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STUDY OF THE COMPARATIVE EFFECT OF FROZEN STORAGE ON THE MICROBIAL PROFILE OF THE MUSCLE OF FRESH WATER FISHES OF HIGH COMMERCIAL VALUE

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ABSTRACT

The present investigation was directed towards the study of the comparative effect of frozen storage on the microbial profile of the muscle of freshwater fishes *Labeo rohita* and *Catla catla* of high commercial value. The current research focuses on the development of an archetype of a new fish preservation method to increase their shelf life for commercial purpose. During the study period, the fish muscles were processed with two types of preservatives including a mix of sodium chloride and ascorbic acid and a blend of sodium chloride and dried citrus fruit (lemon) peel powder and then subjected to frozen storage for a period of 21 days in 02 freezing conditions *viz* at-20±2°C and 0±2°C. The analysis was carried out an interval of 07 days and unprocessed fresh water fish samples of *Labeo rohita* and *Catla catla* were treated as control. The freezing of fish at low temperature after processing with NaCl and Lemon peel powder or NaCl and nontoxic pure ascorbic acid powder may make it less prone to spoilage by decreasing the bacterial activity. As a result of this, freezing at -20±2°C temperature and processing of fishes with both the blends of preservatives are highly recommended as low-cost and eco-friendly preservation technique due to above mentioned reasons for freeze storage of fishes for commercial use.

Keywords: Preservatives, Frozen storage, Archetype, Reduction of Bacterial Activity.

1. INTRODUCTION

With diverse environmental conditions like humid weather and hot summer, there is increased demand of appropriate alternative cost effective frozen storage facilities for fishes in Jabalpur district of Central India to manage the demand-supply ratio. Inappropriate freezing practice and humid conditions favour microbial growth and fish tissue degradation. Frozen storage offers a means of preserving fish however, during frozen storage quality is lost due to a deterioration of texture, flavour and colour especially after long periods of storage when poor freezing practices are employed or when the initial fish quality is low [1].

Thus in context of analysis of frozen storage effect on fishes, the aim of the present comparative study was to assess the variations in the microbial status of the freeze stored processed freshwater fishes of high commercial value in comparison to effect of freezing on microbial profile of unprocessed freshwater fishes (*Labeo rohita* and *Catla catla*) treated as control and kept for 7th,14th and 21st days in 02 different frozen storage conditions *viz*. at -20±2°C (good freezing practice) and at 0±2°C (poor freezing practice). The present study aims to develop an archetype of a novel fish preservation method using two different blends of preservatives to enhance the conditions of fishes for commercial purpose.

2. MATERIAL AND METHODS

2.1. Collection of fish samples and processing of fish tissues

During the study period, untreated freshwater fishes of high commercial value (*Labeo rohita* and *Catla catla*) were collected from Govt. fish farm Ranjhi, Jabalpur. The fish samples were kept immediately with crushed ice and ice packs in thermocool ice boxes and were transported to the lab for further analysis.

2.2. Preparation of Fish Samples

The fishes were washed with large amount of distilled water and triplicates of 65 gms each of muscles of unprocessed freshwater fishes were taken out in 3 parts from the dorsal part of the body. The first triplicate parts of 65 gm of fish muscles were treated as control while second triplicate parts of 65 gm of fish muscles

were treated with a mix of 1gm sodium chloride and 1gm ascorbic acid and the third triplicate parts of 65 gm of fish muscles were treated with blend of 1gm sodium chloride and 1gm dried citrus fruit (lemon) peel. All the 03 triplet pieces of muscles were immediately wrapped in aluminum foil and kept in a tray in 02 freezing conditions (at -20 ±2°C and at 0±2°C) for a period of 21 days followed by their biochemical analyses on 7th, 14th and 21st day of storage.

2.3. Analysis of Microbial quality

Aerobic Plate Count or Total Plate Count (TPC) by Pour Plate Technique was determined according to methods [2, 3]. The standard deviation of mean values (±SD) of the microbial composition of the triplicates of fish muscles were analyzed as per standard method [4].

3. RESULTS AND DISCUSSION

The outcome of the comparative analysis of the microbial composition of the triplicates of fish muscles processed with two different preservatives and frozen for a period of 21 days in 02 freezing conditions (at 0±2°C and at -20±2°C) from August 2017 to July 2019 are as discussed in subsequent fractions.

3.1. Microbial profile of *Labeo rohita* at 0±2°C

During the present study, at 0±2°C the maximum value of TPC was recorded as 3.0±0.20 log10 cfu/g (for control) as 2.9±0.158 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 2.9± 0.264 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-1} dilution for a period of 21days of freezing while the minimum value was found to be 2.4±0.353 log10 cfu/g (for control) as 2.3±0.20 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 2.3±0.30 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-1} dilution for a period of 07 days of freezing in comparison to the TPC on 0 day as 2.15±0.01 log 10 cfu/g at 10^{-1} dilution for fresh muscle (control) of *Labeo rohita*. The maximum value of TPC was recorded as 0.0095±0.00015 log10 cfu/g (for control) as 0.0091± 0.00015 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 0.0094 0.00015 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-4} dilution for a period of 21 days of freezing while the minimum value was found to be 0.0074±0.00020 log10 cfu/g (for control) as 0.0074± 0.00021 log10 cfu/g for muscles treated with blend of NaCl and Ascorbic acid) and as 0.0080±0.00015 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-4} dilution for a period of 07 days of freezing (table 1b) in comparison to the TPC on 0 day as 0.0060±0.001 log10 cfu/g at 10^{-4} dilution for fresh muscle (control) of *Labeo rohita* (table 1a).

3.2. Microbial profile of *Labeo rohita* at -20±2°C

During the present study, at -20±2°C the maximum value of TPC was recorded as 2.8±0.264 log10 cfu/g (for control) as 2.8±0.158 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 2.8±0.158 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-1} dilution for a period of 21 days of freezing while the minimum value was found to be 2.3±0.20 log10 cfu/g (for control) as 2.3±0.158 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 2.2±0.158 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-1} dilution for a period of 07 days of freezing in comparison to the TPC on 0 day as 2.15±0.01 log10 cfu/g at 10^{-1} dilution for fresh muscle (control) of *Labeo rohita*. The maximum value of TPC was recorded as 0.0085±0.00015 log10 cfu/g (for control) as 0.0090±0.00015 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 0.0087±0.00030 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-4} dilution for a period of 21 days of freezing while the minimum value was found to be 0.0068±0.00030 log10 cfu/g (for control) as 0.0080±0.00025 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 0.0074±0.00010 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-4} dilution for a period of 07 days of freezing in comparison to the TPC on 0 day as 0.0060±0.001 log10 cfu/g at 10^{-4} dilution for fresh muscle (control) of *Labeo rohita* (Table-1c).

Table 1a: Mean value of Aerobic Plate Count or Total Plate Count (log10 cfu/g) by Pour Plate Technique of muscle of *Labeo rohita* on 0 day

Parameters	Variations in Total Plate Count (TPC)			
Dilutions	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Control	2.15 ± 0.01	0.17 ± 0.0151	0.012 ± 0.0030	0.0060 ± 0.001

Table 1b: Changes in Mean value of Aerobic Plate Count or Total Plate Count (log10 cfu/g) by Pour Plate Technique of muscle of *Labeo rohita* [kept at 0±2°C]

Parameters	Fish frozen for 7 days				Fish frozen for 14 days				Fish frozen for 21 days			
	Dilutions				Dilutions				Dilutions			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
NaCl + Ascorbic acid	2.3 ± 0.20	0.18 ± 0.03	0.013 ± 0.0021	0.0074 ± 0.00021	2.6 ± 0.158	0.19 ± 0.025	0.032 ± 0.0015	0.0085 ± 0.00015	2.9 ± 0.158	0.24 ± 0.021	0.084 ± 0.0025	0.0091 ± 0.00015
NaCl + Lemon peel powder	2.3 ± 0.30	0.18 ± 0.026	0.012 ± 0.0021	0.0080 ± 0.00015	2.5 ± 0.20	0.18 ± 0.015	0.013 ± 0.0015	0.0084 ± 0.00015	2.9 ± 0.264	0.23 ± 0.015	0.016 ± 0.0026	0.0094 ± 0.00015
Control	2.4 ± 0.353	0.18 ± 0.021	0.014 ± 0.0012	0.0074 ± 0.00020	2.7 ± 0.158	0.20 ± 0.025	0.016 ± 0.0021	0.0082 ± 0.00015	3.0 ± 0.20	0.27 ± 0.015	0.020 ± 0.0030	0.0095 ± 0.00015

Table 1c: Changes in Mean value of Aerobic Plate Count or Total Plate Count (log10 cfu/g) by Pour Plate Technique of muscle of *Labeo rohita* [kept at -20±2°C]

Parameters	Fish frozen for 7 days				Fish frozen for 14 days				Fish frozen for 21 days			
	Dilutions				Dilutions				Dilutions			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
NaCl + Ascorbic acid	2.3 ± 0.158	0.18 ± 0.021	0.012 ± 0.0026	0.0080 ± 0.00025	2.5 ± 0.20	0.16 ± 0.021	0.014 ± 0.0022	0.0085 ± 0.00021	2.8 ± 0.158	0.20 ± 0.030	0.015 ± 0.0021	0.0090 ± 0.00015
NaCl + Lemon peel powder	2.2 ± 0.158	0.14 ± 0.015	0.010 ± 0.0025	0.0074 ± 0.00010	2.3 ± 0.254	0.17 ± 0.030	0.013 ± 0.0025	0.0084 ± 0.00021	2.8 ± 0.158	0.20 ± 0.025	0.014 ± 0.0025	0.0087 ± 0.00030
Control	2.3 ± 0.20	0.17 ± 0.015	0.013 ± 0.0030	0.0068 ± 0.00030	2.6 ± 0.158	0.18 ± 0.010	0.015 ± 0.0026	0.0080 ± 0.00040	2.8 ± 0.264	0.21 ± 0.030	0.016 ± 0.0021	0.0085 ± 0.00015

3.3. Microbial profile of *Catla catla* at 0±2°C

During the present study, at 0±2°C the maximum value of TPC was recorded as 2.9±0.130 log10 cfu/g (for control) as 2.5±0.20 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 2.9± 0.212 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-1} dilution for a period of 21days of freezing while the minimum value was found to be 2.7±0.20 log10 cfu/g (for control) as 2.2±0.13 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 2.4±0.20 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-1} dilution for a period of 07 days of freezing in comparison to the TPC on 0 day as 2.1±0.141 log 10 cfu/g at 10^{-1} dilution for fresh muscle (control) of *Catla catla*. The maximum value of TPC was recorded as 0.0066±0.00025 log10 cfu/g (for control) as 0.0058 ±0.00026 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 0.0054±0.00015 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-4} dilution for a period of 21days of freezing while the minimum value was found to be 0.0062±0.00026 log10 cfu/g (for control) as 0.0040± 0.00026 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 0.0043±0.00021 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-4} dilution for a period of 07 days of freezing (table 2b) in comparison to the TPC on 0 day as 0.0035±0.00020 log10 cfu/g at 10^{-4} dilution for fresh muscle (control) of *Catla catla* (Table-2a).

3.4. Microbial profile of *Catla catla* at -20±2°C

During the present study, at -20±2°C the maximum value of TPC was recorded as 2.6±0.158 log10 cfu/g (for control) as 2.8±0.212 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 2.9±0.130 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-1} dilution for a period of 21 days of freezing while the minimum value was found to be 2.3±0.212 log10 cfu/g (for control) as 2.4±0.212 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 2.2±0.10 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-1} dilution for a period of 07 days of freezing in comparison to the TPC on 0 day as 2.1±0.141 log10 cfu/g at 10^{-1} dilution for fresh muscle (control) of *Catla catla*. The maximum value of TPC was recorded as 0.0066±0.00025 log10 cfu/g (for control) as 0.0059±0.00025 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 0.0069±0.00011 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-4} dilution for a period of 21 days of freezing while the minimum value was found to be 0.0043±0.00015 log10 cfu/g (for

control) as 0.0049±0.00025 log10 cfu/g (for muscles treated with blend of NaCl and Ascorbic acid) and as 0.0054±0.00015 log10 cfu/g (for muscles treated with blend of NaCl and lemon powder) at 10^{-4} dilution for a period of 07 days of freezing (Table-2b) in comparison to the TPC on 0 day as 0.0035±0.00020 log10 cfu/g at 10^{-4} dilution for fresh muscle (control) of *Catla catla* (table 2a).

The quality of fish meat is largely dependent on its microbial contamination. Inquisitive study shows an increasing trend for TPC during the frozen storage period. Initially the values for TPC were 2.18±0.02 log cfu/g and increased to 6.87±0.1 log cfu/g at the end of storage thus crossing the permissible limits of 6 log cfu/g on 20th day of storage [5]. Likewise researchers [6] found an increase in Coliform count with the increasing storage period in frozen Tilapia. Significant statistical increase in total viable counts of whole gutted common sole (*Solea solea)* over the storage period of 24 days is also reported [7].

Table 2a: Mean value of Aerobic Plate Count or Total Plate Count (log10 cfu/g) by Pour Plate Technique of muscle of Control Fish *Catla catla* on 0 day.

Parameters	Variations in Total Plate Count (TPC)			
Dilutions	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Control	2.1 ± 0.141	0.14 ± 0.021	0.015 ± 0.0026	0.0035 ± 0.00020

Table 2b: Changes in Mean value of Aerobic Plate Count or Total Plate Count (log10 cfu/g) by Pour Plate Technique of muscle of *Catla catla* [kept at 0±2°C]

Parameters	Fish frozen for 7 days				Fish frozen for 14 days				Fish frozen for 21 days			
	Dilutions				Dilutions				Dilutions			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
NaCl + Ascorbic acid	2.2 ± 0.13	0.17 ± 0.015	0.023 ± 0.0010	0.0040 ± 0.00026	2.3 ± 0.212	0.18 ± 0.032	0.029 ± 0.0020	0.0044 ± 0.00015	2.5 ± 0.20	0.19 ± 0.042	0.032 ± 0.0015	0.0058 ± 0.00026
NaCl + Lemon peel powder	2.4 ± 0.20	0.12 ± 0.026	0.022 ± 0.0015	0.0043 ± 0.00021	2.6 ± 0.130	0.16 ± 0.025	0.025 ± 0.0020	0.0048 ± 0.00025	2.9 ± 0.212	0.19 ± 0.036	0.033 ± 0.0021	0.0054 ± 0.00015
Control	2.7± 0.20	0.20 ± 0.025	0.026 ± 0.0010	0.0062 ± 0.00026	2.8 ± 0.158	0.26 ± 0.015	0.031 ± 0.0015	0.0064 ± 0.00026	2.9 ± 0.130	0.28 ± 0.026	0.034 ± 0.0020	0.0066 ± 0.00025

Table 2c: Changes in Mean value of Aerobic Plate Count or Total Plate Count (log10 cfu/g) by Pour Plate Technique of muscle of *Catla catla* [kept at -20±2°C]

Parameters	Fish frozen for 7 days				Fish frozen for 14 days				Fish frozen for 21 days			
	Dilutions				Dilutions				Dilutions			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
NaCl + Ascorbic acid	2.4 ± 0.212	0.19 ± 0.025	0.022 ± 0.0010	0.0049 ± 0.00025	2.7 ± 0.264	0.20 ± 0.020	0.034 ± 0.0015	0.0052 ± 0.00022	2.8 ± 0.212	0.22 ± 0.012	0.036 ± 0.0025	0.0059 ± 0.00025
NaCl + Lemon peel powder	2.2 ± 0.10	0.15 ± 0.021	0.020 ± 0.0032	0.0054 ± 0.00015	2.6 ± 0.158	0.39 ± 0.015	0.046 ± 0.0025	0.0065 ± 0.00025	2.9 ± 0.130	0.43 ± 0.021	0.049 ± 0.0020	0.0069 ± 0.00011
Control	2.3 ± 0.212	0.16 ± 0.020	0.018 ± 0.0032	0.0043 ± 0.00015	2.5 ± 0.158	0.16 ± 0.020	0.022 ± 0.0026	0.0050 ± 0.00021	2.6 ± 0.158	0.17 ± 0.025	0.023 ± 0.0025	0.0066 ± 0.00025

During the present study, it was observed that the rate of increase in the value of Aerobic Plate Count or Total Plate Count (TPC) for a period of 21 days of freezing showed less increase in microbial growth in muscles of *Labeo rohita* treated with a blend of NaCl and ascorbic acid powder at 0±2°C while the rate of increase in the Aerobic Plate Count was found to be less in muscles treated with blend of NaCl and lemon powder and -20±2°C. While in case of *Catla catla* the rate of increase in the value of Aerobic Plate Count was less in muscles treated with a blend of NaCl and lemon peel powder at 0±2°C while the rate of increase in the Aerobic Plate Count was found to be less in muscles treated with blend of NaCl and Ascorbic acid powder at -20±2°C. Nevertheless, the growth was less than the permissible limits of log10 cfu/g for a period of 21 days of freezing of fish muscles treated separately with a blend of NaCl and lemon powder and blend of NaCl and Ascorbic acid powder followed by freezing at -20±2°C (good freezing practice) and also at 0±2°C (poor freezing practice).

The minimum mean value of Aerobic Plate Count of both *Labeo rohita* and *Catla catla* at both 0±2°C and at -20±2°C were found to show an increasing trend of microbial growth for a period of 21 days of freezing in comparison to the maximum mean value of Aerobic Plate Count of control fish on 0 day. However, in case of *Labeo rohita* the increase was comparatively more in the sample stored at 0±2°C comparison to the sample stored at -20±2°C in all the samples and in case of *Catla catla,* the increase in microbial growth was comparatively more in the sample stored at -20 ±2°C compared to the samples stored at 0±2°C in all the samples.

4. CONCLUSION

With increase in the duration of frozen period, almost similar growth pattern and changes in mean value of Aerobic Plate Count (log10 cfu/g) of muscle of both *Labeo rohita* and *Catla catla* was observed at both 0±2°C and at -20±2°C when treated with blend of four different dilutions NaCl and Ascorbic acid as well as NaCl and lemon powder. However,-20°C temperature and processing of fishes with cost effective blend of NaCl & Lemon peel powder preservative to keep a check on microbial growth is recommended for freeze storage of fishes for commercial use.

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