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RESEARCH ARTICLE

NUTRITIONAL AND MICROBIAL QUALITY CHANGES IN RAW AND BRINED FROZEN MUSCLE OF *Cyprinus carpio* (Linn.).

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ABSTRACT

The present study was designed to investigate the nutritional and microbial quality changes in raw and brined (20%) frozen muscle of *cyprinus carpio* stored for a period of four weeks. A significant total perecntal decrease ($p \leq 0.05$) in protein, lipid, moisture and ash conetnt was found in both the samples after thirty days of storage. It was 8.95%, 27.5%, 2.61%, 35% in raw samples and 6.69%, 13.26% 4.30 %, 20% in brined samples repectively. However, the microbial load was found to be significantly increased ($p \leq 0.05$) during th entire frozen storage. In raw samples the total plate count was found to increase from 1.05±0.02 log cfu/g to 7.52±0.03 log cfu/g and in brined samples from 0.69±0.09 log cfu/g to 5.77±0.04 log cfu/g.

INTRODUCTION

Fish is one of the most perishable food and its preservation is usually accomplished by combination of different techniques. Contamination with spoilage microorganisms is almost unavoidable because fish is a very good culture media. Therefore, good fish preservation techniques must prevent microbial spoilage of fish without affecting its quality and nutritional value (Ghaly *et al.*, 2010). Spoilage of fish can be due to rapid autolysis by the fish enzymes, and because of less acid reaction of fish flesh that favors microbial growth (Yohanna *et al.*, 2011). Fish, in general, usually spoil more rapidly than other muscle foods; the spoilage process (Rigor mortis) will start within 12 hrs of their catch in the high ambient temperatures of tropics (John, 1994). Fish preservation methods include, salting, drying, chilling, smoking and freezing. Salting is one of the oldest treatments in extending shelf-life. Salt decreases the water activity and causes plasmolysis.

It also alters protein and enzyme states in such a way that proteins become impervious to enzyme action and lose their efficacy. It also has bacteriostatic and bactericidal effects (Ismail and Wootton 1982). Salting is mainly used to preserve products and also to promote important sensorial changes that make the final product appreciated by consumers (Andrés *et al.* 2005b). Salting is usually performed by dry, brine, or injection salting or a combination of these methods. When salt brine or dry salt are used as salting agents, two main simultaneous flows are usually generated; water loss and salt uptake.

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The salt uptake and water loss depend on the contact area and initial weight (Fuentes *et al.* 2007). The properties of fish muscle vary due to changes in water and salt content: the muscle gains salt, whereas water is lost or gained depending on the salting procedure (Thorarinsdottir *et al.* 2002 and Thorarinsdottir *et al.* 2004). Salt uptake depends on many factors including species, muscle type, fish size, fillet thickness, weight, composition (lipid content and distribution), physiological state, salting method, brine concentration, duration of salting step, fish-to-salt ratio, ambient temperature, and freezing and thawing (Wang *et al.* 1998), Jittinandana *et al.* 2002). The rates of the salt and water diffusion are positively correlated with increasing the brine concentration (Poernomo *et al.* 1992, Bellagha *et al.* 2007). The rate of salt uptake is very important with regard to weight change, water holding capacity (WHC) and quality of the final product. This all result in slowing down the rate of spoilage, thus extending the shelf life. Under this background presently an attempt was undertaken to assess the quality changes through biochemical and microbial evaluation in muscle of common carp (*Cyprinus carpio*), a local fish available in the market of Jammu.

MATERIALS AND METHODS

Sample collection

Fresh samples of *Cyprinus carpio* were purchased from local market of Jammu city. They were immediately brought to the lab in polythene bags along with crushed ice.

Sample processing

The viscera of fish were removed and the fish was washed with large amount of water. The fish was cut into pieces and

- To prepare raw sample (control), these pieces were washed and immediately wrapped in aluminium foil, kept in air tight plastic container and stored at -12±2°C (frozen storage).
- To prepare the brined sample, these pieces were immersed in 1 20% brine solution in ratio 1:2 for 2 hours. Brining was conducted in plastic containers at 4±1°C.

After 2 hours, the fish pieces were removed from the brine solution and left as such for half an hour for extract release. Finally the fish pieces were washed and immediately wrapped in aluminium foil, kept in air tight plastic container and stored at -12±2°C (frozen storage). Analytical procedures for biochemical changes were done on 0, 10th, 20th and 30th day of storage and for microbiological changes on 0, 5th, 10th, 15th, 20th, 25th and 30th day of storage in both the samples.

Analyses

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

RESULTS AND DISCUSSION

Chemical Analysis

The proximate composition of fresh and frozen muscle of *Cyprinus carpio* during storage period of four weeks has been shown in the following Table….

Table 1. Proximate composition (wet weight basis) of raw fish muscle of (*Cyprinus carpio*) stored in freezer at -12±2°C during 30 days of storage

DAYS	0	10th	20th	30th
Total Protein (%)	16.86±0.01	16.02±0.2	15.75±0.03	15.35±0.02
Total Lipid (%)	2.00± 0.2	1.82± 0.025	1.67± 0.03	1.54 ± 0.02
Moisture (%)	82.62± 0.02	82.00±0.035	82.54± 0.025	81.46±0.01
Ash (%)	2.20± 0.03	1.93± 0.01	1.75± 0.036	1.43± 0.02

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

Table 2. Proximate composition (wet weight basis) of 20% brined fish muscle of (*Cyprinus carpio*) stored in freezer at -12±2°C during 30 days of storage

Days	0	10th	20th	30th
Total Protein (%)	15.95±0.02	15.46±0.03	15.04±0.2	14.93±0.01
Total Lipid (%)	1.96±0.01	1.89±0.02	1.78±0.1	1.70±0.03
Moisture (%)	80.44±0.02	79.21±0.035	77.92±0.025	76.98±0.01
Ash (%)	3.75±0.03	3.35±0.01	3.15±0.036	3.00±0.02

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

Table 3. proximate composition of raw muscle during frozen storage at -12±2°C from 0 day to 30th day

Days	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
0-10	4.98	- 9.00	- 0.75	- 12.27
0-20	6.58	- 16.50	- 1.30	- 20.45
0-30	8.95	- 27.50	- 2.61	- 35.00

Table 4. Percental decrease in proximate composition of 20% brined muscle during frozen storage at -12±2°C from 0 day to 30th day

Days	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
0-10	- 3.07	- 3.57	- 1.52	- 10.66
0-20	- 5.7	- 9.18	- 3.13	- 16
0-30	- 6.69	- 13.26	- 4.3	- 20

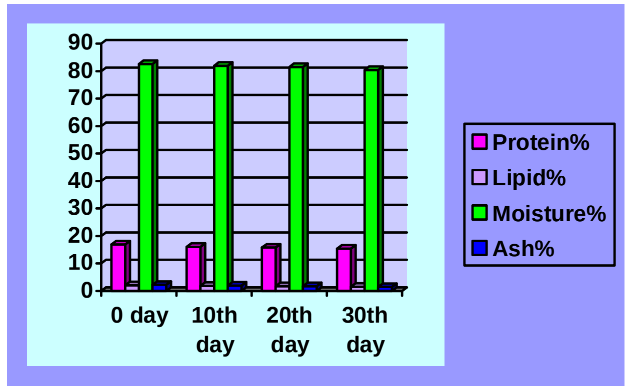

Figure 1: Change in Proximate composition (wet weight basis) of raw muscle (*Cyprinus carpio)* subjected to frozen storage for 30 days.

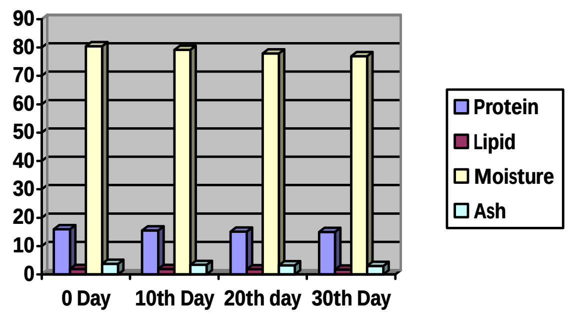

Figure 2: Change in Proximate composition (wet weight basis) of 20% brined muscle (*Cyprinus carpio)* subjected to frozen storage for 30 days.

Protein Content

Results shown in Table 1and 2 revealed that the total protein content of raw and 20% frozen brined muscle showed a decreasing trend with increase in storage period. The total percent decrease was 4.98%, 6.58%, 8.95% in raw and -3.07%, -5.70%, -6.69% in 20% brined muscle on 10th, 20th, 30th days respectively. These results are in accoradnce with Sameul *et al* (2010) who stated that the increased NaCl concetntartion slowed down autolysis in fish muscle of *Clarius gariepinus* which consequently slowed down the protein breakdown. Thorarinsdottir *et al* (2002) while conducting experiment on salted cod (Gadus *morhua*) found that the denaturation of protein leads to increased leaching effect of amino acids and water loss. Presently, the decrease in protein content in raw muscle might be due to the denaturation of fish protein i.e. due to the changes in the proportion of chemical composition and protein breakdown.

Lipid Content

Perusals of Table 1 and 2 revealed that the total lipid content of both raw and 20% brined muscle decreased with increase in storage period. The total percental decrease in total lipid content in 20% brined muscle was found to be less i.e. 13.26% than in raw muscle where it was found to be 27.5%. In support of present findings Unlusayin *et al* (2010) while working on salted cod found that less decrease in lipid content during salting was due to NaCl that slows down lipid hydrolysis. The decrease in both the raw and brined samples was due to fat hydrolysis during storage (Ozogul *et al* 2011). The less percentage decrease in total lipid content in brined muscle might be due to the reason that salt slows down lipid hydrolysis.

Moisture content

Results shown in Table 1 and 2 revealed that the moisture content decreased significantly from 82.62 ± 0.02% to 80.46 ± 0.01% in raw and 80.44 ± 0.15% to 76.98 ± 0.01% in 20% brined muscle on 30th day of storage at -12±2°c. The total percent decrease in moisture content in raw muscle was found to be less i.e. -2.61% than in 20% brined sample i.e. -4.30% on 30th day of storage at -12±2ºc. These results are in accordance with Jittinandan *et al* (2002) and Osibana *et al* (2010) who stated that the rate of salt uptake was in constant ratio to rate of water loss durin salting. The decrease in moisture content may be due to the sublimation of ice in frozen storage and drip loss during thawing process.

Ash Content

Perusals of Table 1 and 2 revealed that the ash content of raw and brined muscle showed a decreasing trend with increase in storage time. Initially, the ash content of brined sample was found to be higher i.e. 3.75± 0.02% than that of raw sample i.e. 2.20 ±0.03%. After that, it showed a decreasing trend. The total percent decrease was found to be 35% in raw samples and 20% in brined samples 0n 30th day of storage. These results are in accordance with those of Jittinandana *et al* (2002), Ahmed *et al* (2010) and Unlusayin *et al* (2010). They stated that the higher ash content in salted samples due to the water losses associated in brining and salt penetration in to fish flesh during salt curing process. Similarly, Okeyo *et al* (2009) in Nile Perch and Emire *et al* (2009) in Nile Tilapia fish (Oreochromis niloticus) found a decrease in total ash content during its frozen storage. The decrease in ash content was associated to the drip loss during thawing process by Beklevik *et al* (2005).

Microbial Analysis

For the determination of microbial quality of fish before and frozen storage, Total Plate Count (TPC) was analysed.

Total Plate Count

Results shown in Fig. 3 and 4 revealed that the Total Plate Count increased with increase in storage period in both the samples. In raw samples, Total Plate Count was found to increase from 1.05±0.02 log cfu/g to 7.52±0.03 log cfu/g and in brined samples from 0.69±0.01 log cfu/g to 5.77 ±0.04 log cfu/g. In present studies, it has been found that the TPC in brined samples was within the permissible limit i.e.6 log cfu/g (ICMSF, 1986) up to 30th day and in raw samples, it crossed the permissible limit after 15th day. In brined samples, the TPC was low as compared to raw samples. These results are in accordance with Osibona *et al.* (2010). This decrease might be due to the fact that in salted products free water bound by the sodium chloride is not readily available for bacterial growth. Similarly, Tsai *et al* (2005) and Ahmed *et al* (2010) reported that the higher salt content (>5%) in salted Mackerel had inhibitory effect on bacterial growth. Obemeata *et al* (2011) showed a significant increase in bacterial count when Tilapia was subjected to frozen storage at -18ºc than at 4ºc. They stated that freezing of fish at -18ºc created unfavourable environmental conditions for the growth and the survival of the micro-organisms.

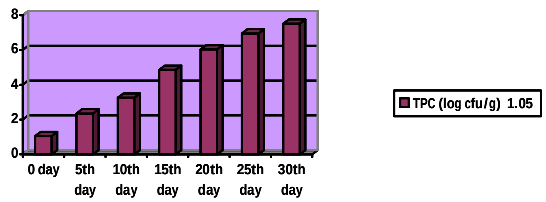

Fig 3: Change in Total Plate Count of raw muscle of Cyprinus carpio stored at12±2ºc for up to 30 days.

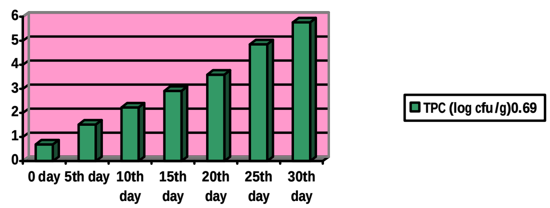

Fig 3: Change in Total Plate Count of 20% brined muscle of Cyprinus carpio stored at -12±2ºc for up to 30 days.

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