidity in the fish fillets (Ulu, 2004). TBA contents can be considered as quality indicators of frozen, chilled, or kept in ice fish (Kuusi *et al*., 1975). It is stated that TBA values and sensory analysis are closely correlated (Barnett *et al*., 1991). According to conducted studies, the acceptable limit of TBA was set between 7-8 mg MDA/kg. However, the limit value for acceptability in a very good and good material should be at least 3 and 5 mg MDA/kg respectively (Bergner *et al*., 1969). The TBA changes in different treatments of trout fillets kept at 4 ℃ for 21 days are shown in Figure 2. Based on the results, the range of TBA changes was between 0.09- 0.11 mg MDA/kg fish that reached 4.3±0.39 -8.3±0.23 mg MDA/kg fish during the storage. The control group exhibited the highest changes, while the combined sample (Ch + Micro treatment containing 3% MT) showed the lowest changes. TBA content of chitosan coating alone (Ch) revealed no significant difference as compared with the control ($P < 0.05$). Nevertheless, the TBA value of Ch treatment did not exceed the threshold value on 21st day, while, such value for the control was slightly higher than the threshold limit. Coated samples contained free MT extract, and protected trout fillets from oxidation by sustaining the TBA values lower than 2.3±0.44 mg MDA/kg fish for the first 7 days of storage and below 5.0±0.57 mg MDA/kg fish over 21 days. Encapsulated treatments, however, protected the sample more effectively from the beginning of the storage by maintaining the TBA value below 4.3± 0.39 mg MDA/kg fish until the end of the storage. The study reveals that MT extract, contain phenolic components mainly silymarin and silybin that are capable of quenching free radical reactions by donating hydrogen to enhance oxidative stability. Encapsulation could protect MT extract from evaporation and decomposition during storage, therefore, the release of phenolic compounds became slower during storage. Coating materials on fish' surface, inhibited the diffusion of oxygen on the surface, hence the oxidation retarded. Pabast *et al*. (2018) reported that *Satureja khuzestanica* EOs could hinder the lipid oxidation of lamb meat.

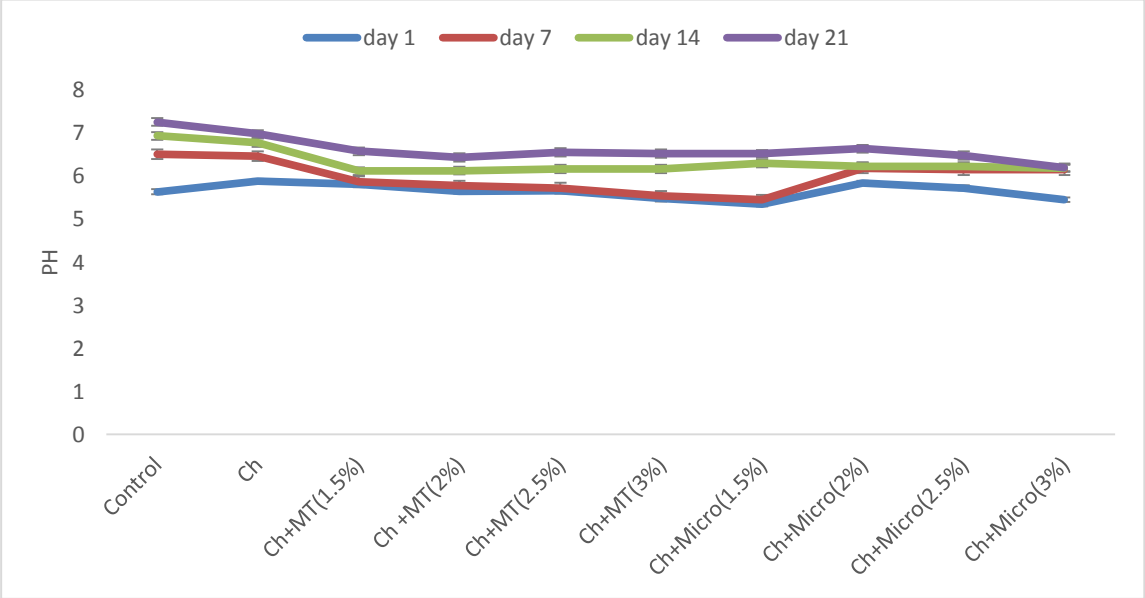

Fig. 1. The pH value of filleted trout samples during 21 days storage at 4 °C (uncoated trout fillet (control), coated with solely chitosan (Ch), coated with free MT (Ch + MT) and microencapsulated MT (Ch + Micro)), Data are means ± SD.

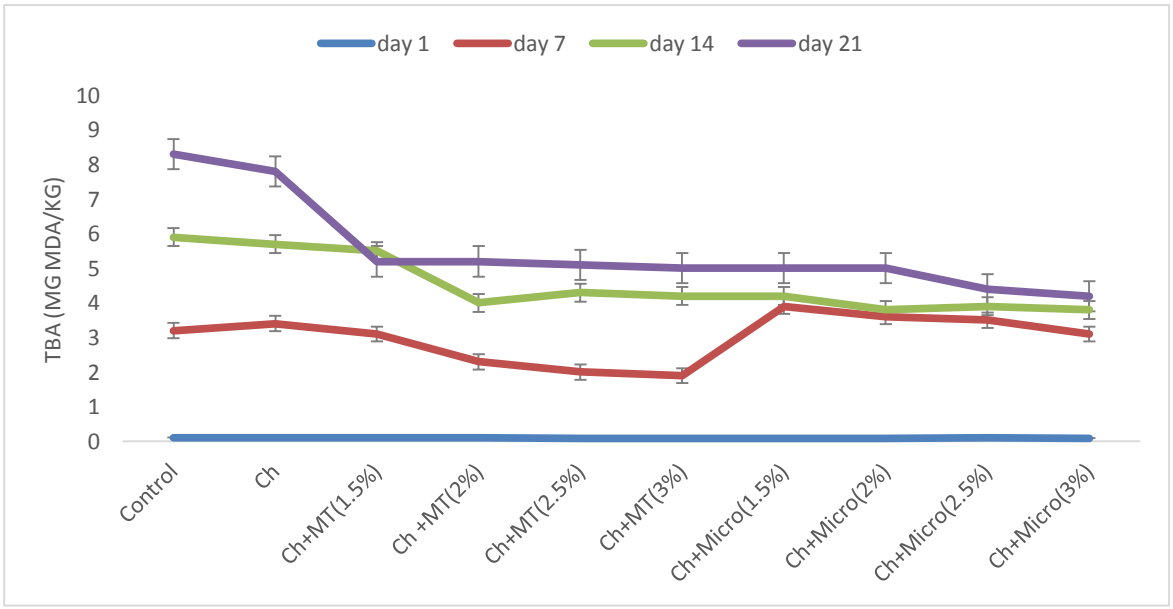

Fig. 2. The TBA value of filleted trout samples during 21 days of storage at 4 °C (uncoated trout fillet (control), coated with solely chitosan (Ch), coated with free MT (Ch + MT) and microencapsulated MT (Ch + Micro)), Data are means ± SD.

- TVB-N changes

TVB-N, as an imperative criterion, is commonly used to evaluate fish spoilage. An increase in the TVB-N indicates the existence of nitrogenous-containing molecules including trimethylamine, di-methylamine and ammonia, which mainly derive from the breakdown of proteins and nonprotein nitrogenous compounds due to the activity of proteolytic bacteria and intracellular enzymes (Yuan *et al*., 2016). According to the proposed criteria by

(Amegovu *et al*., 2012)., the quality of fish and seafood products based on TVB-N values is classified as follows: "high quality" if TVB-N is up to 25 mg/100 g, "good quality" if TVB-N is up to 30 mg/100 g, "marketable" if TVB-N is up to 35 mg/100 g, and "spoiled" if TVB-N exceeds 35 mg/100 g. Meanwhile, (Gimnez et al., 2002) suggested that TVB-N levels of 25 mg/100 g is the highest acceptable level for trout flesh. Figure 3 illustrates the effects of the edible coatings on the total volatile base nitrogen (TVB-N) levels of the fillets during storage. As can be seen, the TVB-N content enhanced during 21-day storage in all groups, particularly in the control which disclosed the highest increase ($P < 0.05$). Indeed, in the control sample, the TVB-N value was above the threshold limit after 14 days which were assessed unpleasant for human consumption, while in the coated samples, TVB-N content was below 25 mg/100 g until day 21 of storage. In the case of Ch treatment, the production of TVB-N was statistically lower than that of the control sample ($P < 0.05$), corresponding to the antimicrobial and polycationic properties of chitosan (Kanatt *et al*., 2013). Our results are in agreements with the results of Duran & Kahve, (2020) and Pabast *et al*., (2018). Moreover, Ch + MT treatments were capable of preserving the TVB-N production under 11±0.87 mg/100 g for the first 7 days of storage and below 22.7±1.01 mg/100 g during the entire storage. While in encapsulated ones, a slow and regular increase of TVBN production was detected from the beginning of storage by sustaining TVB-N value below 13.8±0.75 mg/100 g for the first 7 days of storage and under 20.6 mg/100 g until the end of the storage. These results indicate that encapsulated-loaded MT extract could intensify antimicrobial and antioxidant activities over the free ones by avoiding the bioactive compounds from evaporation and disintegration during storage. In this regard, similar trends were reported by (Ojagh *et al*., 2010; Volpe *et al*., 2015) which confirmed the results of our study.

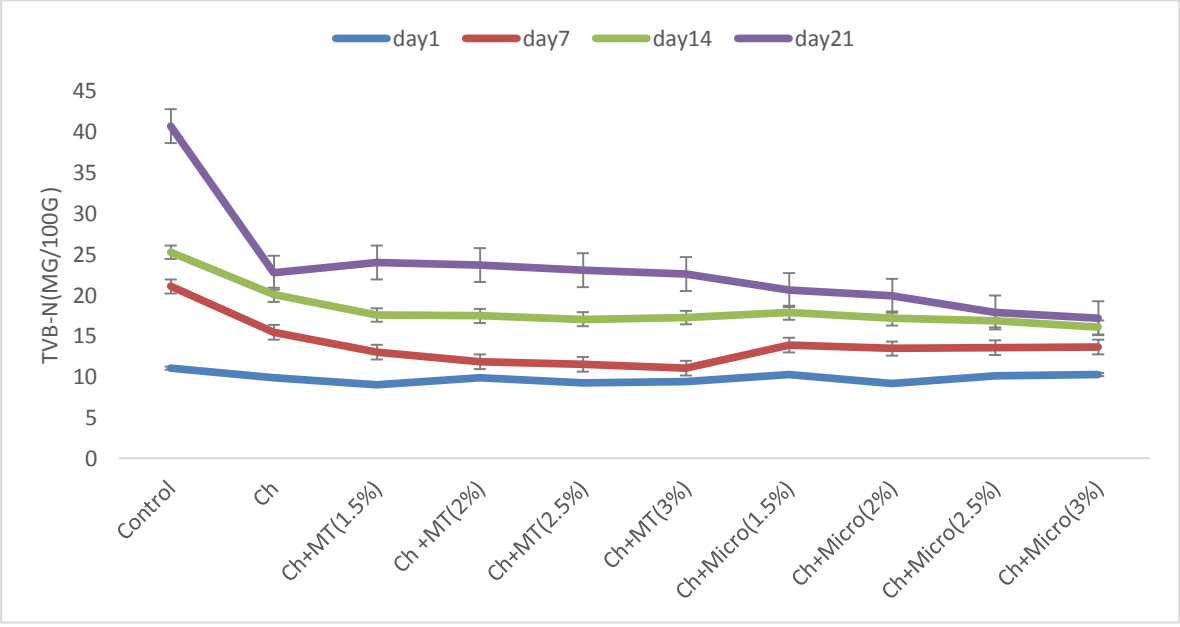

Fig. 3. The TVB-N value of filleted trout samples during 21 days of storage at 4 °C (uncoated trout fillet (control), coated with solely chitosan (Ch), coated with free MT (Ch + MT) and microencapsulated MT (Ch + Micro)), Data are means ± SD.

- Microbiological analysis

Microbiological assessment alongside chemical analysis has been widely employed to appraise the quality and shelf-life of fish. Several studies have indicated the microbial load on the surface of fish varies from 2 $\log_{10}$ CFU g^{-1} to 6 $\log_{10}$ CFU g^{-1} depending on the natural microflora of aquatic environments and temperature (Austin, 2006; Gimnez *et al*., 2002; Novoslavskij *et al*., 2016). In this study, the TVC and TPC of the control and the coated samples were evaluated over 21 days of storage, and the counts are displayed in Figure 4. Regarding the maximal recommended limit of 7 $\log_{10}$ CFU g^{-1} for TVC in fresh fish in which spoilage odor occurs (Ordo ̃nez *et al*., 2000), the TVC value for all treatments did not exceed the acceptability limit during the entire period of storage (Figure 4 A). A similar trend was observed for TPC in all samples (Figure 4 B). However, inhibition of TPC was more pronounced in the trout fillet samples. These values did not exceed 10^5 CFU g^{-1} at 21-day refrigerated storage (Figure 4 B). Compared to the control, however, all chitosan coatings have retarded the bacterial growth which might be related to a film-forming ability or polycationic trait of chitosan which ruptures the bacterial cell (Helander *et al*., 2001; Kanatt *et al*., 2013; Zheng & Zhu, 2003). In the case of coated samples containing free (Ch + MT) and microencapsulated MT (Ch + Micro), bacterial growth was **found to be reduced in parallel with increasing the concentrations of MT extract.** This behavior is likely attributed to the antimicrobial activity of phenolic compounds of MT extract, particularly silymarin and silybin which have been introduced as promising natural antimicrobial and antioxidant compounds (Abouzid & Ahmed, 2013; Dong *et al*., 2003). Concerning encapsulated treatments, Ch + MT treatments showed even slower microbial growth for the first 7 days of storage (mean TVC 2.3 CFU g^{-1} and mean TPC 0.76 CFU g^{-1}), while enhanced up to about 4 CFU g^{-1} at 21 days of storage. Conversely, in coated samples containing encapsulated MT extract (Ch + Micro), the mean value of TVC and TPC were about 2.8 and 1.48 CFU g^{-1} for the first 7 days of storage respectively. However, the mean value of TVC and TPC reached 2.8 and 2.2 CFU g^{-1} over 21 days respectively. The intensified antimicrobial activity of encapsulated loaded MT extract over free ones could be due to the prevention of the MT extract from evaporation, prolongation of its availability and facilitating its interaction with the cell membrane of bacteria (Ghaderi-Ghahfarokhi *et al*., 2017). Improved antimicrobial activity and extended shelf-life for trout fillets treated with essential oils and stored under various chilled conditions have been reported by others which are in good agreement with our findings (Donsì *et al*., 2011; Mazandrani *et al*., 2016; Ojagh *et al*., 2010; Pyrgotou *et al*., 2010; Volpe *et al*., 2015).

- Sensory evaluation

All sensory features were notably affected (P <0.05) by the storage (Table 1). The sensory score of the fish fillet was considered unsuitable for human consumption if it fell below 4 (Fan *et al*., 2008). The control sample along with Ch treatment presumed unacceptable color after day 7. All samples with free (Ch + MT) and encapsulated (Ch + Micro) MT showed a score of 4 after 14 days of storage. Ch + Micro treatment, which contained 3% MT, effectively delayed discoloration and did not reach a score below 4 throughout the storage time

resulting in suitable colors at 21-day refrigerated storage. Both the control and chitosan-coated fish samples received 'unacceptable' scores after the 7th day of storage, which are associated with TBA values well above 2. It is reported that panelists can detect oxidized flavors at a TBA range of 0.6–2.0 (Greene & Cumuze, 1982). All samples with free MT (Ch + MT) and encapsulated MT (Ch + Micro) retarded off-odor formation until day 14. However, Ch + Micro treatment, which contained 3% MT, postponed off-odor development until the end of the storage time, giving a score above 4. Overall, the control and samples treated with encapsulated MT exhibited the lowest and highest overall acceptability in the final days respectively. These **results are consistent** with (Mazandrani *et al.*, 2016; Ojagh et al., 2010).

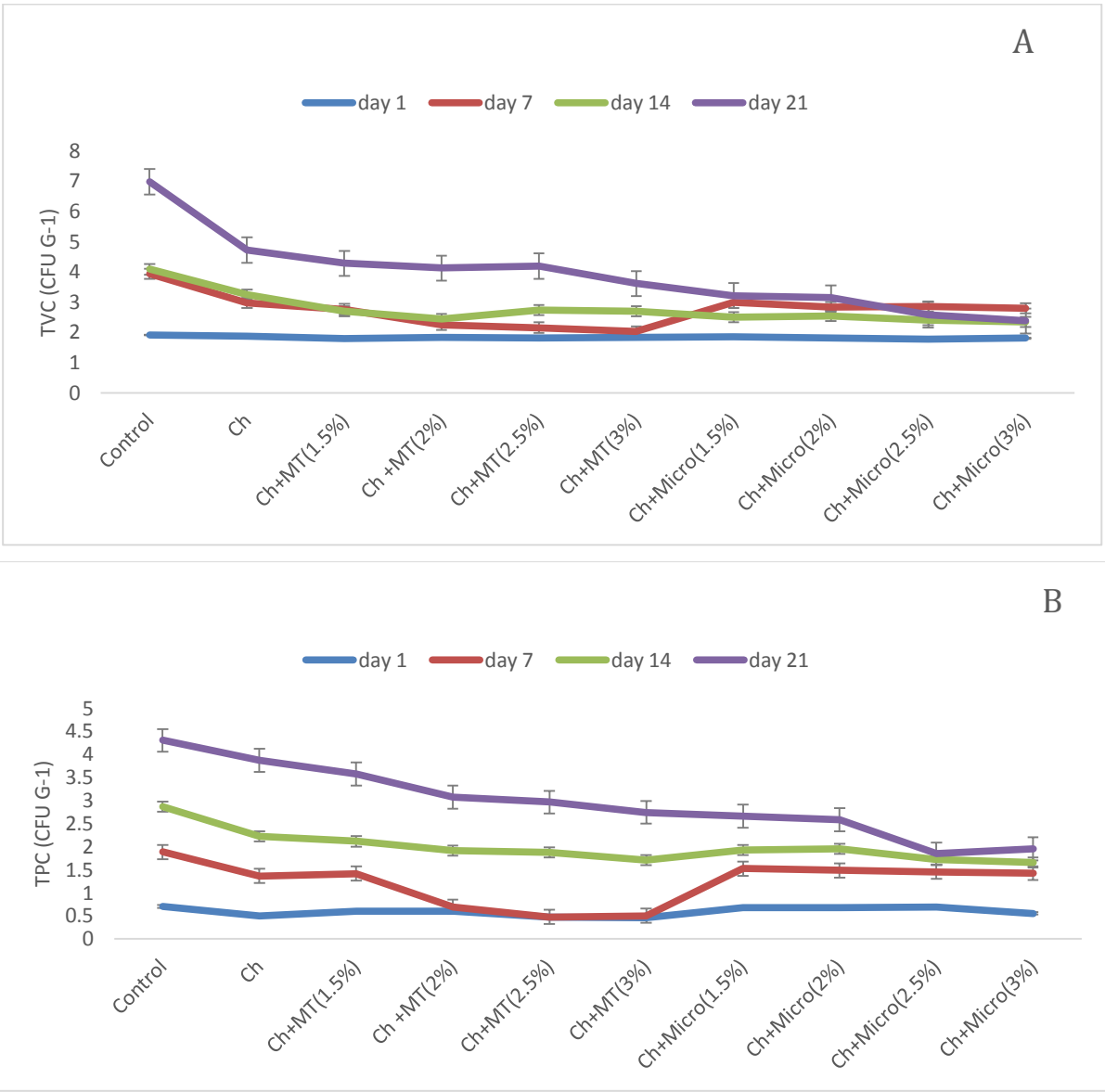

Fig. 4. Changes in (A) total viable count (TVC) and (B) total psychrotrophic count (TPC) of filleted trout samples during 21 days storage at 4 °C (uncoated trout fillet (control), coated with sole chitosan (Ch), coated with free MT (Ch + MT) and microencapsulated MT (Ch + Micro)), Data are means ± SD.

Table 1. Sensory evaluation of cooked filleted trout treated with different concentrations of free and encapsulated MT over 21 storages at 4 °C

Treatments	Color				Odor				Overall acceptability			
	1	7	14	21	1	7	14	21	1	7	14	21
Control	4.80 ± 0.08^{aA}	4.23 ± 0.18^{bC}	2.49 ± 0.01^{cC}	2.01 ± 0.04^{dE}	4.98 ± 0.10^{aA}	4.11 ± 0.11^{bB}	2.39 ± 0.21^{cC}	1.83 ± 0.01^{dD}	4.90 ± 0.10^{aA}	4.23 ± 0.12^{bB}	2.32 ± 0.23^{cD}	1.36 ± 0.32^{dD}
Ch	4.65 ± 0.04^{aA}	4.32 ± 0.19^{bC}	3.71 ± 0.17^{cB}	3.05 ± 0.17^{dD}	4.83 ± 0.13^{aA}	4.23 ± 0.18^{bA}	3.83 ± 0.25^{cB}	3.29 ± 0.05^{dC}	4.88 ± 0.05^{aA}	4.52 ± 0.20^{bA}	3.44 ± 0.13^{cC}	2.89 ± 0.08^{dC}
Ch+MT (1.5%)	4.81 ± 0.04^{aA}	4.60 ± 0.37^{bB}	4.02 ± 0.11^{cA}	3.32 ± 0.18^{dC}	4.83 ± 0.13^{aA}	4.23 ± 0.19^{bA}	3.84 ± 0.25^{cB}	3.21 ± 0.10^{dC}	4.82 ± 0.05^{aA}	4.58 ± 0.20^{bA}	3.48 ± 0.13^{cC}	2.98 ± 0.08^{dC}
Ch+MT (2%)	4.74± 0.03^{aA}	4.50 ± 0.21^{aB}	4.00± 0.10^{bA}	3.34 ± 0.24^{cC}	4.74 ± 0.30^{aA}	4.46 ± 0.13^{bA}	4.05 ± 0.03^{cA}	3.89 ± 0.15^{cA}	4.80 ± 0.10^{aA}	4.63 ± 0.19^{bA}	4.02 ± 0.03^{cB}	3.54 ± 0.02^{dB}
Ch+MT (2.5%)	4.78 ± 0.05^{aA}	4.85 ± 0.12^{aA}	4.42 ± 0.19^{bA}	3.31 ± 0.12^{cC}	4.10± 0.09^{bC}	4.49 ± 0.21^{aA}	4.09 ± 0.10^{bA}	3.69 ± 0.22^{cB}	4.73 ± 0.12^{aA}	4.62 ± 0.15^{aA}	4.07 ± 0.07^{bB}	3.53 ± 0.23^{cB}
Ch+MT (3%)	4.80 ± 0.07^{aA}	4.88 ± 0.12^{aA}	4.38 ± 0.20^{bA}	3.33 ± 0.12^{cC}	4.65 ± 0.28^{aB}	4.4 ± 0.05^{bA}	4.24 ± 0.11^{cA}	3.73 ± 0.05^{dB}	4.62 ± 0.15^{aA}	4.63 ± 0.15^{aA}	4.23 ± 0.12^{bA}	3.52 ± 0.05^{cB}
Ch+Micro(1.5%)	4.75 ± 0.05^{aA}	4.52 ± 0.18^{bB}	4.26 ± 0.17^{cA}	3.53 ± 0.15^{dC}	4.83 ± 0.15^{aA}	4.22 ± 0.18^{bA}	4.13 ± 0.25^{bA}	3.27 ± 0.10^{cC}	4.83 ± 0.05^{aA}	4.55 ± 0.18^{bA}	4.44 ± 0.13^{bA}	3.41 ± 0.08^{cB}
Ch+Micro (2%)	4.70 ± 0.09^{aA}	4.72 ± 0.32^{aA}	4.40± 0.13^{bA}	3.70 ± 0.15^{cB}	4.78 ± 0.40^{aA}	4.46 ± 0.13^{bA}	4.08 ± 0.03^{cA}	3.88 ± 0.11^{dA}	4.80 ± 0.10^{aA}	4.53 ± 0.19^{bA}	4.46 ± 0.03^{bA}	3.51 ± 0.02^{cB}
Ch+Micro (2.5%)	4.75 ± 0.07^{aA}	4.73 ± 0.25^{aA}	4.37 ± 0.10^{bA}	3.72 ± 0.25^{cB}	4.90 ± 0.09^{aA}	4.50 ± 0.21^{bA}	4.12 ± 0.10^{cA}	3.78 ± 0.22 ^{dB}	4.73 ± 0.12^{aA}	4.52 ± 0.15^{bA}	4.45 ± 0.07^{bA}	3.73 ± 0.23^{cB}
Ch+Micro (3%)	4.75 ± 0.06^{aA}	4.82 ± 0.12^{aA}	4.46 ± 0.20^{bA}	4.14 ± 0.13^{cA}	4.62 ± 0.31^{aB}	4.40 ± 0.05^{bA}	4.22 ± 0.12^{cA}	4.10 ± 0.05^{cA}	4.62 ± 0.15^{aA}	4.61 ± 0.15^{aA}	4.41 ± 0.09^{bA}	4.10 ± 0.05^{cA}

Values are the means ± standard deviations (n = 3). Within each column, different lowercase letters indicate significant differences (P < 0.05) among samples. Within each row, different capital letters indicate significant differences (P < 0.05) among average days. (Uncoated trout fillet (control), coated with sole chitosan (Ch), coated with free MT (Ch + MT) and microencapsulated MT (Ch + Micro)),

Conclusion

Chitosan coatings, both alone and with MT, effectively inhibited lipid oxidation and microbial growth in chilled rainbow trout fillets during storage. However, the active edible coating showed greater efficacy in extending shelf life by reducing the microbial growth and lipid oxidation rates. Encapsulation controlled the release of MT extract, resulting in prolonged antimicrobial and antioxidant activity during cold storage. Encapsulated samples showed no significant loss of color, odor, or overall acceptability, making them a promising alternative for maintaining rainbow trout fillet quality and freshness. This study suggests chitosan coating with MT can be used as a safe active packaging for fish preservation during cold storage.

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