Research Article

A Comparative Study on the Effect of Green Preservatives to Increase the Shelf Life of Commercially Important Fish Fillet in Frozen Condition

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ABSTRACT

The present comparative study was carried out to investigate the preservative effect of *Moringa oleifera* and *Sargassum wightii* on fish fillets of commercial important marine fishes *Sardinella longiceps* and *Rastrelliger kanagurta* under frozen storage (4±1ºC). The effect of *Moringa oleifera* and *Sargassum wightii* on biochemical, microbiological and sensory properties of fish fillets stored at 4±1ºC was investigated. The treated fish fillets showed significantly (P<0.05) improved values of biochemical parameters when compared to control throughout the frozen storage period. TVC was also found to be reduced significantly (P<0.05) in treated samples. This assessment also supports the results of sensory assessment that increased in shelf life. From the results obtained from comparative study, it can be concluded that *Moringa oleifera* and *Sargassum wightii* can be used for extending the shelf life of fish fillets during frozen storage (4±1ºC) and in precise *Sargassum wightii* proves best.

Keywords: Natural preservatives, Proximate parameters, Microbial quantity, Frozen storage.

INTRODUCTION

Seafood, including species from non-chordate molluscs till chordate fishes, are excellent sources of protein, fat, vitamins and minerals and they are popular due to their delicacy with high nutritive value. However, Viji *et al*.[1] stated that the shelf-life of seafoods is limited because of the high content of various nutrients, neutral pH and high moisture content. But the rapid microbial and biochemical reactions that occur in seafood immediately after death lead to changes in sensory and nutritional properties that reduce the shelf-life[2]. In general, seafood is abundant in polyunsaturated fatty acids (PUFAs), which make it more prone to lipid oxidation. In 2016, Secci and Parisi[3] stated that formation of unpalatable odor and flavor, loss of nutrition, production of unhealthy molecules, and color changes are mainly the consequences of lipid oxidation in seafood. In addition to proximate changes microbiological changes too contributes to the complexity of seafood spoilage because the transportation period from the harvesting or capturing ground, processing and storage methods too determining the quality and deterioration of seafood. Sriket[4] stated that the initial loss of fish freshness is attributed to indigenous enzymes and chemical reactions, whereas complete spoilage in fish is a function of microbial metabolic activities. Especially in fish which is extremely perishable as a result of rapid microbial growth naturally present in fish or from contamination.

Prevention of nutritional and sensory losses caused by microbiological, enzymatic, or chemical changes, and shelf-life extension of food are usually achieved so far by applying chemical preservatives, such as sodium benzoates, sodium nitrite and sulfurdioxides. Synthetic preservatives were also widely used in fish storage to extend shelf life and maintain quality and safety. But, Ozdemir *et al*.[5] stated that nonetheless, accumulation of these synthetic preservatives in tissues can be detrimental to health. Martínez-Alvarez and Gómez-Guillén[6], suggested treatment with salt is one of the common and oldest natural preservative methods used widely for shelf-life extension of seafood because of its low cost, as well as simplicity can be applicable. However, consumer preferences for natural preservatives and concerns about the safety of synthetic preservatives have prompted the food industry to search natural preservatives. Natural antioxidants have been generally considered safe for human consumption. Since, the potential risk to human health on consuming frozen sea food has not been adequately investigated. The outcome of the present study will provide a necessary basis to enhance and broaden the potential utilization of natural preservatives in the development of novel fish products with improved oxidative stability, flavour quality and nutritional value as well as in health promoting functional food formulations.

MATERIALS AND METHODS

Collection and preparation of preservative pastes

The collected and identified *Sargassum wightii* from Mandapam (9° 16' 48.00" N: 79° 07' 12.00") E., Rameswarm coast, *Moringa oleifera* from local farm were sterilized thoroughly using tap water. Later the cleaned materials were cut into small pieces, shade dried for two weeks and later the samples were made into coarse powder by grinding them in a lab electric mixer grinder and

stored in refrigerator. Whenever required an amount of 100 gm of collected materials were ground for 2-5 mins (each separately) with adding water as solvents and the paste was used for further analysis.

Storage Study

Selected experimental Fishes *Sardinella longiceps* (EF1) and *Rastrelliger kanagurta* (EF2) of little size was purchased from landing center and fish market of Olavakkodu and the fishes were made into a fillets with mean weight about 100-150 g. Then the fillets were covered with paste made from macroalga *Sargassum wightii* and natural *Moringa oleifera* leaves, then the fillets were placed in sterile polythene bags and stored at chilled temperature (4±1 ºC). After 24hrs samples in triplicates were randomly removed from the treatment to evaluate the preservative of macroalga extracts on *Sardinella longiceps* and *Rastrelliger kanagurta* fillets. The fish fillets were stored with and without preservatives in the refrigerator at 4±1°C for a week period (1st, 3rd, 5th and 7th day) of preservation period and analysed for the following parameters as follows.

Wet lab analysis

Proximate composition: For fresh and preserved fish, the proximate composition was determined from the body muscle. Following parameters like ash and moisture of preserved and unpreserved fish samples were evaluated using standard methods of AOAC[7]. The protein content was determined using the Lowry *et al*.[8]. The total carbohydrate was determined using Hedge and Hofreiter[9]. Fat content was determined using Floch *et al*.[10].

Sensory Evaluation: The sensory evaluation was carried out by a group of panelist according to the method described by Potter[11]. A sensory panel formed consist of twelve experienced judges (6 males and 6 females; 30-55 years old). The steam cooked samples of fresh and preserved fish were evaluated for quality attributes include texture, flavour, colour, appearance, general taste and overall acceptability by using 5-point hedonic scales (0 = excellent; 1= very good; 2= good; 3-4 fair; <5 bad) and score 5.0 was considered the borderline of fish acceptability.

Microbial Analysis: The 10 g of fish sample was weighed aseptically and homogenized with 90 mL of physiological saline solution. Appropriate dilutions were made from the 9.0 mL physiological saline and plated onto Potato dextrose agar plate containing antibiotics or tartaric acid solution. The plates were incubated at room temperature for four days and all colonies were counted and the data was reported as Cfu g^{-1}.

Statistical Analysis

Data were analysed using SPSS (Scientific Package of Social Science) version 16.0. The mean, standard deviation (SD) and one-way ANOVA test were performed to compare differences in parameters analysed among selected fish species and a test for the comparison of means ($P < 0.05$).

RESULTS AND DISCUSSIONS

Proximate composition

Ash

Result shown in table 1 revealed that the ash content decreased significantly from 2.28±0.01 to 1.94±0.01 in EF1 similarly 2.29±0.02 to 2.05±0.01 in EF2 on 0th day to 7th day of untreated frozen storage at 4±1ºC. There was a decrease of 2.34±0.03 to 2.08±0.01 in EF1 and 2.37± 0.04 to 2.14±0.02 in EF2 on 0th day to 7th day preserved with *Moringa oleifera* (Table 2). Table 3 showed that initially on day 0 ash content was found to be 2.20±0.03 and it decreased significantly to the value 1.89±0.09 in EF1 and 2.53±2.43 to 2.55±0.15 in EF2 on 0th day to 7th day of storage at 4±1ºC preserved with *Sargassum wightii* (Table 3). A decreasing in ash content of fish during frozen storage which was attributed to the drip loss during thawing process[12,13]. The present results of ash content are in agreement with Beklevik *et al*.[12] while working on sea bass fillets.

Moisture

Initial day of storage (Day-0) of fish muscle without preservative in which the moisture content (% of wet basis) recorded in EF-1 was 72.02±0.05 and 73.02±.09 in EF-2 while in fish muscle preserved with preservative *Moringa oleifera* it was 74.04±0.10 (EF1), 75.05±0.09 (EF2) and preservative *Sargassum wightii* it was 73.23±0.15 (EF1), 74.25±0.18 (EF2). At the end of the storage period (i.e) 7th day the moisture content still got decreased in *Moringa oleifera* treated fish tissue to 71.21±0.05 in EF1 and 78.02±0.02 in EF2 and *Sargassum wightii* treated fish tissue to 68.14±0.33 in (EF-1) and 69.15±0.33 in (EF-2). Decrease in moisture content at the end of frozen storage period in EF-1 and EF-2 fish flesh was noted which may be attributed to the sublimation of ice in frozen storage and drip loss during thawing process. Moisture content in fish was reported to be between 70 and 80% of the total weight[14] which supported the present analysis.

Protein

Perusals of table 1 revealed that protein content was reduced significantly from 0th day (19.15±0.15) to 7th day (13.05±0.15) in EF1 and 21.32±0.18 to 14.02±0.17 in EF2 of untreated fish muscles stored at 4±1ºC. A significant percent increase ($P<0.05$) was found in EF1 and EF2 preserved with *Moringa oleifera* from 0th day 15.5±0.20 (EF1), 17.05±0.25 (EF2) to 7th day 22.15±0.20 (EF1), 25.15±0.21 (EF2) of storage at 4±1ºC (Table 2). Table 3 showed that initially on day 0 protein content was found to be 27.34±1.10 and it increased significantly to the value 45.23±1.21 in EF1 and 28.35±1.15 to 46.32±1.26 in EF2 on 7th day of storage at 4±1ºC preserved with *Sargassum wightii*. Decrease in protein is due to leaching effect of amino acids and water-soluble protein leaching out with melting ice attributed by early workers[12,15-18]. These values showed that the two fish species EF-1 and EF-2 preserved with preservatives contained high levels of proteins at the

end of Day 7 which can be due to the impact of crude protein of preservatives so it can be used preservative on animal proteins source[19,20].

Lipid

The results shown in the table 1 revealed that the lipid content decreased significantly (P<0.05) from 0th day 3.01±0.14 (EF1), 3.52±0.17 (EF2) to 7th day 2.21±0.01 (EF1), 2.48±0.07 (EF2) of untreated fish muscle stored at 4±1ºC. There was increase in lipid content of fish muscle treated with *Moringa oleifera* from 0th day 2.45±0.02 (EF1), 2.53±0.07 (EF2) to 7th day 3.25±0.21 (EF1), 3.92±0.21 (EF2). Table 3 showed that initially on day 0 lipid content was found to be 2.05±0.14 and it increased significantly to the value 2.22±0.06 in EF1 and 2.22±0.17 to 2.43±0.05 in EF2 on the 7th day of storage at 4±1ºC preserved with *Sargassum wightii*. Rasoarahona *et al*.[21] reported that the lipid content of fish differed which could be due to variation of species, diet, geographical origin, age and season which supported the present report on lipid content in EF-1 and EF-2.

Carbohydrate

During the study period on the Initial day of storage (Day-0) the fish muscle without preservative the amount of carbohydrate content (% of wet basis) recorded in EF-1 was 0.41±0.01 and 0.52±0.04 in EF-2 while in fish muscle preserved with preservative *Moringa oleifera* in EF-1 was (0.47±0.01), *Sargassum wightii* (0.30±0.06) and in EF-2, 0.56±0.08 (*M.oleifera*), 0.45±0.08 (*S.wightii*) were recorded respectively. An increase in the value of carbohydrate content 0.77±0.02 and 0.97±0.10 was noted in fish muscle of EF-1 and EF-2 preserved with *Moringa oleifera* on 7th day of storage Similar trend was observed in *Saragassum wightii* (0.82±0.21) in EF-1 and (0.92±0.21) in EF-2 respectively.

Table 1: Proximate chemical composition of marine fishes EF-1 and EF-2 muscle samples (without preservative) under chilled storage condition (4 ±1°C) with respect to storage period

Treatment period (Days)	Chemical composition (g/100g) Without preservative									
	EF-1					EF-2				
	A	M	CP	CL	CC	A	M	CP	CL	CC
0	2.28±0.01	72.02±0.05	19.15±0.15	3.01±0.14	0.41±0.01	2.29±0.02	73.02±0.09	21.32±0.18	3.52±0.17	0.52±0.04
1	2.30±0.02	72.41±0.04	17.28±0.07	2.97±0.12	0.51±0.01	2.38±0.03	73.45±0.03	20.14±0.19	3.01±0.25	0.56±0.07
3	1.98±0.05	73.05±0.11	15.03±0.15	2.95±0.02	0.57±0.03	2.49±0.04	74.02±0.27	18.03±0.32	2.95±0.19	0.64±0.09
5	2.11±0.02	67.98±0.08	14.49±0.21	2.65±0.03	0.54±0.04	2.25±0.02	69.78±0.14	17.50±0.25	2.52±0.05	0.74±0.29
7	1.94±0.01	69.02±0.02	13.05±0.15	2.21±0.01	0.62±0.05	2.05±0.01	70.01±0.05	14.02±0.17	2.48±0.07	0.87±0.05

*Each value is the mean ± SE of three replicates.

Table 2: Proximate chemical composition of marine fishes EF1 and EF2 muscle samples (with preservative-1) under chilled storage condition (4 ±1°C) with respect to storage period

Treatment period (Days)	Chemical composition (g/100g) Preservative-1 (*Moringa oleifera*)									
	EF-1					EF-2				
	A	M	CP	CL	CC	A	M	CP	CL	CC
0	2.34±0.03	74.04±0.10	15.5±0.20	2.45±0.02	0.47±0.01	2.37±0.04	75.05±0.09	17.05±0.25	2.53±0.07	0.56±0.08
1	2.36±0.02	74.41±0.02	16.49±0.24	2.71±0.05	0.52±0.01	2.42±0.03	75.45±0.05	19.50±0.34	2.79±0.13	0.63±0.14
3	2.11±0.04	76.5±0.28	18.3±0.20	3.09±0.01	0.63±0.02	2.51±0.05	78.05±0.38	20.03±0.40	3.22±0.29	0.72±0.21
5	2.27±0.02	70.79±0.14	19.32±0.15	3.13±0.11	0.65±0.02	2.34±0.03	72.89±0.24	23.32±0.19	3.84±0.33	0.86±0.29
7	2.08±0.01	71.21±0.05	22.15±0.20	3.25±0.21	0.77±0.02	2.14±0.02	78.02±0.10	25.15±0.21	3.92±0.21	0.97±0.10

*Each value is the mean ± SE of three replicates.

Table 3: Proximate chemical composition of marine fishes EF1 and EF2 muscle samples (with preservative-2) under chilled storage condition (4 ±1°C) with respect to storage period.

Treatment period (Days)	Chemical composition (g/100g) Preservative-2 (*Sargassum wightii*)									
	EF-1					EF-2				
	A	M	CP	CL	CC	A	M	CP	CL	CC
0	2.20±0.03	73.23±0.15	27.34±1.10	2.05±0.14	0.30±0.06	2.53±2.43	74.25±0.18	28.35±1.15	2.22±0.17	0.45±0.08
1	2.27±0.07	74.83±0.28	31.48±1.31	2.10±0.09	0.39±0.26	2.67±2.53	77.84±0.19	33.51±1.38	2.29±0.09	0.47±0.36
3	2.11±0.01	78.20±0.27	35.71±1.19	2.13±0.16	0.52±0.14	2.47±0.71	80.21±0.11	37.69±1.23	2.32±0.21	0.53±0.24
5	2.17±0.11	71.29±0.20	40.01±1.11	2.20±0.10	0.75±0.12	2.83±0.28	73.22±0.20	41.05±1.17	2.40±0.11	0.87±0.18
7	1.89±0.09	68.14±0.33	45.23±1.21	2.22±0.06	0.82±0.21	2.55±0.15	69.15±0.33	46.32±1.26	2.43±0.05	0.92±0.21

*Each value is the mean ± SE of three replicates.

Table 4: Changes in total viable counts (TVCs) of fish muscle samples during chilling storage (4±1ºC) with respect to storage period

Treatment period (Days)	Number of Colonies (Cfu /ml/gm)					
	Moringa oleifera		*Sargassum wightii*		Without Preservative	
	EF-1	EF-2	EF-1	EF-2	EF-1	EF-2
0	176×10^5	188×10^5	152×10^5	172×10^5	225×10^5	232×10^5
1	100×10^5	104×10^5	72×10^5	96×10^5	232×10^5	249×10^5
3	64×10^5	80×10^5	48×10^5	52×10^5	247×10^5	256×10^5
5	132×10^5	152×10^5	112×10^5	125×10^5	255×10^5	262×10^5
7	145×10^5	175×10^5	130×10^5	158×10^5	269×10^5	276×10^5

Table 5: Quality index score of the selected commercial important marine fishes *Sardinella longiceps* (EF-1) and *Rastrelliger kanagurta* (EF-2) collected from Olavakkode Fish market and kept under ice storage condition (4±1°C) with and without preservatives

Sensory Parameters	Quality Index Score (Initial day till Day 7)					
	EF-1			EF-2		
	WOP	P-1	P-2	WOP	P-1	P-2
Appearance	**2**	**0**	**0**	**3**	**1**	**1**
Odor	3	1	1	3	1	1
Texture	3	1	1	4	1	1
Taste	3	1	1	3	1	1

* 0-Excellent; 1-Very good; 2-Good; 3-Moderate; 4-Bad; 5-unacceptable

Microbial Analysis

The changes in bacterial load in flesh (without skin) of EF-1 and EF-2 fishes during ice storage (4 ±1°C) for a storage period of 0,1, 3,5 and 7 with *Moringa olifera* are shown in Table 4. The TVC in untreated sample increased significantly during ice storage, on compared to the fish muscles stored with preservatives (*Moringa oleifera, Sargassum wightii*) respectively. On 7th day of storage period in EF-2 (*Rastrelliger kanagurta*) the recorded TVCs count in fish flesh stored under chilled condition with preservatives *Moringa oleifera* and *Sargassum wightii* were 175×10^5 cfu g^{-1} and 269 $\times10^5$ cfu g^{-1} which were note to be decrease in TVCs count when compared with the control flesh 276×10^5 cfu g^{-1} stored without preservatives. Li *et al.*[22] in his study stated that the fish spoilage generally occurred either by lipid oxidation, enhanced enzymatic activities, lack of metabolic activities and/or microbial growth.

Sensory Analysis

The organoleptic assessment in this study exhibited that the decline in the score of graded parameters such as: color, texture, freshness, and taste varied with storage duration initial day to day 7 in flesh of fishes EF-1 (*Sardinella longiceps*) and EF-2 (*Rastrelliger kanagurta*) stored without preservative. The reason for this decline in sensory parameters may be due to postmortem handling and storage, the holding temperature, oxygen, endogenous or microbial proteases, moisture can result in detrimental changes in the color, odor, texture, and flavor of fish[4,23]. Fish muscles stored with the selected preservative P-1 (*Moringa oleifera)*, and P-2 (*Sargassum wightii*) the score was 1 i.e. Very good for all the sensory parameters like odour, texture and taste except appearance in EF-1 which was rated 0.

CONCLUSION

Thus, from the present experimental study carried out on commercially important marine fishes *Sardinella longiceps* (EF-1) and *Rastrelliger kanagurta* (EF-2) the flesh stored under frozen temperature 4±1°C without preservative showed reduction in all the proximate parameters like protein, lipid, carbohydrate and moisture content. But in flesh of EF-1 and EF-2 stored with preservatives P-1 (*Moringa oleifera)* and P-2 (*Sargassum wightii*) the results were inversely proportional i.e. proximate parameters like protein, lipid and carbohydrate increased with storage period of 7 Days with decrease in moisture content. The total viable count in untreated samples were observed maximum while less viable counts were found in fish samples treated with P-1 and P-2. Among the experimental fish tissues, the plant preservative treated samples proved best in appearance, smell, colour, texture and taste of both the fishes but decline was found in untreated EF-1 and EF-2. Among the natural preservative used under chilled condition (4±1°C) *Sargassum wightii* (macroalgae) natural preservative proved to be best preservative than *Moringa oleifera*.

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