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Effect of Edible Chitosan Coating Containing *Froriepia subpinnata* Extract on Shelf life of Nile tilapia (*Oreochromis niloticus*) Fillet at Refrigerated Temperature

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Abstract

Natural antimicrobial and antioxidant compounds in extracts and essential oils of plant can be used in the edible coatings for packaging of food product. The aim of this study was to investigate the effect of edible chitosan containing *Froriepia subpinnata* extract on storage time of Nile tilapia (*Oreochromis niloticus*) fille After preparation of fresh fish, except the control group (first treatment), fish fillets were coated with 2% chitosan solution (second treatment), 2% chitosan solution and 1% *F. subpinnata* extract (third treatment) and 2% chitosan solution and 2% *F. subpinnata* extract (fourth treatment) then kept at refrigerator temperature for 21 days. At the beginning of the experiment and at 7th, 14th and 21st days, chemical indexes (pH, total volatile nitrogen bases and thiobarbituric acid), microbial count (mesophilic bacteria, enterobacteriaceae, psychrophile bacteria and mold and yeast) and sensory evaluation of samples were analyzed. Generally, coated treatments showed better quality than the control group in terms of investigated microbial and chemical indexes. Also, the treatment coated with chitosan and 2% of *F. subpinnata* extract showed significant differences (P<0.05) compared to the control group in terms of studied indexes until 21st day, and was known the best treatment beside the other treatments. Coating of tilapia fillets with chitosan containing 2% of *F. subpinnata* extract improves chemical and microbial indexes and increases their shelf life up to 14 days in comparison with control.

Keywords: Chemical indexes, Chitosan, Fish fillet, *Froriepia subpinnata* extract, Microbial indexes

Introduction

Fish are very prone to fat oxidation and loss of quality due to their high content of unsaturated fatty acids and metal ions in their meat. Therefore, methods must be developed for the storage and transfer of them in order to maintain its quality for a longer period of time (Sadeghii *et al.*, 2021). Edible films and coatings in the form of active packaging, with properties such as biodegradability, increasing the quality and level of food safety and increasing the shelf life of food are a good alternative to synthetic polyethylene packaging in various food industries (Gómez-Estaca *et al.*, 2014). Chitosan is a non-toxic, biodegradable, environmentally friendly polysaccharide chitosan with antifungal and antimicrobial properties that is widely used in active packaging and food coatings. Polysaccharides, including chitosan, have the ability to combine with other preservatives and antimicrobials to produce more effective active and preservative coatings (Shokooh Saremi *et al.*, 2018). Essential oils and plant extracts can be

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used as antimicrobials and antioxidants in coatings. *Froriepia subpinnata* is one of the native medicinal plants of Iran and belongs to the Umbelliferae family and its antioxidant and antimicrobial properties have been reported(Bahrami *et al.*, 2021). In the present study, an active coating of chitosan and different amounts of *Froriepia subpinnata* extract were produced for tilapia fish fillets and the effect and preservative capability of this coating were investigated.

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Material and methods

In the first stage, the *Froriepia subpinnata* extract was prepared by dissolving in ethanol and the method of maceration (Mashayekhi *et al.*, 2013). To prepare a 2% chitosan solution, a medium molecular weight chitosan powder was dissolved in a 1% acetic acid solution. Coating with 0, 1 and 2 percent of extract was done by weight/volume (Mehdizadeh *et al.*, 2012). The Iranian National Standard Method No. 1028 (Iranian National Standardization Organization [ISIRI], 2008) was used to measure pH and the combination of malondialdehyde-thiobarbituric acid was used to evaluate the oxidation of fats (Esmaili *et al.*, 2017). Microbial characteristics (mesophilic bacteria, *Enterobacteriaceae,* cold-blooded bacteria, mold and yeast) using specific media and after preparing serial dilutions in test tubes containing 9 mL of peptone water (0.1%) was performed. Results were expressed as Log CFU/g. Sensory evaluation of raw and cooked fish fillets in different treatments using a trained group of seven and 5-point hedonic method was used. Raw and cooked fillets with a score of less than three were defined as unacceptable products (Maghami *et al.*, 2019).

Results and discussion

Based on the data of Fig. (1), the trend of increasing pH in the control sample was more than other treatments. The highest pH in all measurement periods was related to the control treatment and the lowest was observed in the treatment of chitosan with 2% of extract.

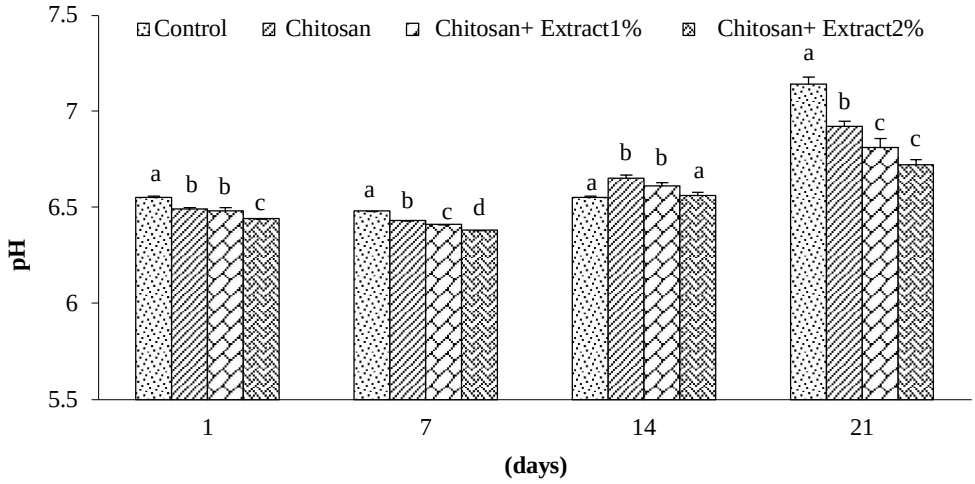

Fig. 1. Change of pH value in fish fillets of different treatments

According to the results obtained from the analysis of total volatile nitrogen bases in different treatments (Fig. 2), in all measurement periods, the lowest and highest values of these bases were observed in chitosan treatment containing 2% of extract and control treatment, respectively. The increase in volatile nitrogen bases in the control treatment is due to the higher activity of bacteria and the production of these compounds. The use of coatings with antimicrobial properties such as chitosan and extract can reduce the production of these compounds by inhibiting the growth of bacteria (Mei *et al.*, 2019).

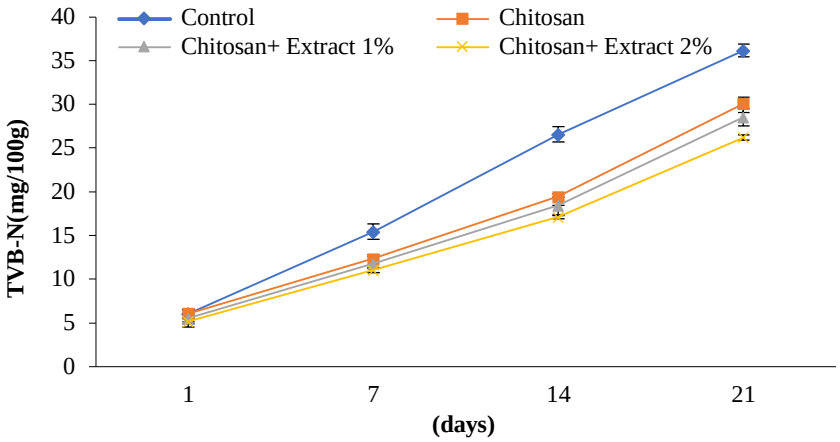

Fig. 2. Changes in total volatile basic nitrogen (TVB-N) of fillets during storage

As lipid oxidation progresses, the primary oxidation products are converted to secondary products such as malondialdehyde due to their instability (Barriuso *et al.*, 2013). According to the results of thiobarbituric acid assay, there was a significant difference (P<0.05) between all treatments compared to the control treatment, while the treatment covered with chitosan containing 2% of extract had the lowest thiobabituric acid content among all treatments in all times (Fig. 3). The results of microbial count showed that the treatment coated with chitosan containing 2% of pomegranate extract at all measurement times had the lowest microbial count among all treatments (Table 1).

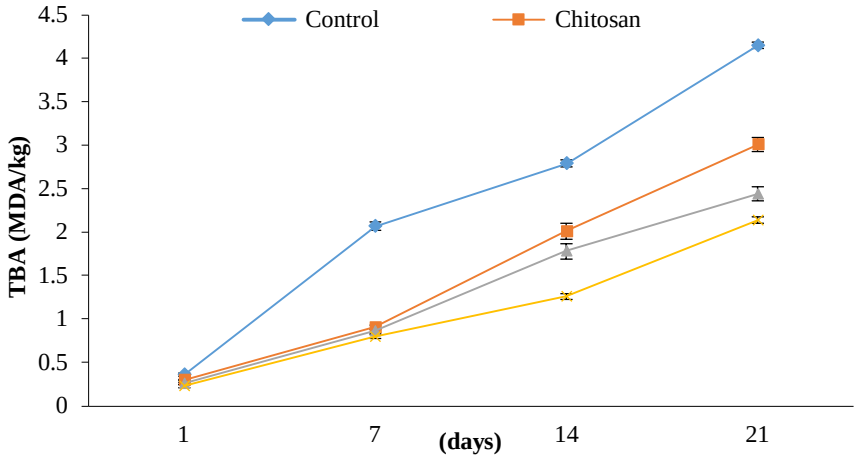

Fig. 3. Changes in thiobarbituric acid values of fish fillet samples during refrigerated storage

Table 1. Comparison of the number of Mesophilic bacteria and Psychrotrophic bacteria in fish fillets of different treatments

Treatments	Microbial characterestics							
	Mesophilic bacteria (log CFU/g)				Psychrotrophic bacteria (log CFU/g)			
	1 (day)	7 (day)	14 (day)	21 (day)	1 (day)	7 (day)	14 (day)	21 (day)
Control	5.11±0.15^{a}	6.47±0.26^{a}	7.20±0.15^{a}	9.50±0.40^{a}	5.17±0.18^{a}	7.06±0.06^{a}	8.72±0.17^{a}	8.79±0.26^{a}
Chitosan	4.75±0.10^{b}	5.30±0.21^{b}	5.47±0.22^{b}	6.01±0.15^{b}	3.73±0.17^{c}	3.84±0.24^{b}	5.29±0.23^{b}	8.65±0.14^{a}
CH+Extract1%	2.04±0.22^{c}	2.30±0.15^{c}	2.74±0.13^{c}	3.50±0.05^{c}	3.05±0.16^{b}	3.72±0.14^{b}	3.82±0.19^{c}	4.78±0.11^{b}
CH+Extract2%	1.95±0.17^{c}	2.29±0.22^{c}	2.61±0.21^{c}	2.80±0.13^{c}	3.12±0.21^{b}	4.83±0.10^{c}	3.69±0.31^{c}	3.45±0.16^{c}

Results are displayed as mean ± standard deviation. Different uppercase letters in each column indicate a statistically significant difference at the level of 5% (P <0.05).

The phenolic active groups in the plant extract disrupt various enzyme systems, produce cellular energy and synthesize structural components, and damage the genetic material of bacteria and prevent their growth and proliferation in fish meat (Savoia, 2012). The increase in the number of psychrotrophic bacteria during the storage period in this study is consistent with the results of Etemadi *et al.* (2008) who investigated the effect of parsley extract on the shelf life of silver carp fillet at 4 °C (Table 2). In general, the sensory properties of fish fillets are related to lipid oxidation conditions, hemoglobin and myoglobin protein oxidation conditions, non-enzymatic activities between lipid oxidation products and protein amine groups, and microbial spoilage (Ghaly *et al.*, 2010). In the present study, the results of raw and cooked fish samples (Tables 3 and 4) showed that chitosan coatings and extract show an acceptable role in maintaining the quality of tilapia fillets over time. It is shown that the treatment covered with chitosan containing 2% of extract, with the highest score on days 7 (for cooked samples) and 14 (for raw samples), significantly (P<0.05) has shown the most protective role.

Table 2. Comparison of *Enterobacteriaceae* Bacteria, Mold and Yeast in Fish Fillets of Different Treatments

Treatments	Microbial characterestics							
	Enterobacteriaceae Bacteria (log CFU/g)				Mold and Yeast (log CFU/g)			
	(day) 1	(day) 7	(day) 14	(day) 21	(day) 1	(day) 7	(day) 14	(day) 21
Control	3.19±0.11^{a}	5.03±0.09^{a}	7.57±0.07^{a}	7.74±0.05^{a}	3.93±0.24^{a}	4.24±0.17^{a}	7.03±0.40^{a}	8.70±0.30^{a}
Chitosan	2.05±0.25^{b}	2.30±0.14^{b}	3.57±0.24^{b}	2.68±0.13^{b}	3.71±0.24^{b}	3.74±0.31^{b}	7.01±0.04^{a}	8.16±0.24^{a}
CH+Extract1%	1.31±0.14^{c}	1.92±0.17^{c}	2.26±0.13^{c}	1.29±0.15^{c}	3.84±0.16^{c}	3.92±0.09^{c}	5.63±0.41^{b}	6.67±0.29^{b}
CH+Extract2%	1.35±0.15^{c}	1.29±0.09^{c}	1.78±0.17^{d}	2.36±0.14^{c}	3.83±0.17^{c}	3.89±0.19^{c}	5.59±0.41^{b}	6.25±0.33^{b}

Results are displayed as mean ± standard deviation. Different uppercase letters in each column indicate a statistically significant difference at the level of 5% (P <0.05).

Table 3. Sensory evaluation of raw and cooked samples of fish fillets of different treatments

	Treatments	Samples			
		(day) 1	(day) 7	(day) 14	(day) 21
Color	Control	5.00 ± 0.00^{a}	4.50 ± 0.14^{a}	2.66 ± 0.92^{a}	D
	Chitosan	5.00 ± 0.00^{a}	4.66 ± 0.14^{a}	3.33 ± 0.64^{ab}	D
	CH+Extract1%	5.00 ± 0.00^{a}	4.62 ± 0.05^{a}	4.00 ± 0.84^{b}	D
	CH+Extract2%	5.00 ± 0.00^{a}	5.00 ± 0.00^{b}	4.50 ± 0.55^{b}	D
Smell	Control	5.00 ± 0.00^{a}	4.53 ± 0.12^{a}	2.75 ± 0.64^{a}	D
	Chitosan	5.00 ± 0.00^{a}	4.48 ± 0.15^{a}	2.75 ± 0.52^{a}	D
	CH+Extract1%	5.00 ± 0.00^{a}	4.75 ± 0.07^{b}	4.00 ± 0.52^{b}	D
	CH+Extract2%	5.00 ± 0.00^{a}	4.75 ± 0.04^{b}	4.33 ± 0.32^{b}	D
Texture	Control	5.00 ± 0.00^{a}	4.55 ± 0.11^{a}	2.78 ± 0.87^{a}	D
	Chitosan	5.00 ± 0.00^{a}	4.48 ± 0.11^{a}	3.25 ± 0.75^{ab}	D
	CH+Extract1%	5.00 ± 0.00^{a}	5.00 ± 0.00^{b}	4.00 ± 0.55^{b}	D
	CH+Extract2%	5.00 ± 0.00^{a}	5.00 ± 0.00^{b}	4.50 ± 0.70^{b}	D

Results are displayed as mean ± standard deviation. Different uppercase letters in each column for each index indicate a statistically significant difference at the level of 5% (P <0.05).

* Scoring is from 1 to 5, and samples with a score of less than 3 are considered unacceptable and are marked with the letter D (Discarded).

Table 4. Sensory evaluation of raw and cooked samples of fish fillets of different treatments

Treatments	Samples			
	1 (day)	7 (day)	14 (day)	21 (day)
Control	5.00 ± 0.00^{a}	4.50 ± 0.13^{a}	D	D
Chitosan	5.00 ± 0.00^{a}	4.48 ± 0.13^{a}	D	D
CH+Extract1%	5.00 ± 0.00^{a}	4.78 ± 0.06^{b}	D	D
CH+Extract2%	5.00 ± 0.00^{a}	4.91 ± 0.04^{c}	D	D

Results are displayed as mean ± standard deviation. Different uppercase letters in each column indicate a statistically significant difference at the level of 5% (P <0.05).

* Scoring is from 1 to 5, and samples with a score of less than 3 are considered unacceptable and are marked with the letter D (Discarded).

Conclusions

Increasing the shelf life of tilapia fish kept at fridge temperature using the combination of chitosan coating and *Froriepia subpinnata* plant extract with a concentration of 1 and 2% was examined in the study. According to the study findings, chitosan and *Froriepia subpinnata* plant extract slows down the increase in pH, volatile nitrogen bases, thiobarbituric acid, microbial load, mold and yeast in tilapia fillets with their antioxidant and antimicrobial properties compared to the control group, whereas the control treatment became unusable on the 7th day. However, the treatment covered with chitosan containing 2% of *Froriepia subpinnata* extract had a better state and prevented the spoilage of tilapia fillets for at least 2 weeks compared to the control group. Thus, using chitosan coating and 2% *Froriepia subpinnata* plant extract is suggested to keep tilapia fillets in fridge temperature and prevent its spoilage.

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