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Research Article

STUDIES ON THE EFFECT OF VACUUM PACKAGING ON BIOCHEMICAL AND MICROBIAL QUALITY OF FROZEN STORED MUSCLE OF COMMON CARP, *CYPRINUS CARPIO*

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ABSTRACT

Presently, an attempt was made to study the quality changes in raw and vacuum packaged muscle of *Cyprinus carpio* stored in freezer at -12±2ºC, for a period of 35 days. An experiment was carried out at the interval of 7 days. A significant total percental decrease ($p \leq 0.05$) in protein, lipid, moisture and ash content was found in both the samples after 35 days of storage. It was 30.92%, 48.21%, 4.24%, 65.76% in raw samples and 26.69%, 43.49%, 4.31%, 53.81% in vacuum packaged samples respectively. However, Free Fatty Acid (FFA), Thiobarbituric Acid (TBA) and pH showed an increasing trend with increase in storage period in both the samples. Similarly, the bacteriological studies revealed that the Total Plate Count (TPC) in both the samples also showed an increasing trend during the entire storage period. In raw samples, the TPC was found to increase from 1.10±0.04 log cfu/g to 12.41±0.17 log cfu/g and in vacuum packaged samples from 0.51±0.01 log cfu/g to 7.98±0.07 log cfu/g. In raw samples, TPC was found within the permissible limits up to 14th day (TPC= 6.09 log cfu/g) and in vacuum packaged samples, up to 28th day (TPC= 6.12±0.09 log cfu/g). Thus, it revealed that Vacuum packaging (VP) extended the shelf life of fish by two weeks.

INTRODUCTION

Fisheries sector has a vital role in income enhancement, poverty alleviation, and food security. Fish provides 20%of animal protein intake to about 2.6 billion people globally and at least 50% of animal protein intake for over 400 million in Asia and Africa. But, in developed countries, it provides only 13% of animal protein intake (FAO, 2008 and Gandotra *et al.* 2012). Fish is considered to be low fat high quality protein diet having omega-3 fatty acid. Omega-3 fatty acid decrease triglycerides, lowers blood pressure, reduce blood clotting, boost immunity and improve arthritis symptoms, keep skin youthful and reduce the risk of coronary heart disease and atherosclerosis, as well as preventing certain form of cancer and in children may improve learning ability. It contain high contents of polyunsaturated fatty acids (PUFA's), among which eicosapentaenoic acid and docosahexaenoic acid are the most abundant (Ackman, 1989) which contributes to essential fatty acid requirement. Moreover, Fish is also a good source of minerals, like calcium, phosphorus, iron and sodium that are vital to our health. It also provides several other nutrients such as Vitamin A, E, K, B_6 and B_{12}, which help in producing RBC's, antibodies and maintain central Nervous System (Gandotra *et al*. 2015).

Although the fish is highly nutritious, but it is one of the most highly perishable commodities and public has always required continuous reassurance about its quality. Being perishable, it cannot be kept for longer period for human consumption. This is due to its short shelf life. Short shelf life of fishery product is due to its rapid spoilage. The spoilage process begins immediately after capture. The development of spoilage is due to combination of chemical, autolytic and microbial activities (Srinivasan *et al.* 1997). Lipid oxidation represents a major loss of quality during refrigerated and frozen storage of fish. It leads to the development of off-flavour and off- odours in edible oils and fat-containing foods, called oxidative rancidity (Nawar, 1996).

Shelf life extension can be achieved by various preservation methods, i.e., refrigeration, freezing, salting, brining, proper packaging, icing, smoking, glazing, steaming drying and frying etc. Refrigeration and freezing help in preserving fish by lowering temperature. At low temperature, micro-organism became inactive thus biochemical activities decreases. Consequently, the fish remain free from spoilage for longer duration.

The 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). Freezing and frozen storage have been widely employed to retain fish properties before it

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consumed. Further, Vacuum packaging is an appropriate method for delaying lipid oxidation by omitting oxygen molecule (Taheri *et al.* (2012), Kocatepe *et al.* (2015), Rashidi *et al.* (2014)). As reported by Anelich *et al.* (2001), packaging under vacuum has positive effect on extended shelf life of fish fillets.

Keeping this in mind, the present work has been designed to generate information on the changes in biochemical and microbiological composition of raw and vacuum packaged muscle of a fish (*Cyprinus carpio*) available in the local market of Jammu, stored in freezer (-12±2ºC) so as to determine their quality change during storage period of 35 days. This study has immense importance to satisfy consumer's query relating to how long fish muscle can be stored without any deterioration in domestic refrigerator for the betterment of the public health. The present study will also help us to impart knowledge about fish storing and preservation technique with an aim to maximize their shelf life without compromising with their quality.

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 were divided into 2 groups

- First group: These pieces were washed and immediately wrapped in aluminium foil, kept in plastic container and stored at -12±2°C (frozen storage).
- Second Group: The fish muscles were deboned, grind in grinder and then packed in vacuum packaged bags and were stored at -12±2^{0}C.

Analytical procedures for chemical and microbiological changes were done on 0th, 7th, 14th, 21th, 28th and 35th day of storage.

Analysis: The proximate composition (ash and moisture) of the fishes 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). Thiobarbituric acid (TBA) value of fish muscle during frozen storage was determined by using the method of Witte *et al.* (1970). Free Fatty Acid (FFA) was determined by method of US Army laboratories (Natick) described by Koniecko. The pH of the fish muscles was determined by the method of Keller *et al.* (1974). 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 program.

RESULTS AND DISCUSSION

Proximate Composition: The proximate composition of raw and vacuum packaged muscle of *Cyprinus carpio* during storage period of 35 days has been shown in the following tables….

Protein Content: Perusals of table 1and 2 clearly revealed that the total protein content of raw and vacuum packaged muscle showed a decreasing trend with increase in storage period. Initially, the protein content of vacuum packaged sample was found to be higher i.e. 20.98±0.30% than that of raw sample i.e. 17.59±0.77 %. After that, it showed a decreasing trend. The total percent decrease was found to be 30.92% in raw samples and 26.69 % in vacuum packaged samples on 35th day of storage. These results are in accordance with Rashidi *et al.* (2014) also observed decrease in protein content from 20.5% to 18.05% in the vacuum packed muscle of jinga shrimp after 18th day of refrigeration. Manju (2005) while working on the effect of vacuum packaging on the shelf life of pearlspot and black pomfret during chill storage found that Vacuum packaging achieves its preservative effect by maintaining the product in an oxygen deficient environment. In support with the present studies Keyvan *et al.* (2008) in Caspian white fish (*Rutilus frisi kutum*), El-Deen and El- Shamrey (2010) in Gahsh (*Lethrinus elongates*), Gandotra *et al.* (2012) (2015) in *Labeo rohita*, Gandotra *et al.* (2014) in *Wallugu attu*, Gandotra *et al.*(2015)a in *Cyprinus carpio* and Aberoumand (2013) in various Iranian fishes also found a protein loss during frozen storage. They related this protein loss to the denaturation of proteins and loss of nitrogen as volatile bases and nitrogen substances formed by bacterial decomposition that escaped from tissue during frozen storage.

Table 1 Proximate composition of raw muscle of *Cyprinus carpio* stored in freezer at -12±2°C during 35 days of storage

DAYS →	0 day	7th day	14th day	21st day	28th day	35th day
TOTAL PROTEIN(%)	17.59±0.77^{a}	16.01±0.31^{b}	15.59±0.64^{c}	13.00±0.16^{d}	12.65±0.50^{e}	12.15±0.34^{f}
TOTAL LIPID(%)	2.80±0.21^{a}	2.65±0.40^{b}	2.21±0.86^{c}	1.99±0.10^{d}	1.78±0.59^{e}	1.45±0.11^{f}
MOISTURE(%)	81.93±0.10^{a}	80.45±0.02^{b}	80.15±0.25^{c}	79.67±0.32^{d}	79.18±0.07^{e}	78.45±0.42^{f}
ASH(%)	2.95±0.35^{a}	2.73±0.19^{b}	2.26±0.34^{c}	1.83±0.29^{d}	1.39±0.50^{e}	1.01±0.17^{f}

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

Table 2 Proximate composition of Vacuum packaged muscle of *Cyprinus carpio* stored in freezer at -12±2during 35 days of storage.

DAYS →	0 day	7th day	14th day	21st day	28th day	35th day
TOTAL PROTEIN(%)	20.98±0.30^{a}	19.54±0.25^{b}	18.82±0.04^{c}	17.47±0.42^{d}	16.01±0.12^{e}	15.38±0.19^{f}
TOTAL LIPID(%)	3.15±0.27^{a}	2.98±0.32^{b}	2.78±0.12^{c}	2.49±0.31^{d}	1.98±0.37^{e}	1.78±0.07^{f}
MOISTURE(%)	79.07±0.40^{a}	78.75±0.15^{b}	78.21±0.47^{c}	77.45±0.24^{d}	76.52±0.19^{e}	75.66±0.53^{f}
ASH(%)	2.99±0.15^{a}	2.78±0.27^{b}	2.37±0.55^{c}	1.88±0.012^{d}	1.65±0.08^{e}	1.38±0.43^{f}

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

Table 3 Percental decrease in proximate composition of raw muscle of *Cyprinus carpio* during frozen storage at -12±2°C from 0 day to 35th day.

DAYS	TOTAL PROTEIN(%)	TOTAL LIPID(%)	MOISTURE(%)	ASH(%)
0-7	8.98	5.35	1.80	7.45
0-14	11.37	21.07	2.17	23.38
0-21	26.09	28.92	2.75	37.96
0-28	28.08	36.42	3.35	52.88
0-35	30.92	48.21	4.24	65.76

Table 4 Percental decrease in proximate composition of vacuum packaged muscle of *Cyprinus carpio* during frozen storage at -12±2°C from 0 day to 35th day.

DAYS	TOTAL PROTEIN(%)	TOTAL LIPID(%)	MOISTURE(%)	ASH(%)
0-7	6.86	5.39	0.40	7.02
0-14	10.29	11.74	1.08	20.73
0-21	16.73	20.95	2.35	37.12
0-28	23.68	37.14	3.22	44.81
0-35	26.69	43.49	4.31	53.81

Lipid Content: In present investigation, a decreasing trend was observed in Total lipid content of both raw and vacuum packaged samples for a period of 35 days. Perusals of table 1 and 2 revealed that initially the lipid content of vacuum packaged muscle was found to be higher i.e. 3.15±0.27% than that of raw sample i.e. 2.80±0.21%. After that, it showed a decreasing trend. The total percent decrease was found to be 48.21% in raw samples and 43.49% in vacuum packaged samples on 35th day of storage. In support of present findings, Ozogul *et al.*(2011) in Common sole (*Solea solea*) found significant increase in peroxide value(PV) and decrease in lipid content during 24 days of storage. The decrease in lipid content was associated to the fat hydrolysis. Taheri *et al.* (2012) reported in cobia fillets (*Rachycentron canadum*) treated by vacuum packaging lowest rate of peroxide formation (8.65) and highest (18.65) in control samples during frozen storage. It was concluded that vacuum packaging treatment has significant effect on delaying lipid oxidation. Similarly Siddique *et al.* (2011) found that total lipid content decreased during frozen storage of three species of *Puntius*.

Moisture Content: The moisture content of fish sample decreased from 81.93±0.10 % to 78.45±0.42 % in raw and 79.07±0.40% to 75.89±0.53% in vacuum packaged sample on 21st day of storage. The total percental decrease was 4.21% and 4.24% in vacuum packaged and raw samples respectively. Present findings are in accordance with Randell *et al.* (1997) who observed that Baltic herring fillets in over-wrap packages (polystyrene or wood fibre) had lower drip than vacuum packed herring fillets during storage at 2ºC. Similarly, Ozogul *et al.* (2004) observed that an increase in drip loss lowered the sensory quality of vacuum packed sardines stored at 4ºC. Akter *et al*. (2012), Ehsani *et al.* (2012), Gandotra *et al.* (2012), Aberoumand (2013), Gandotra *et al.* (2013), Gandotra *et al.* (2015) and Mahmoud Sharaf (2013) also observed decreasing trend in moisture content of fish muscle under frozen storage conditions. A decrease of 2.53% in moisture content was also reported by Beklevik *et al.* (2005) for sea bass (*Dicentrarchus labrax*) filets during 60 days of storage under frozen condition.

Ash Content: The ash content of fish sample decreased from 2.95±0.35 % to 1.01±0.17 % in raw and 2.99±0.15% to 1.65±0.43% in vacuum packaged sample on 21st day of storage. The total percental decrease was 53.81% and 65.76% in vacuum packaged and raw samples respectively. In the favour of present findings, Okeyo *et al.* (2009) in Nile Perch, Bekelvik et al. (2005) in sea bass (*Dicentrarchus labrax*) 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. Similar results were obtained by Gandotra *et al*. (2012), Gandotra *et al.* (2013), Gandotra *et al.* (2015) and Mahmoud Sharaf (2013).

Biochemical Composition

Thiobarbituric acid (TBA): The TBA value is an index which measures the malondialdehyde (MDA) content and is a widely used method for assessment of degree of lipid oxidation. MDA is formed through hydroperoxides, which are the initial reaction products of polyunsaturated fatty acids with oxygen. The present study showed a progressive increase in TBA value (secondary oxidation product) with increase in storage period under frozen conditions. The values rose from 0.12±0.014 on day 0 to17.25±0.12 in control and 0.48±0.18 to 9.11±0.02 mg MA/kg in vacuum packaging on 35th day of frozen storage period. Vacuum packaging has been found to substantially reduce oxidative deterioration in frozen fish and fishery products (Taheri *et al.* 2012). Giménez *et al.* (2002) suggest that lower concentrations of malondialdehyde (MDA) in the muscle of rainbow trout than in fish stored in air are the consequence of a low O2 content. The sharp decline of peroxides in simple packaging resulted in an increase in TBA due to the presence of oxygen. . Similar finding were also reported by Manju *et al.* (2007) in baleen fish, Gandotra *et al* (2015) in *Labeo rohita*, Jezek and Buchtova (2014) in rainbow trout. They concluded that vacuum packaging treatment has significant effect on delaying lipid oxidation.

Table 4 Chemical composition of raw muscle of *Cyprinus carpio* stored in freezer at -12±2°C during 35 days of storage.

DAYS	0 day	7th day	14th day	21st day	28th day	35th day
TBA(mgMA/kg)	0.12 ± 0.014^{a}	4.25 ± 0.04^{b}	8.12 ± 0.22^{c}	12.51 ± 0.42^{d}	14.91 ± 0.50^{e}	17.25 ± 0.12^{f}
FFA(%)	0.98 ± 0.09^{a}	3.65 ± 0.20^{b}	5.85 ± 0.04^{c}	7.76 ± 0.03^{d}	9.81 ± 0.20^{e}	12.11 ± 0.39^{f}
pH	6.5 ± 0.35^{a}	6.7 ± 0.07^{b}	7.2 ± 0.10^{c}	7.4 ± 0.04^{d}	7.6 ± 0.17^{e}	7.8 ± 0.07^{f}

Table 5 Chemical composition of Vacuum packaged muscle of *Cyprinus carpio* stored in freezer at -12±2°C during 35 days of storage

DAYS	0 day	7th day	14th day	21st day	28th day	35th day
TBA(mgMA/kg)	0.48 ± 0.18^{a}	2.21 ± 0.32^{b}	4.28 ± 0.15^{c}	5.69 ± 0.17^{d}	9.11 ± 0.31^{e}	11.11 ± 0.02^{f}
FFA(%)	0.21 ± 0.04^{a}	1.81 ± 0.12^{b}	3.21 ± 0.56^{c}	4.99 ± 0.43^{d}	5.37 ± 0.12^{e}	7.14 ± 0.50^{f}
pH	6.8 ± 0.73^{a}	6.9 ± 0.32^{b}	7.1 ± 0.05^{c}	7.2 ± 0.12^{d}	7.4 ± 0.07^{e}	7.6 ± 0.10^{f}

***Free fatty acids (FFA)**:* The values for Free Fatty Acids (FFA) were 0.98±0.09% on day 0 and it rose to12.11±0.39% in control and 7.14±0.50% in vacuum packaging samples on 35th day of frozen storage respectively. The results thus clearly depicts, that there was a gradual increase in the FFA content with increasing storage time. The levels had also direct correlation with pH (Table) showing that it could act as a good indicator for the assessment of the freshness of all the three forms of stored fish muscles. Balev *et al.* (2011) reported that at the end of storage the total FFA concentration of air packaged and vacuum packaged samples increased of 1.17 and 0.85g/kg fresh fish weight respectively in Russian Sturgeon during frozen storage. Their results showed that vacuum packaging significantly (P<0.05) delayed lipolysis of lipids. Present findings are in line with that of Ozogul *et al.* (2011) who carried out experiment on Common sole (*Solea solea*) and reported that the release of FFA significantly increased from the initial value of 9.86 (expressed as % of oleic acid) to the final value of 13.25 (P<0.05) during the storage period. They associate this increase with the hydrolysis of fat and oil which in turn resulted in loss of freshness. They further reported there is a relationship between FFA release and loss of freshness. Similar findings were also reported by Gandotra *et al.* (2014), Taheri *et al.* (2012) and Gandotra *et al*. (2015).

pH: The pH values also showed an increasing trend with increase in frozen period. The pH values ranged from 6.5±0.35 on day 0 to 7.8±0.07 in control and 7.6±0.10 in vacuum packaging on 30th day. Decrease or constant levels of pH might be attributed to increasing solubility of CO2 at storage time, effecting on 2 growth of aerobic microflora (Mahmoudzadeh *et al*. 2010), Taheri *et al.* (2012).

Microbial Analysis

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

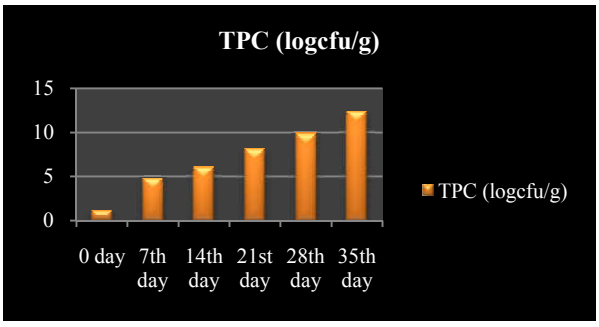

Fig 1 Change in Total Plate Count of raw muscle of *Cyprinus carpio* stored at12±2ºc for up to 35 days.

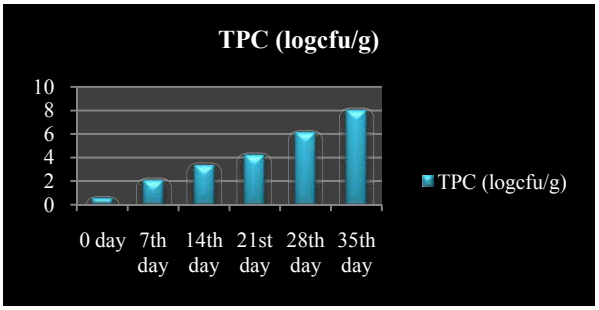

Fig 2 Change in Total Plate Count of vacuum packaged muscle of *Cyprinus carpio* stored at12±2ºc for up to 35 days.

Total Plate Count

Results shown in fig. 1 and 2 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 1.10±0.04 log cfu/g to 12.41±0.17 log cfu/g and in vacuum packaged samples from 0.51±0.01 log cfu/g to 7.98±0.07 log cfu/g. In present studies, it has been found that the TPC in vacuum packaged samples was within the permissible limit i.e.6 log cfu/g (ICMSF, 1986) up to 28th day and in raw samples, it crossed the permissible limit after 14th day. The complete removal of oxygen from a pack of fresh meat ensures longer preservation against microbial deterioration than packaging in oxygen. Present findings are in accordance with Ozpolat *et al.* (2014) who observed the effect of vacuum -packing on the shelf – life of *Capoeta umbla* sausages and found that Total Plate Counts (TPC) (log cfu / g) increased from an initial level of 2.61±0.3 to 6.86±0.5 during 0-14 days period in air; to 6.58±0.2 during 0-56 days period in fish sausage packed in vacuum. The acceptable TPC limit was exceeded in air conditions on 14th and vacuum conditions on 56th day. The results of the present study confirm the earlier finding of Leung *et al.* (1992), Huang *et al.* (1994), Lyon and Reddmann (2000), Ozogul *et al.* (2000, 2004) and Gandotra *et al.* (2015) in that bacteria grew most quickly in fish stored in air compared to vacuum pack (VP) at 0ºC.

CONCLUSION

The freezing of fish at low temperature makes it less prone to spoilage by decreasing the bacterial activity. However, it was observed that there was a decrease in the nutritional parameters while an increase was observed in biochemical composition and microbial count during frozen storage. Vacuum packaging fits into an important area of preservation where shelf life is extended without the loss of important nutritional parameters of fishes. Thus, Present findings revealed that usage of Vacuum Packaging had positive influence on delaying lipid oxidation and increasing shelf life of fish.

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