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CHANGES IN BIO-CHEMICAL, MICROBIAL AND SENSORY QUALITIES OF RAW AND VALUE ADDED PRODUCT (FISH CUTLET) OF SILVER CARP (*HYPOPHTHALMICHTHYS MOLITRIX*) STORED UNDER FROZEN CONDITIONS.

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ABSTRACT

A comparative study was undertaken to evaluate shelf life of value added product (fish cutlet) and raw muscles of Silver carp on the basis of bio-chemical, microbial and sensory quality during 30 days of frozen storage. Results of the bio-chemical analysis thiobarbituric acid (TBA), Free fatty acid (FFA), pH revealed a higher percental increase ($p \leq 0.05$) in raw muscles as compared to the fish cutlets. In raw muscle samples the permissible limits for TBA crosses on 20th day and FFA on 10th day while the, the fish cutlets maintained the acceptable values for these parameters till the end of experimentation upto 30 days. Similarly, the bacterial count Total Plate Count (TPC), Coliform count (CC), Psychrophillic count (PC) in fish cutlets shows lower values i.e.(3.75 log cfu/g in TPC, 2.10log cfu/g in CC and 1.75log cfu/g in PC) as compared to raw muscle samples (9.96 log cfu/g in TPC, 5.65log cfu/g in CC and 7.35log cfu/g in PC) at the end of storage period. Sensory scores revealed that fish cutlets possessed the overall acceptability till the end of storage period i.e. upto 30 days whereas the raw muscle lost their sensory acceptability after 10th day. Hence, shelf life extension of fish muscles could be achieved by adding value to it i.e. spices and frying in the cutlet form.

KEYWORDS: silver carp, value-added, bio-chemical, microbial, sensory and frozen.

INTRODUCTION

Fish is a dietary source of protein, has a high content of water and lipid soluble vitamins, minerals and polyunsaturated fatty acids (PUFAs) of n-3 family. It is believed that Omega-3 fatty acids from fish can lower body triglycerides thus reducing the chances of heart diseases. Fish also reduces blood pressure by small but significant amounts and improve blood clotting regulation. But also, fish is a very perishable commodity, more than cattle, sheep and poultry. So, it must be properly preserved before it is disposed off. Major factors responsible for its perishable nature are the microbial growth and oxidation of lipids which influence the colour, texture, nutrition, safety along with flavour. Although many damaging processes are inhibited by low temperature storage methods, but the undesirable reactions associated with lipids and proteins are shown to occur, leading to the detrimental changes in nutritional and sensory properties. All these negative changes limit the marketing process of fish products and hence to satisfy the consumer demand, it is necessary to produce good quality and safe fish.

Consumer habits have changed greatly in recent years due to increasing urban, dynamic lifestyle, and moreover, the remoteness from the level of primary production has led to a lack of interest in or even dislike of fresh whole fish. Hence, easy-to-use value added products which only take a short time to cook and serve have become the choice of today.

Silver carp is one of the most important freshwater fish, considered worldwide for its taste and nutritious value. However, the high intramuscular bone in Silver carp makes it less economical for consumption purposes. Hence, there comes a need to develop some convenient value added product which besides utilizing the carp meat economically, also adds to its taste and shelf life. Value addition involves addition of various natural antioxidant and antimicrobial ingredients (garlic, ginger, onion, peppers, turmeric etc.) which have positive effects in the human (Aron *et al.* 2008 and Ghosh *et al.* 2009). Thus, the objective of present work is to evaluate the bio-chemical (TBA, FFA, pH), microbial (TPC, CC, PC) and sensory quality changes in raw and value added (fish cutlets) samples of Silver carp during 30 days of frozen storage period.

MATERIALS AND METHOD

Collection of fish samples: Fresh samples of Silver carp (*Hypophthalmichthys molitrix*) with an average weight of 1000-1200 g were purchased from local market of

Jammu city. They were immediately transported to the lab within 20 minutes in polythene bags along with crushed ice. The head, viscera and skin of fish were removed, washed with large amount of water and then divided into two groups. One group (H_R) was kept raw, used as control sample, packed in aluminum foil and kept in freezer at -12±2˚C. The second group (H_{FC}) samples were deboned after steaming; the flesh was minced and then made into fish cutlets after mixing with some natural ingredients.

Preparation of fish cutlets: The fish cutlets were made by mixing of minced fish muscle (75%) with refined oil (6%), onion (1.5%), ginger (2%), garlic (2.5%), black pepper (0.6%), red pepper (0.5%) salt (3%), cumin (0.4%), coriander (0.5%). It was then mixed with starch (8%) and then made into various shapes. The cutlets were then flash fried in vegetable oil. They were packed in aluminum foil, kept in air- tight containers and stored in freezer at -12 ±2^{o}C. Analytical procedures for biochemical and microbiological changes were done on 0, 10th, 20th and 30th day of storage period.

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). Thiobarbituric acid value of fish muscle during storage was determined using the method of Witte *et al.* (1970). Free Fatty Acid (FFA) was determined by method of US Army laboratories (Natick) described by Koniecko (1979). Extract Release Volume (ERV) was determined as per the method of Strange *et al.*(1977). The pH of 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. Values <0.05 were considered as significant and p values <0.001were considered as highly significant.

3. RESULTS AND DISCUSSIONS

Chemical changes

Thiobarbituric acid (TBA)

The TBA value is an index of lipid oxidation measuring malondialdehyde (MDA) content and widely used for assessment of degree of lipid oxidation. MDA is formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen. (Sallam, 2007). On day 0, the values for TBA in raw (H_R) and fish cutlet (H_{FC}) was found to be 0.44±0.02 and 0.05±0.01 respectively. On 10th day, the values increased upto 6.72 ±0.01 in raw muscle and 0.77±0.08 in fish cutlets. On 20th day, the TBA value crossed the acceptable limit of 8 mg MA/kg in raw muscle i.e 12.04±0.05 mg MA/kg while in fish cutlets the value was only 1.1±0.04%. Further, the values increased to 17.67±0.04 in raw and 1.45±0.3 in fish cutlets on 30th day of storage.

Our results are in concordance with Asgharzadeh *et al.* (2010) who proposed that increase in TBA in frozen minced muscle of Silver carp (*Hypophthalmichthys molitrix*) is due to lipid hydrolysis and ice crystal formation which results in release of pro-oxidants causing lipid oxidation. Vanitha *et al.* (2013) observed an increase in TBA from 0.47 to 0.8 and 0.29 to 0.67 mg MA/kg in fish cutlet and fish burger, respectively at the end of 90 days storage period. Similar results have been proposed by Boran and Cose (2007) in fish ball, Ninan *et al*., (2010) in fish cutlets, Vanitha *et al.*, (2013) in mince based products from *Catla* and Khanipour *et al*., (2014) in breaded kilka and Talab (2013) who related it to the interaction of decomposition product of protein with malonaldehyde to give tertiary products. Also, the lower TBA value in fish cutlets could be attributed to the peroxide scavenging enzyme activity of added spices like garlic, onion and ginger which could reduce unsaturated fatty acid and thus prevent lipid oxidation (Nuutila *et al*., 2003, Gokoglu, 2011, Kumolu-Johnson and Ndimele (2011), Rakshit and Ramalingam (2013), Coban, 2013 and Frank *et al*., 2014).

Table 1: Chemical composition of fish cutlets of *Hypophthalmichthys molitrix* (Silver carp) stored under frozen conditions at -12±2 ˚C.

DAYS	TBA (mg Mal/kg)*		FFA (%)**		p H	
	Raw(H_R)	Fish cutlet (H_{FC})	Raw (H_R)	Fish cutlet (H_{FC})	Raw (H_R)	Fish cutlet (H_{FC})
0 day	0.44^{a} ±0.02	0.05^{a}±0.01	1.14±0.01	0.0^{a}±0.05	6.3^{a} ±0.05	6.3^{a} ±0.02
10th day	6.72^{b}±0.01	0.77^{ab} ±0.08	5.23±0.02	0.21^{a} ±0.05	6.8^{b} ±0.01	6.4^{b} ±0.01
20th day	12.04^{c} ±0.05	1.1^{c}±0.04	7.44±0.02	1.05^{b} ±0.05	7.1^{c}±0.01	6.5^{b} ±0.05
30th day	17.67^{d}±0.04	1.45^{cd} ±0. 3	12.65±0.05	1.50^{c} ±0.44	7.3^{d}±0.01	6.5^{b}±0.01

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

*Thiobarbituric acid-8mgMA/kg

**Free fatty acid-5%

Free fatty acid (FFA)

Perusals of Table-1, the increase in FFA values was 1.14±0.01% to 12.65±0.05% in raw muscle (H_R), thus, crossing the acceptable limits of 5% on 10th day. However, fish cutlets (H_{FC}) reported a lower increase i.e. from 0% to 1.50±0.44% and were within acceptable limits till the end of 30 days of frozen storage period. Our observations coincide with the results of Ninan *et al.*, (2010) in Tilapia fish cutlets, Pawar *et al.* (2013) in Catla fish cutlets, Rathod and Pagarkar, (2013) in

Pangasius fish cutlets, Vanitha *et al.*(2013) in minced based products of Catla and Goswami (2014) in ginger, garlic and turmeric treated chicken mince who proposed that oxidative hydrolysis of lipids during frozen storage result in the formation of FFA, thus deteriorating the quality of meat. However, lower FFA values in fish cutlets as compared to raw muscle can be attributed to the deactivation of lipase due to heating effect which prevents the release of FFA in cooked samples. (Zhang *et al.*, 2013 and Al saghir *et al.*, 2004).

pH: The values for pH in raw muscle (H_R) and fish cutlet (H_{FC}) on day 0 was found to be similar i.e.6.3. Progressively, these values reported an increasing trend i.e.6.8 in H_R and 6.4 in H_{FC} on 10^{th} day, 7.1 in H_R and 6.5 in H_{FC} on 20^{th} day and on 30^{th} day the value reached to 7.3 in H_R while in H_{FC} it remained the same i.e. 6.5 only. Our results are in line with those of Talab (2013) who reported an increase in pH of fish cutlets during 4 months of frozen storage period which was associated with the production of basic components induced by growth of bacteria. Pawar (2013) also reported the similar increasing trend in *Catla catla* fish cutlets. Coban (2013) in refrigerated *Sarda sarda* cutlets where he observed a lower pH in ginger oil treated cutlets as compared to control samples. Dhanpal *et al*., (2012) associated this increased pH to the breakage of hydrogen bond and electrostatic interactions. Amany (2010) related the pH increase in garlic, thyme and lemon grass oils treated minced beef to the activation effect of microbial load which may cause protein hydrolysis with the appearances of alkyl groups. Goswami (2014) supported the similar view in chicken mince.

Microbial changes

The initial values for Total plate count (TPC), Coliform count (CC) and Psychrophillic count (PC) on day 0 were found to be 3.05±0.01, 2.09±0.02 and 2.76±0.02 log cfu/g raw fish and 1.95±0.02, 1.07±0.02 and nil log cfu/g in fish cutlets respectively. Further, these values rose to 1.95±0.02, 1.07±0.02 and 0 log cfu/g in raw fish and 1.95±0.02, 1.07±0.02 and 0 log cfu/g in fish cutlets on 30^{th} day of storage for TPC, CC and PC respectively.

The increase in microbial load during frozen storage is related to growth promoting effect of moisture during frozen storage or due to growth of microorganisms during thawing as suggested by Liu *et al.* (2010) in Tilapia, Sharma (2012) in *Mystus* seenghala, Gandotra *et al.*(2013) in *wallago attu* and Genc *et al.* (2015) in Meagre fillets.

However, the low increase of bacterial count in our value added product (fish cutlet) is due to the antimicrobial nature of various additives in it as proposed by Guinares *et al.* (2014) who observed significant reduction in the microbial counts of the garlic- treated sardine samples as compared to control during frozen storage. Allicin present in garlic results in partial inhibition of DNA and protein synthesis and total inhibition of RNA synthesis as a primary target, thus reducing microbial growth as suggested by Ranjan *et al.* (2012). Vanitha *et al.* (2013) related the reduction in microbial load of Catla fish cutlets to freezing and antimicrobial nature of various additives. The high antimicrobial nature of Allium crops was related to the sulphur component present in them by Erkan *et al.* (2015).

Table 2: Bacteriological changes in value added product of Silver carp stored under frozen conditions at -12±2 ˚C.

DAYS	TPC(log cfu/g) *		CC(log cfu/g) **		PC(log cfu/g) ***	
	Raw (H_R)	Fish cutlet (H_{FC})	Raw(H_R)	Fish cutlet (H_{FC})	Raw(H_R)	Fish cutlet (H_{FC})
0 day	3.05±0.01	1.95 ^{a} ±0.02	2.09±0.02	1.07 ^{a} ±0.02	2.76±0.02	Nil
10^{th} day	6.22±0.01	2.5 ^{a}b±0.05	2.95±0.05	1.35 ^{b} ±0.05	4.88±0.03	Nil
20^{th} day	8.12±0.04	3.5 ^{c} ±0.07	4.12±0.06	1.95 ^{c} ±0.06	5.87±0.01	1.02 ^{a} ±0.4
30^{th} day	9.96±0.05	3.75 ^{cd}±0.08	5.65±0.04	2.10 ^{d} ±0.02	7.35±0.05	1.75 ^{b}±0.02

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

*Total plate count-6 log cfu/g **Coliform count-2.69 log cfu/g ***Psychrophillic count-4.6 log cfu/g.

SENSORY ANALYSIS

Table-5 shows the changes in sensory scores of raw muscle and fish cutlets during 30 days of frozen storage. Sensory qualities of fish were evaluated in terms of appearance and colour, flavour, juiciness, texture, odour and overall acceptability. Initial sensory score for overall acceptability in raw muscle is 6.6 while in fish cutlets it is above 8. Sensory sessions were conducted upto 10^{th} day in raw muscle and upto 30 days in fish cutlets as the cutlets did not reach organoleptic spoilage within this time frame. Addition of various ingredients which have antioxidant and antimicrobial properties might have protected the cutlets from developing off-flavours during storage.

Table 3: Sensory scores of raw muscles of Silver Carp (H_R) stored under frozen conditions.

DAYS / SENSORY	0	10^{th}	20^{th}	30^{th}
APPEARANCE & COLOUR	6.1±0.2	4.4±0.03	-	-

FLAVOUR	6.7±0.05	4.1±0.01	-	-
JUICINESS	6.8±0.2	5.1±0.03	-	-
TEXTURE	6.8±0.1	3.9±0.05	-	-
OVERALL ACCEPTABILITY	6.6±0.4	3.12±0.03	-	-

Table 4: Sensory scores of value added product of Silver carp (H_{FC}) stored under frozen conditions.

DAYS / SENSORY	**0**	**10th**	**20th**	**30th**
APPEARANCE & COLOUR	8±0.1	7.2±0.02	6.5±0.02	6.3±0.05
FLAVOUR	8.8±0.03	8±0.06	7.4±0.03	7.1±0.02
JUICINESS	8.1±0.1	7.4±0.3	6.2±0.2	5.9±0.2
TEXTURE	8.2±0.5	7.6±0.05	7.1±0.05	6.4±0.03
OVERALL ACCEPTABILITY	8.27±0.4	7.55±0.2	6.8±0.01	6.4±0.05

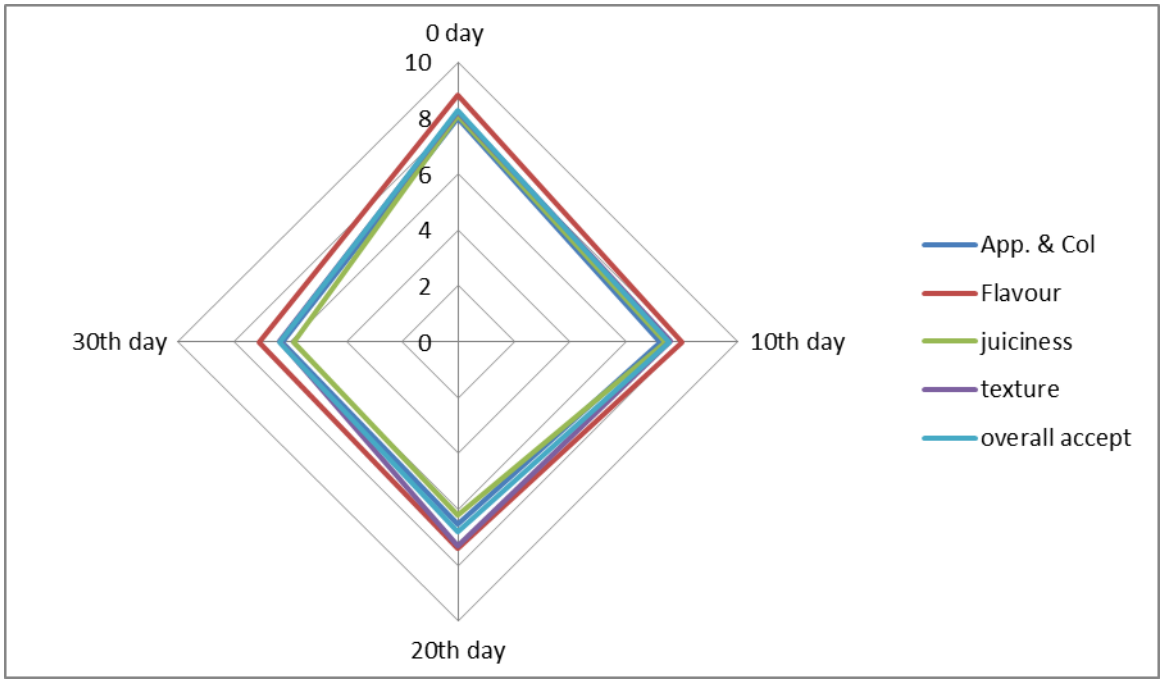

Fig.- Sensory evaluation of H_{FC} during 3o days of frozen storage.

CONCLUSION

The paper described quality changes of value added product (fish cutlet) based on evaluation of biochemical parameters (TBA, FFA and pH, microbial and sensory qualities while stored under frozen display unit (-12^{O}C). The rate of quality deterioration accelerated with the passage of storage time in both S_R and S_{FC} but were still under acceptable limits in S_{FC} during the end of storage period (30 days). The low deterioration of our value added product is attributed mainly to the antioxidant and antimicrobial nature of various additives (garlic, ginger, onion, pepper etc.). Scores for sensory parameters appearance, colour, taste, odour and overall acceptability were also good in S_{FC}. Hence, the addition of various additives can be regarded as a boon during preparation of ready to cook and eat products. These additives apart from enhancing flavour also add to their shelf life.

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