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Application of Green Bio-Preservatives in Extending the Shelf Life of Commercially Important Fishes *Sardinella Longiceps* and *Rastrelliger Kanagurta*

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ABSTRACT

The aim of the present study was to look at the preservative effect of *Coleus aromaticus* and *Sargassum wightii* on fish fillets of *Sardinella longiceps* and *Rastrelliger kanagurta* under chilled storage (4±1ºC). The effects of *Coleus aromaticus* and *Sargassum wightii* on proximate, microbiological, and sensory characters of fish fillets stored at 4±1ºC were investigated. The treated fish fillets showed significantly ($P<0.05$) improved values of proximate parameters as compared to control throughout the chilled storage period. TVC was also found to be reduced significantly ($P<0.05$) in treated samples. This assessment also supports the results of sensory property that increased shelf life. From the present study, it can be concluded that *Coleus aromaticus* and *Sargassum wightii* showed best preservative effect on fish fillets during chilled storage (4±1ºC).

Keywords: *Chilled storage, Coleus aromaticus, Preservation, Rastrelliger kanagurta, Sardinella longiceps, Sargassum wightii.*

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INTRODUCTION

Fish, the primary dietary source [1-3] with high protein and omega-3 fatty acid [4] is well known for its protective and anti-inflammatory properties since ancient times. But the shelf-life of such prime seafood is limited because of its high contents of various nutrients, neutral pH, high moisture content, etc. According to WHO, half of the seafood consumed by the consumers is fresh and live fish. Moreover, the consumers are very keen on the freshness which is associated with safety, reassurance, and superior taste. Anvari *et al*. (2013) stated that 70% of the total fish catch in developing countries were preserved using various basic food preservation techniques like freezing, chemical preservation, salting, and smoking to improve the microbial safety and increase the shelf life of fish and shellfish [5]. But Stolyhow *et al*. (2006) reported that these basic techniques not only increased the lipid oxidation but also decreased the nutritional quality of the fish [6]. So, synthetic preservatives were widely used in fish storage to extend shelf life and maintain quality and safety to prevent the nutritional and sensory losses caused by microbiological, enzymatic, or chemical changes, and shelf-life extension of food are usually achieved so far by chemical preservatives, such as sodium benzoates, sodium nitrite, and sulfur-di-oxide. Nonetheless, the accumulation of these synthetic preservatives in tissues can be detrimental to health. However, the use of these preservatives has been linked to potential health hazards. In this regard, natural preservatives with excellent antioxidant and antimicrobial properties have been extensively searched and

implemented as safe alternatives in seafood processing, with the sole purpose of extending shelf-life. Natural preservatives from microorganisms, plants, and animals have been shown potential in replacing the chemical and synthetic antimicrobials. Plant extracts have been recently received considerable attention in seafood processing as alternatives for synthetic preservatives [7, 8].

Pragmatically loss of freshness of fish is the consequence of post-mortem biochemical, physicochemical, and microbiological processes as well as several extrinsic factors such as the handling onboard and on land, and technological processing for which natural antioxidants have been generally considered safe for human consumption. The potential risk to human health on consuming frozen seafood has not been adequately investigated. With the view to help humans to overcome malnutrition problems and fish protein demand, the present study was undertaken with the objectives to assess the effect of frozen storage period - a week i.e. 0-7 days - at (4±1ºC) temperature on the fish fillet of selected marine fishes with and without preservation.

MATERIALS AND METHODS

Collection and preparation of preservative paste: The collected and identified *Sargassum wightii* from Mandapam (9°16'48" N: 79°07'12" E), Rameswaram coast, *Coleus aromaticus* from a local farm were washed thoroughly using tap water. Later the cleaned materials were cut into small pieces, shade dried for two weeks, powdered by grinding them in a lab electric mixer grinder, and stored in a refrigerator. Whenever required, 100 gm of collected materials were ground for 2-5 mins (each separately) with adding water as a solvent and the paste was used for further analysis.

Storage Study: Selected experimental fishes *Sardinella longiceps* (EF1) and *Rastrelliger kanagurta* (EF2) of little size were purchased from the landing center and fish market of Olavakkodu, Kerala, and fillets were made with a mean weight of 100-150 g. Then, the fillets were covered with a paste made from macroalga *Sargassum wightii* and natural *Coleus aromaticus* leaves, the fillets were placed in sterile polythene bags and stored at chilled temperature (4±1 ºC). After 24hrs, the 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 (1st, 3rd, 5th, and 7th day) of the preservation period and analyzed for the following parameters.

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 such as AOAC (2000) [9]. The protein content was determined by the methods described by Lowry *et al*. (1951) [10]. The total carbohydrate was determined using Hedge and Hofreiter methods (1962) [11]. Fat content was determined using Floch *et al*. (1957) [12].

Sensory Evaluation: The sensory evaluation was carried out by a group of panelists according to the method described by Potter (1968) [13]. A sensory panel was formed consisting 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 including texture, flavor, color, 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: 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 the 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 analyzed 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 analyzed

among selected fish species and a test for the comparison of means ($P < 0.05$).

RESULTS AND DISCUSSION

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 2.25±0.01 to 2.10±0.04 in EF1 and an increment of 2.75±0.21 to 4.05±0.08 in EF2 on 0th day to 7th day preserved with *Coleus aromaticus* (Table 2). Table 3 showed that initially on day 0 ash content was 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). The increase in ash contents of fish products during frozen storage might be attributed to the loss recorded in the concentration of protein and fat content, which reflected the increasing, found in ash contents. A decrease in ash content of fish during frozen storage was attributed to the drip loss during the thawing process [14]. The present results of ash content are in agreement with Beklevik *et al*., (2005) on sea bass fillets.

Moisture: In the initial day of storage (Day-0) of fish muscle without a preservative, 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 *Coleus aromaticus* it was 74.40±0.11 (EF1), 77.57±0.27 (EF2) and with preservative *Sargassum wightii* it was 73.23±0.15 (EF1) and 74.25±0.18 (EF2). At the end of the storage period (i.e 7th day), the moisture content still got decreased in *Coleus aromaticus* treated fish tissue to 70.5±0.18 in EF1 and 81.05±0.57 in EF2 and *Sargassum wightii* treated fish tissue to 68.14±0.33 in EF-1 and 69.15±0.33 in EF-2. The moisture content of the ice stored fish increased from the initial value till 3rd day which could be due to the absorption of melted ice water by the fish muscle. The later gradual decrease was observed on the 7th day. It might be due to the evaporation of moisture from the surface, which relies on various factors like geometric shape, the chemical composition of the product, storage temperature, and relative humidity, and these findings were supported by Dhanapal *et al*. (2013) [15].

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 EF-1 and 21.32±0.18 to 14.02±0.17 in EF-2 of untreated fish muscles stored at 4±1ºC. A significant percent increase ($P<0.05$) was found in EF-1 and EF-2 preserved with *Coleus aromaticus* from 0th day 25.44±0.16 (EF1) and 28.44±0.05 (EF2) to 7th day 39.24±0.20 (EF1) and 40.00±0.93 (EF2) of storage at 4±1ºC (Table 2). Table 3 showed that initially on day 0 protein content was 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*. The decrease in protein is due to leaching effect of amino acids and water-soluble protein leaching out with melting ice attributed by early researchers [14, 16-19]. 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 as a preservative on animal protein sources [20, 21].

Lipid: The results in the table 1 revealed that the lipid content decreased significantly ($P<0.05$) from 0th day 3.01±0.14 (EF1) and 3.52±0.17 (EF2) to 7th day 2.21±0.01 (EF1) and 2.48±0.07 (EF2) of untreated fish muscle stored at 4±1ºC. There was an increase in lipid content of fish muscle treated with *Coleus aromaticus* from 0th day 2.08±0.11 (EF1) and 2.28±0.32 (EF2) to 7th day 2.27±0.09 (EF1) and 2.95±0.82 (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*. (2005) 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 [22].

Carbohydrate: During the study period on the initial day of storage (Day 0) of the fish muscle without preservative, the amount of carbohy-

drate 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 *Coleus aromaticus* was 0.65±0.02, and preserved with *Sargassum wightii* was 0.30±0.06 in EF-1. In EF-2, it was 0.52±0.02 for *Coleus aromaticus* and 0.45±0.08 for *Sargassum wightii*. An increase in the value of carbohydrate content (0.93±0.10 and 0.55±0.48) was noted in the fish muscle of EF-1 and EF-2 preserved with *Coleus aromaticus* on 7th day of storage. Similar trend was observed in *Sargassum wightii* in EF-1 (0.82±0.21) EF-2 and (0.92±0.21).

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^{a}	72.02±0.05^{c}	19.15±0.15^{bc}	3.01±0.14^{a}	0.41±0.01^{c}	2.29±0.02^{b}	73.02±0.09^{ab}	21.32±0.18^{b}	3.52±0.17^{c}	0.52±0.04^{ac}
1	2.30±0.02^{c}	72.41±0.04^{de}	17.28±0.07^{ab}	2.97±0.12^{b}	0.51±0.01^{c}	2.38±0.03^{ed}	73.45±0.03^{c}	20.14±0.19^{a}	3.01±0.25^{b}	0.56±0.07^{ac}
3	1.98±0.05^{de}	73.05±0.11^{ab}	15.03±0.15^{cd}	2.95±0.02^{ab}	0.57±0.03^{ed}	2.49±0.04^{cd}	74.02±0.27^{ab}	18.03±0.32^{cd}	2.95±0.19^{d}	0.64±0.09^{ed}
5	2.11±0.02^{gf}	67.98±0.08^{cd}	14.49±0.21^{d}	2.65±0.03^{cd}	0.54±0.04^{d}	2.25±0.02^{ab}	69.78±0.14^{gf}	17.50±0.25^{cd}	2.52±0.05^{gf}	0.74±0.29^{ab}
7	1.94±0.01^{b}	69.02±0.02^{a}	13.05±0.15^{c}	2.21±0.01^{c}	0.62±0.05^{a}	2.05±0.01^{ac}	70.01±0.05^{a}	14.02±0.17^{c}	2.48±0.07^{bc}	0.87±0.05^{b}

*Each value is the mean ± SE of three replicates. Mean values followed by different letters in the same column were significantly different (P<0.05)

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 the storage period.

Treatment period (Days)	Chemical composition (g/100g) -Preservative-1 **(***Coleus aromaticus*)									
	EF-1					**EF-2**				
	A	**M**	**CP**	**CL**	**CC**	**A**	**M**	**CP**	**CL**	**CC**
0	2.25±0.01^{bc}	74.40±0.11^{ef}	25.44±0.16^{ab}	2.08±0.11^{a}	0.65±0.02^{bc}	2.75±0.21^{ab}	77.57±0.27^{b}	28.44±0.05^{de}	2.28±0.32^{ab}	0.52±0.02^{a}
1	2.29±0.05^{ab}	75.16±0.10^{a}	27.39±0.12^{bc}	2.13±0.08^{ab}	0.72±0.09^{bc}	3.24±0.34^{a}	79.46±0.32^{b}	30.39±0.11^{ab}	2.29±0.42^{ab}	0.65±0.14^{bc}
3	2.15±0.08^{b}	76.1±0.05^{g}	32.29±0.19^{a}	2.19±0.07^{d}	0.81±0.13^{a}	3.17±0.57^{g}	80.01±0.43^{b}	35.65±0.24^{c}	2.54±0.56^{f}	0.74±0.21^{a}
5	2.20±0.10^{fg}	72.3±0.15^{a}	35.25±0.21^{f}	2.23±0.10^{fg}	0.89±0.05^{g}	3.58±0.93^{f}	68.07±0.16^{a}	38.27±0.35^{g}	2.79±0.67^{g}	0.85±0.37^{f}
7	2.10±0.04^{c}	70.5±0.18^{b}	39.24±0.20^{c}	2.27±0.09^{b}	0.93±0.10^{a}	4.05±0.08^{c}	81.05±0.57^{a}	40.00±0.93^{b}	2.95±0.82^{a}	0.55±0.48^{b}

*Each value is the mean ± SE of three replicates. Mean values followed by different letters in the same column were significantly different (P<0.05)

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 the 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^{a}	73.23±0.15^{b}	27.34±1.10^{d}	2.05±0.14^{a}	0.30±0.06^{c}	2.53±2.43^{b}	74.25±0.18^{a}	28.35±1.15^{e}	2.22±0.17^{d}	0.45±0.08^{e}
1	2.27±0.07^{bc}	74.83±0.28^{ac}	31.48±1.31^{ef}	2.10±0.09^{cd}	0.39±0.26^{ab}	2.67±2.53^{cd}	77.84±0.19^{bc}	33.51±1.38^{ac}	2.29±0.09^{ab}	0.47±0.36^{ac}
3	2.11±0.01^{a}	78.20±0.27^{c}	35.71±1.19^{a}	2.13±0.16^{c}	0.52±0.14^{a}	2.47±0.71^{c}	80.21±0.11^{d}	37.69±1.23^{a}	2.32±0.21^{e}	0.53±0.24^{d}
5	2.17±0.11^{b}	71.29±0.20^{a}	40.01±1.11^{c}	2.20±0.10^{e}	0.75±0.12^{b}	2.83±0.28^{a}	73.22±0.20^{c}	41.05±1.17^{e}	2.40±0.11^{d}	0.87±0.18^{c}
7	1.89±0.09^{ac}	68.14±0.33^{ac}	45.23±1.21^{d}	2.22±0.06^{cd}	0.82±0.21^{ab}	2.55±0.15^{c}	69.15±0.33^{ab}	46.32±1.26^{ac}	2.43±0.05^{ae}	0.92±0.21^{e}

*Each value is the mean ± SE of three replicates. Mean values followed by different letters in the same column were significantly different (P<0.05)

Table-4. The 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)					
	Coleus aromaticus		***Sargassum wightii***		**Without Preservative**	
	EF-1	**EF-2**	**EF-1**	**EF-2**	**EF-1**	**EF-2**
0	198×10^5	218×10^5	152×10^5	172×10^5	225×10^5	232×10^5
1	112×10^5	121×10^5	72×10^5	96×10^5	232×10^5	249×10^5
3	78×10^5	88×10^5	48×10^5	52×10^5	247×10^5	256×10^5
5	148×10^5	180×10^5	112×10^5	125×10^5	255×10^5	262×10^5
7	163×10^5	228×10^5	130×10^5	158×10^5	269×10^5	276×10^5

Table 5. The quality index score of the selected commercial important marine fishes *Sardinella longiceps* (EF-1) and *Rastrelliger kanagurta* (EF-2) collected from Olavakkode Fishmarket.

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**
Odour	3	1	1	3	1	1
Texture	3	1	1	4	1	1
Taste	3	1	1	3	1	1

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

Microbial analysis: Both the preservatives used in this experimental study showed antibacterial activity in EF1 and EF2 fishes during ice storage (4±1ºC) for a storage period of 0, 1, 3, 5 and 7 days (Table 4). The TVC in the untreated sample increased significantly during ice storage, compared to the fish muscles stored with preservatives (*Coleus aromaticus* and *Sargassum wightii*). On 7th day of the storage period in EF1 (*Sardinella longiceps*) and EF-2 (*Rastrelliger kanagurta*), the observed TVCs count in fish flesh stored under the chilled condition with preservatives *Coleus aromaticus* and *Sargassum wightii* were 163×10^5cfu g^{-1}, 130×10^5cfu g^{-1}; 228×10^5cfu g^{-1}, and 269 $\times10^5$cfu g^{-1} respectively, which decreased in TVCs count when compared with the control flesh (276×10^5cfu g^{-1}) stored without preservatives. Li *et al*. (2011) 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 [23]. Lugasi *et al*. (2007) reported that plant extracts preserve the frozen minced and filleted fish product storage quality.

Sensory analysis: The results of the sensory quality parameters in *Sardinella longiceps* (EF-1) and *Rastrelliger kanagurta* (EF-2) during the refrigerated condition can be seen in Table 7. A decline was observed in the sensory parameters like appearance, odor, texture, and taste from the initial day to the 7th day of storage period without preservative. The reason for this decline in sensory parameters may be due to post mortem 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 [24, 25]. Dhanapal *et al*. (2013) reported that organoleptic scores of Migala stored in ice decreased gradually with increasing storage time similar to the results of the present analysis score of fishes stored without preservatives. For the fish muscles stored with the selected preservative P-1 (*Coleus aromaticus)*, and P-2 (*Sargassum wightii*) the score was 1, i.e. very good for all the sensory parameters like odor, texture, and taste except appearance in EF-1 which was rated 0.

CONCLUSION:

The use of selected preservatives P-1 (*Coleus aromaticus*) and P-2 (*Sargassum wightii*) showed the best prospective use in food production with maximum potential towards the production of safer and healthier foods, with more efficient preservation of the environment. Commercially important experimental fishes *Sardinella longiceps* (EF-1) and *Rastrelliger kanagurta* (EF-2) showed a reduction in all the proximate parameters like protein, lipid, carbohydrate, and moisture content when stored under frozen temperature 4±1°C without preservative. But the proximate parameters like protein, lipid,

and carbohydrate increased with a storage period of 7 Days with a decrease in moisture content in flesh of EF-1 and EF-2 stored with preservatives P-1 (*Coleus aromaticus*) and P-2 (*Sargassum wightii*). The total viable count in untreated samples was observed maximum while less viable counts were found in fish samples treated with P1 and P2. The organoleptic assessment proved that plant preservative-treated samples showed the best appearance, smell, color, texture, and taste of both the fishes but a decline was found in untreated EF1 and EF2. Among the natural preservatives used under chilled condition (4±1ºC) *Sargassum wightii* proved to be a better preservative than *Coleus aromaticus*. Therefore, as the developments in food preservation are focusing on the implementation of natural antimicrobials and antioxidants to replace synthetic preservatives with natural substances. The present bio-preservatives were proved to be efficient in maintaining the quality attributes of the stored fish muscles.

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