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Effect of two different storage temperatures (-12 ^{0}C and -20 ^{0}C) on the proximate and microbial quality of *Labeo rohita* muscles

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Abstract
The present study was aimed to investigate the proximate and microbial profile of muscles of *Labeo rohita* kept under storage at two different temperatures viz. -12 ^{0}C and -20 ^{0}C for a period of 4 weeks. The analysis was carried out at an interval of 7 days. Initially, the proximate composition of fresh samples (at 0 day) revealed 16.00±0.1% protein, 3.55±0.01% lipid, 82. 28±0.04% moisture and 1.45±0.04% ash. However, after 4 weeks of frozen storage, there was a significant percentile decrease ($p \leq 0.05$) i.e. 36%, 60.2%, 8.53%, 27.5% in protein, lipid, moisture and ash content, respectively in -12 ^{0}C storage samples and in -20 ^{0}C storage sample the percentile decrease was comparatively less, i.e. 17.87% in protein, 24.50% in lipid, 5.28% in moisture and 20.68% in ash. The microbial load of fresh samples was found to be 2.10±0.2 log cfu/g (TPC i.e. Total Plate Count), 1.20±0.15 log cfu/g (CC i.e. Coliform Count), 2.12±0.02 log cfu/g (PC i.e. Psychrophilic Count). These values increased significantly ($p \leq 0.05$) during 4 weeks of frozen storage. The study further revealed that, TPC was within the acceptable limit (6 log cfu/g) on 21st day in samples stored at -20 ^{0}C whereas at -12 ^{0}C it crosses the limit on 7th day of storage, beyond which the quality of fish deteriorates thus, making it unfit for human consumption. Thus, storage of fish fillets at -20 ^{0}C extends their shelf life by 2 weeks as compared storage at -12 ^{0}C.

Keywords: Low storage temperature, proximate composition, microbial count, *Labeo rohita*.

1. Introduction
Fish is a food of excellent nutritional value, acting as a source of animal protein for people worldwide. In addition to this, fish has a high content of water and fat soluble vitamins (A, D, E, K), minerals and polyunsaturated fatty acids (PUFAs) of n-3 family. Although fish is highly nutritious, it is a highly perishable commodity and therefore cannot be kept for long. This is because of its short shelf life which in turn is attributed to its high rate of spoilage due to its high susceptibility to chemical and microbial activity. Shelf life can be defined as a period during which a perishable product under a given set of conditions can be stored until it is considered unfit for human consumption. Hence there is a need to carefully handle the fish and preserve it with appropriate methods so as to increase its shelf life.
Freezing of fish is an effective way of long term preservation and it has been shown that fish stored for up to three months under ideal conditions cannot be distinguished from fresh fish regarding color, taste and texture (Cappeln *et al*. 1999; Nielsen and Jessen, 2007 [1, 2]. Freezing, when done properly, can preserve the greatest quantity of nutrients. Low temperature limits the growth of food poisoning and food spoilage microorganisms. By making micro-organisms inactive and slowing down the enzymatic activity, freezing thus causes a decline in the biochemical activities. As a result of this, the fish remains free from spoilage for longer duration. In our country due to far location of major fishing grounds from consuming sites and seasonal fishing, fresh fish is not always available to the consumers. Thus, freezing and frozen storage have largely been employed to retain sensory and nutritional properties of fish flesh. Moreover the quickness and convenience with which fish can be preserved by freezing makes it an even more popular preservation method.
Keeping this in mind, the present study was conducted with an aim to extend the shelf life i.e. keeping quality of edible *Labeo rohita* muscle by storing them in two different temperatures i.e. -12 ^{0}C and -20 ^{0}C by evaluating their proximate and microbial composition during storage.

2. Materials and Methods

2.1 Collection of fish samples

Fresh samples of *Labeo rohita* were purchased from local market of Jammu city. They were immediately brought to the laboratory in polythene bags with crushed ice. The fish was degutted, washed with large amount of water and then subjected to filleting. The fillets were wrapped in aluminium foil, and divided into two groups: first group (G1) was stored at a temperature of -12 ^{0}C while the second group (G2) was stored at a temperature of -20 ^{0}C.

The complete experiment was carried out for a period of 4 weeks. Analytical procedures for proximate and microbiological changes were done on 0, 7th, 14th, 21st and 28th day of storage.

2.2 Analysis

The proximate composition (ash and moisture) of the fish samples were evaluated using the standard AOAC procedure (AOAC, 1995) [3]. The protein content was determined using the Lowry *et al.* (1951) [4]. The fat content was determined using Folch *et al.* (1957) [5]. The microbiological profile was determined according to APHA method (1984) [6]. Data was expressed as mean ± SD and were analyzed by one-way ANOVA test using SPSS statistical programme.

2.3 Statistical Analysis

Mean and standard errors were calculated for different parameters. The data analyses were performed using SPSS software (12.0 for Windows). Differences between treatments were analyzed using independent-measures one-way ANOVA. Post-hoc comparisons were conducted using Duncan's test. The values were expressed as mean ± SE. p values <0.05 were considered as significant and p values <0.001were considered as highly significant.

3. Results and Discussions

3.1 Proximate Composition

The proximate composition of muscles of *Labeo rohita* stored at -12 ^{0}C and -20 ^{0}C temperature for a period of four weeks has been shown in the following tables:

Table 1. Proximate composition (wet weight basis) of raw fish muscle of (*Labeo rohita*) stored in freezer at -12±2 °C during 4 weeks of storage

Days of storage	0 day	7th day	14th day	21st day	28th day
Total Protein (%)	16.14^{a} ±0.06	14.92^{b} ±0.10	13.01^{c} ±0.14	11.98^{d} ±0.49	10.24^{e} ±0.33
Lipid (%)	3.65 ^{a} ±0.43	3.01 ^{b} ±0.08	2.71^{c} ±0.14	2.18 ^{d} ±0.21	1.41 ^{e} ±0.17
Moisture (%)	82.52 ^{a} ±0.08	80.55 ^{b} ±0.01	78.5 ^{c} ±0.42	76.24 ^{d} ±0.002	75.26 ^{e} ±0.11
Ash (%)	1.48 ^{a} ±0.57	1.38 ^{b} ±0.03	1.26 ^{c} ±0.05	1.18 ^{d} ±0.50	1.05 ^{e} ±0.07

Table 2: Proximate composition (wet weight basis) of raw fish muscle of (*Labeo rohita*) stored in freezer at -20±2 °C during 4 weeks of storage

Days of storage	0 day	7th day	14th day	21st day	28th day
Total protein (%)	16.24 ^{a} ±0.09	15.14 ^{b} ±0.19	14.59 ^{c} ±0.44	13.95^{d} ±0.09	13.14^{e} ±0.28
Total lipid (%)	3.84 ^{a} ±0.12	3.54 ^{b} ±0.34	3.03 ^{c} ±0.10	2.94^{d} ±0.03	2.68^{e} ±0.16
Moisture (%)	82.59 ^{a} ±0.50	81.11 ^{b} ±0.40	79.16 ^{c} ±0.02	78.45^{d} ±0.24	77.93^{e} ±0.17
Ash (%)	1.45 ^{a} ±0.11	1.41 ^{b} ±0.003	1.35 ^{c} ±0.32	1.26^{d} ±0.29	1.15^{e} ±0.77

Mean SD with different superscripts in a row differs significantly (P<0.05)

Table 3: Percentile decrease in proximate composition of raw fish muscle of (*Labeo rohita*) stored in freezer at -12±2 °C during 4 weeks of storage

Days	Total Protein (%)	Total Lipid (%)	Moisture (%)	Ash (%)
0-7	7.55	17.53	2.38	6.75
0-14	19.39	25.75	4.87	14.86
0-21	25.77	40.27	7.61	20.27
0-28	36.55	61.36	8.79	29.05

Table 4: Percentile decrease in proximate composition of raw fish muscle of (*Labeo rohita*) stored in freezer at -20±2 °C during 4 weeks of storage

Days	Total Protein (%)	Total Lipid (%)	Moisture (%)	Ash (%)
0-7	6.77	7.81	1.79	2.75
0-14	10.16	21.09	4.15	6.89
0-21	14.10	23.43	5.01	13.10
0-28	19.08	30.20	5.64	20.68

3.1.1 Protein Content

Perusals of Table 1 and 2 depict that the total protein content of -12 ^{0}C stored as well as -20 ^{0}C stored samples followed a decreasing trend with increasing storage period. In support of present findings Emire *et al.* (2009) [7] in Nile Tilapia (*Oreochromis niloticus*)**,** Siddique *et al.* (2011) [8] in *Puntius sps* and Gandotra *et al.* (2014) [9] in *Tor tor* reported a significant decrease in protein content during frozen storage. This decrease in protein content was attributed to denaturation of fish protein. Denaturation of protein involves the destruction of its secondary, tertiary and quaternary structures, reducing the protein to a simple polypeptide chain (Careche and Li-Chan 1997) [10]. Gupta (2017) [11] Also observed a decline in the protein content of *Wallago attu* fillets stored at -12±2 ^{0}C for a period of 30 days. Table 3 and 4 reveal that the percent decrease was 7.55%, 19.39%, 25.77%, 36.55%, in samples stored at -12 ^{0}C while 6.77%, 10.16%, 14.10%, 19.08% and in samples stored at -20 ^{0}C on 7th, 14th, 21st and 28th day of storage respectively. Thus, the percentile decrease is less in the sample stored at -20 ^{0}C as compared to the sample stored at -12 ^{0}C.

3.1.2 Lipid content

Results shown in Table 1 and 2 depict that the lipid content decreased in both the -12 ^{0}C and -20 ^{0}C stored samples with increase in storage days. The total percentile decrease in Rohu fillets kept at -12 ^{0}C was found to be 17.53%, 25.75%, 40.27%, 61.36% while it was found to be 7.81%, 21.09%, 23.43%, 30.20% in samples stored at -20 ^{0}C. In favor of present findings Arannilewa *et al* (2005) [12] in Tilapia found a significant loss in total lipid content when stored at low temperature. Similarly, Alam *et al.* (2012) [13] observed a sharp decrease in lipid percentage during storage of fillets of *Labeo rohita* at -18 ^{0}C.

3.1.3 Moisture content

Results shown in Table 1 and 2 reveal that the total moisture content of fish fillets stored in -12 ^{0}C decreased from 82.52±0.50% on day 0 to 75.26±0.11% on day 28. Also, in case of fillets stored at -20 ^{0}C the moisture content showed a decreasing trend with an initial value of 82.59±0.50% on day 0 to 77.93±0.17% on day 28. Devadasan *et al.* (1978) [14] also reported that a definite decrease in the moisture content was observed during storage of fillets at -18 ^{0}C. These results are also supported by the findings of Le-blanc and Le-blanc (1992) [15] El-deen and El-shamrey (2010) [16] and Aberoumand (2013) [17] who proposed that decrease in moisture is due to condensation of water during frozen storage. Similarly, Bakhiet *et al.* (2017) [18] registered a decline in the moisture content of muscle of Nile Tilapia (*Oreochromus niloticus*) kept in frozen storage.

3.1.4 Ash content

The ash content of both the -12 ^{0}C and -20 ^{0}C stored muscles showed a decreasing tend. The total percentile decrease was found to be 29.05% in fish fillets stored at -12 ^{0}C whereas 20.68% in samples kept at -20 ^{0}C, on 28th day of storage. The decrease in ash content was associated to the drip loss during thawing process (Beklevik *et al.* 2005) [19] Similarly, Okeyo *et al.* (2009) [20] in Nile Perch, and Gandotra *et al.* (2015) [21] in *L. rohita* found a decrease in total ash content during its frozen storage.

3.2 Microbial Analysis

The quality of fish flesh is largely depicted by its microbial contamination. For the determination of microbial load of fish muscle during storage at -12 ^{0}C and -20 ^{0}C, Total Plate Count (TPC), Coliform Count (CC) and Psychrophilic Count (PC) was analyzed for a period of 4 weeks.

Table 5: Microbial load of raw fish muscle of (*Labeo rohita*) stored in freezer at -12±2 °C during 4 weeks of storage

Days of storage	0 day	7th day	14th day	21st day	28th day
TPC*	2.10^a ±0.2	**6.79^b ±0.11**	8.17^c ±0.07	9.95^d ±0.02	12.11^e ±0.30
CC**	1.20^a ±0.15	1.98^b ±0.1	2.85^c ±0.07	4.11^d ±0.2	6.19^e ±0.02
PC***	2.12^a ±0.2	3.36^b ±0.04	4.84^c ±0.1	5.98^d ±014	6.21^e ±0.11

*Total Plate Count(log10cfu/g) **Coliform Count(log10cfu/g) ***Psychrophillic Count(log10cfu/g)

Table 6: Microbial load of raw fish muscle of (*Labeo rohita*) stored in freezer at -20±2 °C during 4 weeks of storage

Days of storage	0 day	7th day	14th day	21st day	28th day
TPC*	2.14^a ±0.09	4.01^b ±0.11	4.30^c ±0.07	5.10^d ±0.02	6.20^e ±0.01
CC**	1.08^a ±0.15	1.92^b ±0.2	2.04^c ±0.1	3.08^d ±0.07	4.30^e.±0.4
PC***	2.12^a ±0.2	3.12^b ±0.04	3.98^c ±0.1	5.12^d ±0.05	6.09^e ±0.11

*Total Plate Count(log10cfu/g) **Coliform Count(log10cfu/g) ***Psychrophillic Count(log10cfu/g)
Mean SD with different superscripts in a row differs significantly (P<0.05)

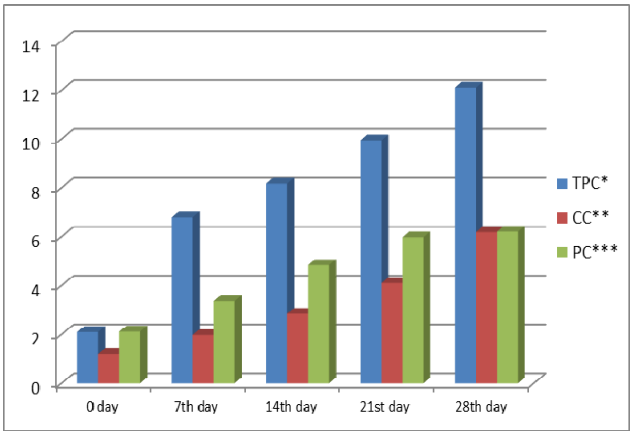

Fig 1: Changes in Total Plate Count (TPC), count (PC) of fish muscle stored at −12±2 ^{0}C count (PC) of fish muscle stored at -20±2 ^{0}C for up to 28 days

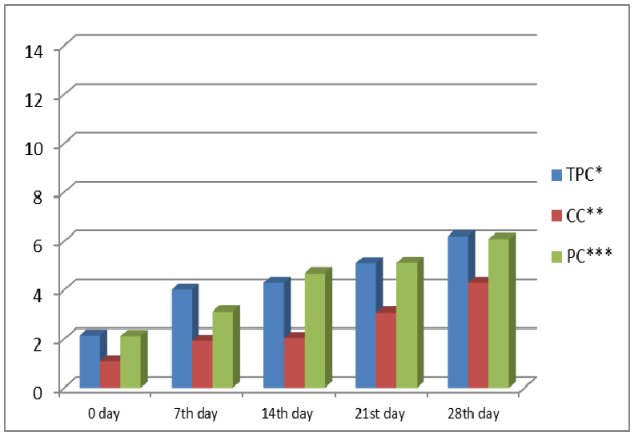

Fig 2: Changes in Total Plate Count (TPC), count (PC) of fish muscle stored at −12±2 ^{0}C count (PC) of fish muscle stored at -20±2 ^{0}C for up to 28 days

Inquisitive study of Table 5 and 6 depicts that the values of TPC, CC and PC increased with increase in the storage period. This increase in microbial count is attributed to growth promoting effect of moisture during frozen storage.

3.2.1 Total Plate Count (TPC)

The initial value of TPC was found to be 2.10±0.2log cfu/g which increased to 12.11±0.30 log cfu/g in the samples kept under storage at -12 ^{0}C and 6.20±0.30 log cfu/g in the samples stored at -20 ^{0}C on the last day (28th day) of storage. Thus, in -20 ^{0}C stored fillets the permissible limit for TPC (6 log cfu/g) was crossed on 28th day while in the fish samples stored at -12 ^{0}C this limit was crossed on 7th day of storage. Ozogul *et al.* (2011) [22] also reported a significant statistical increase in total viable counts of whole gutted common sole (*Solea solea)* over the storage period of 24 days. Similarly, Gandotra *et al.* (2016) [23] found that in raw samples of *Cyprinus carpio* kept at -12 ^{0}C, the TPC was found to increase from 1.10±0.04 log cfu/g to 12.41±0.17 log cfu/g.

3.2.2 Coliform Count (CC)

The value of CC also showed an increasing trend with increase in storage period in both the -12 ^{0}C stored and -20 ^{0}C stored fillets of rohu. CC was within acceptable limits (2.69 log cfu/g) on 14th day in sample stored at -20 ^{0}C but crossed this limit on 21st day whereas in sample stored at -12 ^{0}C, CC crossed the acceptable limit on 14th day. Likewise, Gandotra *et al.* (2014) [24] also reported an increasing trend of CC and PC in both control and ice glazed samples of *L. rohita* during frozen storage. Koul (2017) [25] also observed an increase in the Coliform Count (CC) in muscles of *M. seenghala* stored in frozen condition.

3.2.3 Psychrophilic Count (PC)

The PC bacteria were found to grow exponentially during the entire storage period. Results shown in Table 5 and 6 reveal that PC crossed the acceptable limit (4.6 log cfu/g) on 21st day in sample stored at -20 ^{0}C where as in the sample stored at -12 ^{0}C this limit was crossed on 14th day of storage. Similarly, Ola and Oladipo (2004) [26] and Liu *et al.* (2010) [27] found an increasing trend for psychrotrophs during storage period. The results are also supported by the findings of Dhanpal *et al.* (2013) [28] who reported increasing trend of PC with increasing storage period in ice stored rohu fish muscle.

4. Conclusion

Thus, these results clearly indicate that the lower the temperature of storage the more effective is the retardation of growth of microorganisms in the fish muscle along with the extension of its shelf life.

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