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To study the microbial stability of catla (*Catla catla*) fillets under refrigerated storage using least chemical preservatives

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Abstract
In the present study an attempt has been made to prepare ready to eat fish fillets which were packed in stand up pouches and its shelf life was studied at 4°C i.e. refrigerated storage after an interval of 4 days each. Methods of cooking showed non-significant effects on bacterial load with the mean value of 4.33 log cfu/g in conventional oven cooking and 4.42 log cfu/g in microwave oven cooking. Proliferation of microbes was noted during storage and Total Plate Count increased significantly on 12th day of storage which is within acceptable range for spiced products i.e. 6.44log cfu/g and 6.19 log cfu/g in conventional and microwave oven respectively but non-spiced products deteriorated completely. The mean value of Total Plate Count increased from 2.13log cfu/g on 0 day to 6.44 log cfu/g on 12 day. The increase in coliform count up to 4th day of storage was non-significant The present investigation was therefore undertaken to assess the microbial stability of the products, in terms of total plate count (TPC) and coliform counts on different treatments.

Keywords: bacterial load, coliform count, fish fillets, shelf life, refrigerated storage

Introduction
Health, nutrition and convenience are the major factors driving the global food industry. Fish products have attracted considerable attention as a source of protein, vitamins, minerals, fats and rank third among the food categories with fastest overall growth worldwide. As fish is highly perishable, proper processing and packaging helps in maintaining the quality of fish [1]. Consumers demand high quality processed foods with minimal changes in nutritional, microbial and sensory properties. Alternative or novel processing technologies are being explored and implemented to provide safe, fresher-tasting, nutritive foods without the use of heat or chemical preservatives [2]. Sea foods are highly perishable and usually spoil faster than other muscle foods. Freshly caught fish undergoes quality changes as a result of autolysis and bacterial activity. Extent of these changes with time determines shelf life of the product. Proper storage conditions are essential to prevent the spoilage of fish and fishery products. Many emerging technologies have the potential to extend the shelf life. Few of the emerging technologies that have application in fish processing are High Pressure Processing, Irradiation, Pulsed light technology, Pulsed Electric Field, Microwave Processing, Radio frequency, Ultrasound etc. Packaging technologies like Modified Atmosphere, Active and Intelligent packaging also plays an important role in fish preservation [3,4].There are not many studies about catla fish fillets stored under refrigerated conditions. Previous studies were about shelf life of refrigerated raw anchovy (*Engraulis encrasicholus*) patties [5], determination of quality parameters of fish balls prepared from raw catla fish [6] and quality changes in fish patties produced from anchovy during refrigerated storage [7].
The aim of this study was to produce catla fish fillets, investigate the quality changes in catla and determine the shelf life of catla fillets during refrigerated (4 ^{0}C) storage. The microbial evaluation was done to make the present findings a wholesome one in order to know the microbial load of spiced and non-spiced fillets cooked conventionally and in microwave oven.

Materials and Methods
Sample preparation: Fresh catla (*Catla catla*) were obtained from local fish market, Hisar, Haryana (India). After purchase, the fish were transferred to the laboratory in polystyrene

boxes with crushed ice within 1 h. On arrival at the laboratory, quality control analyses of the fish were performed and balls were prepared. The fish were gutted, filleted and washed. Ingredients were added to the fish fillets and were mixed properly. The mixture was kept in a refrigerator at 4 ºC for 1 h. Fillets were then cooked in conventional and microwave oven.

Microbiological evaluation: Microbiological condition of the product prepared by conventional cooking and microwave cooking using fish meat after addition of additives and spices was evaluated at 0, 4, 8 and 12 days of storage at 4 ± 1 °C. Total plate count and coliform count was done according to the method prescribed by [8].

Results and Discussion

Changes in Total Plate Count on storage

The highest value of TPC at 0 day of cooking was found in P0S1M1 (2.28 log cfu/g) followed by P0S0M0 (2.22 log cfu/g), P0S0M1 (2.21 log cfu/g), P0S1M0 (2.13 log cfu/g). TPC of fillets in both kinds of cooking did not differ significantly. Proliferation of microbes was noted at storage intervals and TPC increased significantly on 12th day of storage. At 4th day of storage highest count was found in P0S0M1 (4.55 log cfu/g) and lowest was observed in P0S1M1 (3.18 log cfu/g). Non-spiced fillets whether cooked conventionally (P0S0M0) or in microwave (P0S0M1) showed non-significant difference i.e. 4.29 and 4.56 log cfu/g respectively on 4th day. Spiced fillets whether cooked conventionally (P0S1M0) or in microwave (P0S1M1) were also showing significantly similar with much closer counts (3.27 and 3.18 log cfu/g) which is much less than the non-spiced fillets. While comparing the spice effect, conventionally cooked spiced fillet (P0S1M0) possessed lower (3.27 log cfu/g) count than non-spiced fillet (4.55 log cfu/g).

Table 1: Effect of different treatments as affected by spices and method of cooking on total plate count (log cfu/g) of fish fillets during storage

Treatment	Total plate count (log cfu/g)				
	Storage period (days)				
	0	4	8	12	Mean
P0S0M0	$_{a}2.22^{c}\pm0.65$	$_{a}4.29^{b}\pm0.73$	$_{a}6.47^{a}\pm0.68$	-	4.33
P0S0M1	$_{a}2.21^{c}\pm0.63$	$_{a}4.55^{b}\pm0.77$	$_{a}6.49^{a}\pm0.77$	-	4.42
P0S1M0	$_{a}2.13^{d}\pm0.87$	$_{b}3.27^{c}\pm0.80$	$_{b}4.82^{b}\pm0.83$	$_{a}6.44^{a}\pm0.84$	4.17
P0S1M1	$_{a}2.28^{d}\pm0.67$	$_{b}3.18^{c}\pm0.89$	$_{b}4.90^{b}\pm0.71$	$_{a}6.19^{a}\pm0.60$	4.14
Mean	2.19	3.84	5.63	6.35	
CD at 5%	p×s×M×D = 0.829, Duration (mean) = 0.030, p×s×M (mean) = 0.414				

P0S0M0 (Fillets without Spice cooked in Conventional oven)
P0S0M1 (Fillets without Spice cooked in Conventional oven)
P0S1M0 (Fillets with spice cooked in Conventional oven)
P0S1M1 (Fillets with Spice cooked in Microwave oven)
Mean values are the average of 6 replications
Similar superscripted letters along the row and subscripted letters along the column depicts non-significant difference

The trend in variation of TPC in fillets was almost identical with respect to method of cooking and addition of spice mix. At 8th day of storage, both the methods for preparation of fillets with or without spice exhibited almost similar trend as that of 4th day. Whereas at 12th day of storage, the TPC level of non- spiced fillets increased enormously to an immeasurable extent leading to spoilage, and therefore studies up to 8th day were possible. The TPC of spiced fillets cooked by either of the method are of the same level as that for 8th day in non-spiced ones. Excluding the non-spiced products which were completely spoiled at 12th day the highest count (6.44 log cfu/g) was found in P0S1M0 and lowest (6.19 log cfu/g) in P0S1M1. Spiced microwave cooked fillet contained lower TPC (6.19 log cfu/g) than conventional oven cooked spiced fillet (6.44 log cfu/g). As the storage duration increased TPC also increased significantly in each treatment from 0 to 12 days of storage. The mean value for TPC increased from 2.13 log cfu/g (P0S1M0) on zero day to 6.44 log cfu/g (P0S1M0) on 12 days of storage.

Table 1 illustrated that method of cooking had non-significant effect on TPC throughout the storage duration and it is evident that at 0 day, microwave cooked fillets possessed higher TPC than conventionally cooked (non-significant).One of the related research also reported non-significant difference in TPC in chicken patties cooked by conventional and microwave oven [9]. Similar finding was also reported in spiced and non-spiced fish balls cooked in microwave and conventional oven [6]. However in another research higher TPC in microwave cooked than gas oven cooked chicken meat was observed [11]. At 0 day higher TPC was found in spiced products, which might be due to initial microbial load of spices which contributed to higher TPC in spiced fillets and balls. Whereas, due to extended period of storage stock non spiced products were found to have higher TPC than spiced products; particularly at 4th day where non-spiced fillets exhibited highest counts, thereby confirming the antimicrobial action of the spice ingredients owing various microbial inhibitory substances like cinnamic aldehyde in cinnamon and eugenol in cloves. While taking into consideration effect of spices, various degrees of inhibition against microbial contamination and growth when essential oils and oleoresins from most of the spices were used in product formulation was observed [1]. They also observed that spices either in powder or oils are known to control microbial spoilage. The increase in TPC with the increase in storage duration was also reported, who found that initial total count of fresh chicken patties was less than 10^4 /g and after storage of 10 days at 3 ˚C count increased to 7 x 10^6/g [12]. Proliferation of microbes was noted during storage and TPC increased significantly on 12th day of storage ranging from 6.19 (P0S1M1) to 6.44 log cfu/g (P0S1M0) which is still confined for the acceptable range was also in agreement with the finding of Sharma [9]. Additives and spice mix used in the study for preparation of fillets and balls helped to extend their shelf life at 4±1 ºC temperature by their antimicrobial action.

Changes in Coliform during storage

The perusal of data regarding changes in coliform count (log cfu/g) of fish fillet as affected by method of cooking, spice mix and duration of storage are presented in Table 2. At 0 day of product preparation highest coliform count was found in non-spiced products i.e. P0S0M1 (0.82 log cfu/g) followed by P0S0M0 (0.80 log cfu/g), P0S1M1 (0.67 log cfu/g) and P0S1M0 (0.64 log cfu/g). This indicates that non spiced fillets exhibited higher coliform count than spiced fillets. Although there was a non-significant difference in coliform count of all the treatments at zero days of storage, and similar results were obtained at 4th and 8th day. At 4th day of storage highest count (1.29 log cfu/g) was found in P0S0M0 and P0S0M1 possessing equal value and lowest (1.09 log cfu/g) in P0S1M0. Therefore, spices produced their antimicrobial effects leading to lowest count in spiced conventional oven cooked fillet and highest in non-spiced microwave cooked ball. At 8th day, highest count (1.97 log cfu/g) was observed in P0S0M1 and lowest (1.30 log cfu/g) in P0S1M1 and at 12th day, non-spiced products get deteriorated completely indicating that spices must have shown their antimicrobial effects in the treatments containing spice mix. With the increase in storage duration coliform count increased non-significant till 4th day of storage and significant difference was observed from 8th day of storage in two treatments i.e. P0S1M0 possessing value (1.38 log cfu/g) along with P0S1M1 (1.30 log cfu/g). At 12th day non spiced products were completely spoiled and spiced products contained a moderate amount of coliform counts. The mean values for coliform count increased from 0.64 (P0S1M0) at zero day to 1.97 (P0S0M1) on 8th day of storage at refrigerated condition (4±1 °C).

Table 2 suggests that the increase in coliform count up to 4th day of storage was non-significant. This indicates that raw fish procured was of acceptable quality and the hygienic conditions of the laboratory were also adequately maintained during product preparation. Application of heat processing (conventional and microwave oven) stopped the increase in coliform counts. Our results are also in agreement with the findings of some researchers who showed that the coliforms are killed during heat processing of products leading to lower counts [6, 13]. The reason for the spoilage of non-spiced product at 12th day of storage could be higher proliferation of bacteria where spices were not utilized as ingredients. Additives and spice mix used in this study for preparation of fillets and balls helped to extend shelf life by their antimicrobial action.

Table 2: Effect of different treatments as affected by spices and method of cooking on coliform count (log cfu/g) of fish products during storage

Treatment	Coliform count (log cfu/g)				
	Storage period (days)				
	0	4	8	12	Mean
P0S0M0	$_{a}0.80^{ab}\pm0.09$	$_{a}1.29^{ab}\pm0.03$	$_{a}1.87^{a}\pm0.06$	-	1.32
P0S0M1	$_{a}0.82^{ab}\pm0.06$	$_{a}1.29^{ab}\pm0.07$	$_{a}1.97^{a}\pm0.06$	-	1.36
P0S1M0	$_{a}0.64^{ab}\pm0.07$	$_{a}1.09^{ab}\pm0.06$	$_{a}1.38^{ab}\pm0.04$	$_{a}1.94^{a}\pm0.06$	1.27
P0S1M1	$_{a}0.67^{ab}\pm0.07$	$_{a}1.15^{ab}\pm0.07$	$_{a}1.30^{ab}\pm0.04$	$_{a}1.86^{a}\pm0.05$	1.25
Mean	0.69	1.22	1.64	1.89	
CD at 5%	p×s×M×D = 0.743, Duration (mean) = 0.263, p×s×M (mean) = 0.372				

P0S0M0 (Fillets without Spice cooked in Conventional oven)
P0S0M1 (Fillets without Spice cooked in Conventional oven)
P0S1M0 (Fillets with spice cooked in Conventional oven)
P0S1M1 (Fillets with Spice cooked in Microwave oven)
Mean values are the average of 6 replications
Similar superscripted values along the row and subscripted values along the column depicts non-significant difference

Conclusion

Spice mix application was found to be most desirable in enhancing the microbiological quality of fish products by reducing the microbial load. Methods of cooking and products showed non-significant effects on bacterial load. Proliferation of microbes was noted during storage and TPC increased significantly on 12th day of storage which is within the acceptable range for spiced products but non spiced products deteriorated completely. Based on microbial evaluation the shelf life of catla fillets without spice was up to 8 days of storage and for spiced one is up to 12 days during refrigerated storage. Microbial inhibitory substances present in spice mix due to the presence of cinnamom and eugenol were proved to be really effective. The increase in coliform count up to 4th day of storage was non-significant indicating hygienic conditions of laboratory along with acceptable quality of raw fish procured. Application of heat processing restricted the increase in coliform count. Additives and spice mix used in the study for preparation of fillets helped to extend shelf life by their antimicrobial action. Although the shelf life of catla fillets can further be enhanced by effective packaging (modified atmosphere or vacuum packaging).

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