

Qualitative and quantitative changes of fried fish steaks and fish steak curry of catla (*Catla catla*) during frozen storage

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Article history

Received: 19 October 2014

Received in revised form:

14 February 2015

Accepted: 5 March 2015

Keywords

Catla

Fried fish steaks

Fish steak curry

Frozen storage

Abstract

The shelf life of ready to eat fish curry packed with fried fish steaks and fish steak curry of Catla (*Catla catla*) during frozen storage at -20°C were studied in terms of sensory, bacteriological and biochemical changes. Based on sensory evaluation, frying time of 10 minutes at 160°C was optimum to get good quality fried fish steaks. The proximate composition of the fried fish steaks and fish steak curry showed marginal variation throughout the period of study. The chemical changes in fried fish steaks during frozen storage, TVB-N, NPN, PV and FFA were 26.8 ± 0.04 mg/100 g, 50.76 ± 0.12 mg/100 g, 12.40 ± 0.20 meq O_2/kg and 4.58 ± 0.42 % oleic acid at 0th day were changed to 30.5 ± 0.85 mg/100 g, 56.8 ± 0.87 mg/100 g, 56.2 ± 0.71 meq O_2/kg and 18.27 ± 0.75 % oleic acid at 90th day, respectively. But, the chemical values for fried fish steak curry during frozen storage, TVB-N, NPN, PV and FFA were 25.6 ± 0.81 mg/100 g, 56.55 ± 0.54 mg/100 g, 12.40 ± 0.20 meq O_2/kg and 3.91 ± 0.36 % oleic acid at 0th day were changed to 30.1 ± 0.85 mg/100 g, 59.4 ± 0.38 mg/100 g, 21.98 ± 0.45 meq O_2/kg and 6.81 ± 0.21 % oleic acid at 90th day, respectively. Total bacterial counts were decreased over the period of storage in fish steaks and fish steak curry. Fish steak curry exhibited longer shelf life (86 days) when compared to the shorter shelf life (51 days) exhibited by fried fish steaks during frozen storage at -20°C .

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Introduction

Global fish production has grown steadily during the last five decades and with the total world production of 147 MT to 158 MT with the aquaculture contribution of 49.9 MT to 66.6 MT (both in terms of inland and marine) during 2007 to 2012, respectively (FAO, 2014). Aquaculture in India is almost synonymous to carp culture, since the latter alone contributes to more than 80% of the total aquaculture production of the country. The carp culture mainly involves two groups, i.e. the three Indian Major Carps (IMC) such as catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*), and three domesticated exotic carps such as silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) (Jena and Das, 2006). Farming of IMC has been yielding a production of 6-8 tonnes/ha/year (Ayyapan and Diwan, 2006). The current annual production of the three IMC species is about 2 MMT. IMC are currently consumed mostly in the

fresh form, especially in the eastern parts of India. While value addition of marine fishery products has received tremendous attention, freshwater fish species are generally neglected in this respect (Bawa and Jayathilakan, 2002). Processing of IMC can help to enhance its marketability and availability to consumers far away from the production centers.

Frying process is complex and its mathematical models require thorough knowledge of transport mechanism of heat and water in the product (Hallstrom, 1990). The oil used to transfer heat energy is responsible for delicious flavor, odor and texture associated with the fried food (Latha and Tewari, 1999). Mass transfer during frying is due to movement of oil into food and water from product in the form of vapour. During frying heat is transferred from oil to product. Some research observations were observed on the heat and mass transfer kinetics of *Catla catla* fish during frying (Pandey *et al.*, 2008). However, several workers have studied the moisture and fat variation in deep-fat frying. But, the present investigation aims at making a comparative study of

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the frozen storage characteristics of fried fish steaks and fish steak curry prepared from catla. Since, catla is the widely cultured and therefore an ideal choice as raw material for the development of ready to serve products such as fried fish steaks and fish steak curry for both domestic and international markets.

Material and Methods

Fish

Freshly harvested catla (*Catla catla*) was procured from nearby freshwater fish culture ponds of Purusottampur village of Ganjam district, Odissa and were transported in iced condition in the ration of 1:1 to the laboratory. The fishes were first washed with potable water to remove slime and dirt. Then the fishes were scaled, finned, gilled, gutted and were cut to steaks of about 40 g each (6 x 4 x 3 cm). The steaks were washed thoroughly to remove any visceral organ and peritoneal membrane. Spices includes turmeric (2 g), chilli powder (7 g) and salt (10 g) were smeared on the fish steaks (1000 g) and mixed with it thoroughly. The spiced steaks were kept at room temperature for 30 minutes for the spices to penetrate into the steaks after which they were fried.

Standardisation of frying time and preparation of fish steaks

Commercially available refined sunflower oil was used as frying medium. Fish steaks were fried at 160°C for 2, 4, 6, 8, 10, 12, 14 and 16 minutes. The temperature of the frying medium was observed by a thermometer. Frying oil was agitated intermittently using a stirrer to maintain uniform temperature throughout the frying medium.

The fried fish steaks were analysed for proximate composition (moisture, total protein, total lipids and ash), Biochemical parameters viz., Total volatile base nitrogen (TVB-N), non protein nitrogen (NPN), peroxide value (PV) and free fatty acids (FFA) and organoleptic properties (appearance, color, odor, taste, flavor, texture and overall acceptability). The fish steaks were prepared based on the organoleptic analysis revealed that, the optimum time of frying is 10 minutes.

Preparation of fish steak curry

The ingredients used to prepare fish curry were obtained from the local supermarket. Fish steak curry was prepared in Odissa-style using the fried fish steaks following standard method. The ingredients were used for the preparation of 1000 g of fish curry includes vegetable oil (100 ml) garlic (50 g), ginger (50 g), onion (250 g), tomato (200 g), mustard seed (5

g), sugar (5 g), chilly powder (4 g), turmeric powder (1 g), curry powder (4 g), salt (10 g), water (2000 ml).

Preparation of curry

Raw onion, garlic and ginger were peeled and cut into small pieces followed by grinding in a kitchen grinder so as to get fine pastes. Fresh tomatoes were cut into small pieces. On a gas stove, oil was heated in a bowl (kadai) till appearance of smoke. Immediately sugar was put and allowed to foam and float on the oil surface. Sugar was used for development of red color of the gravy through non-enzymatic browning (Maillard reaction). Mustard seed was put and allowed to crack. Quickly the onion paste was put and fried continuously in the oil for a long time till its color became golden brown. Then ginger paste and garlic paste were put in the oil and frying continued till the mixture became sticky. Then cut pieces of tomatoes were added and stirred thoroughly. Other spices like chili powder, turmeric powder and curry powder were added sequentially with thorough stirring. Finally salt was added and frying continued. Then water was added to the mixture and stirred to get slurry. While still on the stove, the fried fish steaks were put in the slurry and allowed to boil for 10 minutes so as to get the fish steak curry.

Packing, freezing and storage

The two different products like fried fish steaks and fish steak curry prepared from the fried fish steaks of catla were packed in 250 g capacity pouches made of polypropylene (vacuum metalized with aluminum) having a thickness of 0.05 mm. The packs were heat sealed using a heat sealing machine. The pouches were labeled properly and subjected to freezing and frozen storage at -20°C in a quick freezer.

Biochemical parameters

Moisture content of fish meat was determined by the standard hot air oven method described in AOAC (2006). The crude protein content (total protein × 6.25) of the meat was determined by estimating total nitrogen by Kjeldahl method (AOAC, 2006). The total lipid content of the meat was determined by Soxhlet extraction method (AOAC, 2006). Total ash content of the meat was determined by the method described in AOAC (2006).

Non-Protein Nitrogen (NPN) of the samples was determined by estimating the nitrogen content in aliquots of TCA extract following the procedure described earlier (Srikar and Chandru, 1983). NPN was expressed as mg/100g meat. Total Volatile Base Nitrogen (TVB-N) content of the samples was determined by Conway's microdiffusion method as

described by Beatty and Gibbons (1937) using the TCA extract. The values were expressed as mg/100 g meat. Peroxide Value (PV) was estimated by the method described by Jacobs (1958) and expressed as millimoles of oxygen per kilogram of fat. Free fatty acids (FFA) content of the samples was estimated by improved titrimetric method of Ke *et al.* (1976) and expressed as percentage of total lipid as oleic acid.

Microbiological analyses

The total plate count (TPC) was estimated by the spread plate technique recommended by APHA (1992). Ten grams of meat was macerated aseptically with 90 ml of sterile physiological saline (0.85% NaCl) for 3 min. and appropriate dilutions were made from the macerate in 0.85% saline and plated on sterile plate count agar (PCA) plates. The plates were incubated at 37°C for 48 h in an inverted position. After incubation, the individual bacterial colonies were counted. The average number of colonies on the plates and were expressed as CFU/g of the sample. All the experiments were carried out in triplicates.

Sensory analysis

Sensory quality of the products (fried fish steaks and fish steak curry) was assessed by a group of 12 experienced panelists using a 5-point hedonic scale based on changes in appearance, color, odor, taste, flavor, texture, and overall accessibility and were asked to assign a score of 1 to 5 (1=not acceptable, 2=acceptable, 3=fair, 4=good, 5=excellent).

Statistical analysis

The IBM SPSS (V 20.00 for windows, SPSS Inc., Chicago, IL, USA) statistical package was used for analysis of the experimental results. Correlation coefficient between the variables was analyzed using Pearson correlation coefficient and the significance of correlation was tested using student's 't' test. The results were expressed as mean \pm standard deviation. Duncan Multiple Regression Test (DMRT) was used to assess statistical significance ($P < 0.05$) between fried fish steaks and fish steak curry during frozen storage period. The shelf-life of the products were determined from the respective regression equations of mean overall acceptability scores on storage period using "3" as the limit below which the products were not marketable as value added products of prime quality.

Results and Discussion

Physical, Proximate, Chemical and Microbial Characteristics of Fresh Fish

The physical characteristics of fresh fish were found to have a length of 26.85 cm and a weight of 750.67 g. The proximate analysis contained 78.36% moisture, 19.65% total protein, 1.31% total lipid, and 0.9% ash. The results of the proximate composition compares well with those obtained by Gopakumar (1997). Nair and Suseela (2000) have analysed the proximate composition of catla. They have reported that the moisture content is 76.30%; protein content is 19.60%; total lipids content is 1.30% and ash content is 0.90%. The slight difference in the values may be attributed to seasonal and size variation of the fish selected. Proximate composition of fish differs with species, sex, body, size, season, environmental factors, nutritional status and even on the type of muscle sample (Love, 1974).

The chemical characteristics with respect to TVBN, NPN, PV and FFA indicate that the values are within the acceptable range. The TVBN content in the fresh meat was found to be 10.46 mg/100g. In general, fishes with TVBN value below 30 mg/100g can be considered as suitable for edible purposes (Hall and Ahmed, 1992). In fresh kurusa (*Labeo gonius*), the TVBN content was found to be 11.37 mg/ 100g (Kumar *et al.*, 2013). Gopakumar (2002) recommends a TVBN value of 35-40 mg/100g for a good quality fish and value of 50-70 mg/100g as the upper limit beyond which fish is considered inedible. However, according to Mathew (2003) a TVBN value of 35-40 mg/100g of muscle is usually regarded as the limit of acceptability beyond which the fish can be considered as spoiled. The NPN content in the fresh meat was found to be 24.12 mg/100g respectively. The NPN values of fresh marine fishes like mackerel and pink perch have been reported to be 76.26 mg/100g and 116.08 mg/100g respectively (Khuntia, 1990). The lower values of NPN in the present study as compared to these values may be attributed to the fact that fresh water fishes like the ones used in the present study lack of TMAO in their tissue, which is an important component of NPN. According to Ninan (2003) the NPN content of seafood is significantly higher than that in other food myosystems, i.e., 9-18% of total nitrogen in teleosts and 33-38% in elasmobranches. The PV of fresh meat was found to be 4.64 milli moles of O₂ / kg fat respectively. In fresh mackerel and pink perch, PV values of 4.62 and 3.69 milli moles of O₂/kg fat respectively have been reported (Khuntia, 1990). According to Gopakumar (2002), for a good fish, the PV value should be much below 10 milli moles O₂/kg fat and at a PV value above 20 milli moles of O₂/kg fat most fishes smell rancid. Mathew (2003) have described PV values above the level of 10-20 milli

Table 1. Changes in proximate and chemical parameters of fried fish steaks during frying time standardisation

Parameters	Frying time (minutes)								
	Fresh fish	2	4	6	8	10	12	14	16
Moisture	78.36 ± 0.17	76.56 ± 0.12	72.39 ± 0.16	70.21 ± 0.16	68.20 ± 0.12	65.84 ± 0.02	61.26 ± 0.05	58.04 ± 0.15	53.18 ± 0.24
Protein	19.65 ± 0.28	19.23 ± 0.31	22.24 ± 0.21	24.09 ± 0.50	26.21 ± 0.25	27.74 ± 0.21	29.80 ± 0.74	32.26 ± 0.54	36.28 ± 0.25
Total Lipid	1.31 ± 0.18	2.89 ± 0.12	4.06 ± 0.74	4.88 ± 0.28	5.01 ± 0.24	5.26 ± 0.34	6.93 ± 0.41	7.23 ± 0.48	9.06 ± 0.24
Ash	0.93 ± 0.03	1.32 ± 0.02	1.44 ± 0.09	1.42 ± 0.01	1.56 ± 0.05	1.94 ± 0.05	2.81 ± 0.01	2.97 ± 0.06	3.14 ± 0.08
TVBN (mg/100g)	10.46 ± 0.11	26.80 ± 0.04	26.23 ± 0.06	26.84 ± 0.07	27.41 ± 0.02	27.93 ± 0.21	26.05 ± 0.32	26.3 ± 0.44	27.42 ± 0.51
NPN (mg/100g)	24.12 ± 0.52	50.76 ± 0.28	50.74 ± 0.43	51.21 ± 0.25	50.29 ± 0.37	50.96 ± 0.41	52.20 ± 0.45	52.30 ± 0.74	53.31 ± 0.52
PV (meq of O ₂ /kg fat)	4.64 ± 0.12	12.81 ± 0.12	12.90 ± 0.24	12.10 ± 0.31	12.43 ± 0.17	12.40 ± 0.20	13.21 ± 0.36	13.28 ± 0.42	13.65 ± 0.51
FFA (% oleic acid)	3.10 ± 0.05	4.56 ± 0.04	4.58 ± 0.47	4.51 ± 0.12	4.53 ± 0.09	4.61 ± 0.17	4.64 ± 0.56	4.61 ± 0.51	4.66 ± 0.08

Values indicate mean ± standard deviation

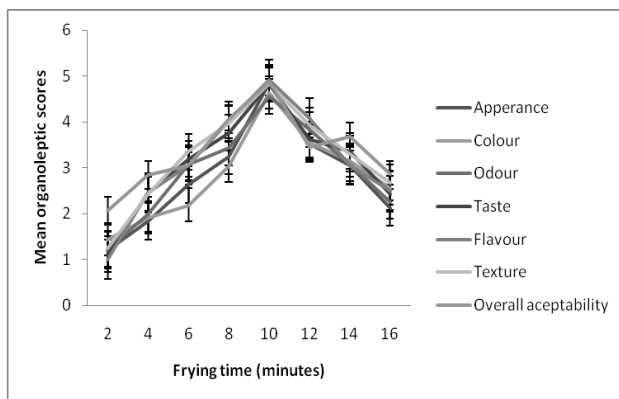


Figure 1. Changes in the mean organoleptic scores of fried fish steaks during frying time standardization

moles of O₂/kg fat to impart rancid smell and taste in all probability. The present results suggest that the fish are in good condition throughout the storage period based on values of 10-20 meq/kg of oil as recommended by Connell (1995).

Free fatty acid content in the fresh meat was used in the present study were 3.10% of TL as oleic acid per kg of meat respectively. Khuntia (1990) has reported FFA values of 3.13 and 1.81 % of TL as oleic acid per kg of meat for fresh mackerel and pink perch respectively. Fresh grass carp meat has also been reported to contain FFA of 2.03% of TL as oleic acid per kg of meat (Pal, 2004). The chemical parameters indicate the quality of fish used in the present study was good, since all the parameters are in below the range of acceptable limit.

Total plate count (TPC) as determined by serial dilution agar plating technique were found to be

8.18 x 10⁵ colony forming units (CFU) per gram of meat. Similar observations have been made during iced storage of common murrel (Perigreen *et al.*, 1987) and tilapia fishes (Dhanapal *et al.*, 2010). The microorganisms present in a fishery product may be 'natural' present in gut, gills, skin, etc. or 'incidental' which enter into the product during post-harvest processing (Abraham *et al.*, 1992).

Frying time standardisation

For frying time standardisation, the fish steaks were fried at 160° C for 2, 4, 6, 8, 10, 12, 14 and 16 minutes with product to oil ratio of 1:10 (w/v). The products were analysed for proximate composition, quality parameters and organoleptic scores (Table 1). Results indicated that, frying times of 10 minutes at 160°C were optimum to get good quality fried fish. This is indicated by sensory evaluation. The sensory evaluation was based on the attributes like appearance, color, odor, taste, flavor, texture and overall acceptability. The mean organoleptic scores for all the attributes in the present investigation increased from frying for 2 to 10 minutes and then decreased from frying for 10 to 16 minutes (Figure 1). Similar works have been reported in catla during frying (Pandey *et al.*, 2008).

Moisture content decreased throughout the period of frying (0 -16 minutes) from 78.36% to 53.18% (Table 1). The initial protein content of the fresh fish steaks were 19.65% and the protein content increased constantly during frying to 36.28% at the end of sixteen minutes of frying. The total lipids of

Table 2. Changes in biochemical parameters of frozen fried fish steaks during frozen storage

Parameters	Frozen Storage Period (days)									
	0	10	20	30	40	50	60	70	80	90
Moisture	65.84 ± 1.66	65.82 ± 1.46	64.89 ± 1.09	64.63 ± 1.06	64.93 ± 1.35	65.88 ± 1.33	65.81 ± 1.19	65.62 ± 1.5	64.38 ± 1.47	64.24 ± 1.13
Total Protein	27.74 ± 0.21	27.91 ± 0.98	26.89 ± 0.36	28.02 ± 0.25	28.12 ± 0.46	27.81 ± 0.52	27.9 ± 0.54	27.86 ± 0.28	27.8 ± 0.29	28.91 ± 0.34
Total lipid	5.26 ± 0.24	5.21 ± 0.26	4.65 ± 0.35	5.81 ± 0.24	5.21 ± 0.38	5.53 ± 0.21	4.32 ± 0.34	4.98 ± 0.21	5.21 ± 0.23	5.05 ± 0.67
Ash	1.94 ± 0.02	1.74 ± 0.04	1.8 ± 0.03	1.76 ± 0.01	1.79 ± 0.12	1.68 ± 0.06	1.79 ± 0.13	1.88 ± 0.11	1.84 ± 0.14	1.8 ± 0.21
TVBN (mg/100g)	26.8 ± 0.04	27.2 ± 0.06	24.84 ± 0.07	27.4 ± 0.02	30.9 ± 0.21	30.05 ± 0.32	29.3 ± 0.44	26.4 ± 0.51	26.5 ± 0.32	30.5 ± 0.85
NPN (mg/100g)	50.76 ± 0.12	50.1 ± 0.71	50.28 ± 0.14	50.4 ± 0.45	51.6 ± 0.74	55.4 ± 0.10	54.9 ± 0.23	54.3 ± 0.25	55.1 ± 0.54	56.8 ± 0.87
PV (meq of O ₂ /kg fat)	12.40 ± 0.20	14.01 ± 0.12	15.84 ± 0.32	18.4 ± 0.17	20.64 ± 0.65	20.83 ± 0.39	36.80 ± 0.58	40.1 ± 0.85	42.5 ± 0.74	56.2 ± 0.71
FFA (% oleic acid)	4.58 ± 0.42	5.11 ± 0.75	5.92 ± 0.47	5.82 ± 0.16	5.96 ± 0.25	5.91 ± 0.09	10.82 ± 0.78	12.13 ± 0.45	17.26 ± 0.71	18.27 ± 0.75

Values indicate mean ± standard deviation

the steaks during frying time standardisation noticed steady increase from 1.31% in fresh steaks to 9.06% at the end of 16 minutes of frying. Ash content showed gradual increase from 0.93% to 3.14%, after 16 minutes of frying. The decrease in moisture content of steaks during frying might have resulted from the loss of moisture during frying in oil. According to Zaitsev *et al.* (1969) fish meat undergoes chemical, physical and histological changes during frying. At a temperature of 100°C, the proteins coagulate and moisture is released, but with a further rise in temperature, the proteins hydrolyse and become denatured leading to enhanced release of moisture. The increasing in fat content throughout the period of frying in all the three species may be attributed to the absorption of fats during frying then in the hot oil. According to Jena and Das (2006) the flesh of lean fish absorbs about 7% to 9% of oil during frying while fatty fishes absorb about 3% to 5%. Higher the temperature of the frying oil (180°C-190°C), lower is its absorption by the product (Dhanapal *et al.*, 2010).

TVBN showed only an initial increase during the first 2 minutes. During subsequent frying for 16 minutes it did not show much variation. TVBN content increased from 10.46 mg/100g in fresh fish steaks to 26.80 mg/100g after 2 minutes of frying. The highest TVBN content (27.93 mg/100g) was noticed at 10 minutes. The NPN values of 24.12 mg/100g were found in the fresh steaks and the values increased to 50.76 mg/100g. The PV values initially increased from 4.64 to 12.81 milli moles of O₂/ kg of fat during the first 2 minutes of frying. During subsequent frying, the PV values showed a

marginal variation having a highest value of 13.28 milli moles of O₂/ kg of fat after 14 minutes of frying. FFA showed an increasing trend during the first 2 minutes of frying which stabilized during subsequent frying up to 16 minutes. Such increase in the values of the quality parameters in the first two minutes may be attributed to the high rate of chemical reaction at the high temperature of the frying oil. The subsequent stabilisation may be due to exhaustion of the chemical precursors of these parameters. Adoga *et al.* (2010) observed during ice storage of tilapia fish (*Oreochromis niloticus*) that the TVB-N value increased from 5.51 mg/100g to an acceptable value of 22.53 mg/100g in 15 days and finally to a rejection value of 38.75 mg/100g at the end of 21 days storage period. In freshwater fish (*Gudusia chapra*), at the end of storage the TVBN value was increased from the initial value of 21.43 mg/100 g to 98.24 mg/100 g for dry salted products, 25.06 mg/100g to 114.54mg/100 g for wet salted products and 32.48 mg/100g to 148.30 mg/100g for unsalted products, respectively (Sharma *et al.*, 2013).

Quantitative and qualitative changes of fried fish steaks during frozen storage

The changes in proximate composition of fried steaks during frozen storage at -20°C have been given in Table 2. During frozen storage, the moisture content of the fried fish steaks and fish steak curry of all did not show much variation throughout the period of study except minor decreases. It decreased from 65.84 to 64.24%. It is usual to find reduction in moisture content of fish and fishery products during

Table 3. Changes in biochemical parameters of frozen fish steak curry during frozen storage

Parameters	Frozen Storage Period (days)									
	0	10	20	30	40	50	60	70	80	90
Moisture	71.35 ± 0.56	71.81 ± 0.45	71.43 ± 0.65	71.41 ± 1.32	71.84 ± 1.56	72.09 ± 1.74	71.08 ± 1.54	71.36 ± 1.78	70.89 ± 1.69	70.34 ± 1.74
Total Protein	21.65 ± 1.02	21.78 ± 1.32	22.09 ± 1.06	22.87 ± 1.25	22.61 ± 1.64	22.09 ± 0.90	22.87 ± 0.08	22.87 ± 0.63	22.56 ± 0.86	22.89 ± 0.95
Total Lipid	5.84 ± 0.14	5.69 ± 0.18	4.71 ± 0.56	5.83 ± 0.20	5.37 ± 0.10	4.69 ± 0.45	5.81 ± 0.25	4.81 ± 0.65	5.28 ± 0.74	5.01 ± 0.24
Ash	1.84 ± 0.01	1.83 ± 0.02	1.09 ± 0.01	1.81 ± 0.20	1.78 ± 0.10	1.56 ± 0.23	1.87 ± 0.01	1.84 ± 0.14	1.82 ± 0.09	1.86 ± 0.25
TVBN (mg/100g)	25.6 ± 0.81	25.4 ± 0.54	23.2 ± 0.65	24.5 ± 0.27	29.15 ± 0.48	29.5 ± 0.78	29.91 ± 0.98	29.8 ± 0.71	28.57 ± 0.57	30.1 ± 0.85
NPN (mg/100g)	56.55 ± 0.54	56.68 ± 0.25	59.2 ± 0.75	56.0 ± 0.67	57.8 ± 0.75	58.3 ± 0.45	58.0 ± 0.71	56.8 ± 0.91	59.1 ± 0.45	59.4 ± 0.38
PV (meq of O₂/kg fat)	12.40 ± 0.20	12.11 ± 0.09	13.12 ± 0.05	16.13 ± 0.41	16.15 ± 0.24	20.91 ± 0.14	20.09 ± 0.31	20.27 ± 0.63	20.15 ± 0.56	21.98 ± 0.45
FFA (% oleic acid)	3.91 ± 0.36	4.10 ± 0.24	4.20 ± 0.01	4.61 ± 0.09	4.61 ± 0.21	5.12 ± 0.17	6.14 ± 0.17	6.40 ± 0.19	6.66 ± 0.74	6.81 ± 0.21

Values indicate mean ± standard deviation

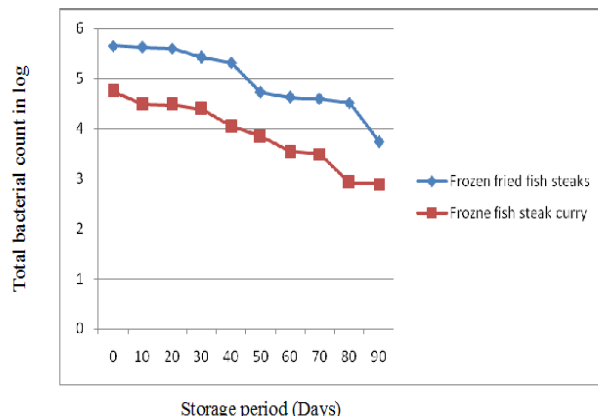


Figure 2. Changes in total plate count (TPC) of fried fish steaks and fish steak curry of catla during frozen storage at -20°C

frozen storage because of dehydration (Vanitha *et al.*, 2013). Moisture slowly leaves the product with the increasing period of storage. Similar observations were observed in frozen mackerel flakes curry and mackerel steaks curry (Rao, 1989). The decreased variation in moisture content in the present study may be attributed to the packaging of the products in vapor proof packaging materials, which prevented moisture reduction by dehydration. Such minor decrease in moisture content may be attributed to cell damage caused by the ice crystals formed during freezing (Reddy *et al.*, 2011).

The total protein content of the fried fish steaks and fish steak curry showed marginal increase in all the three species throughout the period of frozen storage for 90 days. In case of protein content of fried

fish steaks showed an increasing trend from 27.74 to 28.91%. Such marginal increase in protein content may be delineated to its relative increase resulting from the marginal decrease in moisture content. The total lipid content of the fried fish steaks and fish steak curry during frozen storage remained more or less stable. No significant change was noted in the fat content. The ash content, which is a measure of total mineral in the fish meat, has shown slight increase over the period of frozen storage. Similar observations have been reported in frozen storage of fish steak curry from sardine and mackerel (Rao, 1989). Similar trend of marginal increase of fat content have been reported in mackerel during frozen storage (Lakshmisha *et al.*, 2008).

The changes in the TVBN content of the fried fish steaks during the period of frozen storage have been presented in Table 2. Though the values showed fluctuation over a prolonged period of time, towards the end they have showed an increasing trend in the products. The TVBN content of fried steaks was 30.5 mg/100g at the end of the 90 days. NPN values showed slight fluctuation throughout the period of storage in case of fried fish steaks. However, there was an overall increase and increased from 50.76 mg/100g in the 0 day to 56.80 mg/100g in 90 days of storage. The PV registered a gradual increase up to 50 days after which it increased steeply up to 90 days. The initial value of 12.40 milli moles of O₂ / kg fat in 0 day, it gradually increased to 20.83 milli moles of O₂ / kg fat in 50 days and then steeply increased

to 56.20 milli moles of O_2 / kg fat in 90 days. FFA content was increased during 90 days storage period. However, the increase was slow initially up to 40-50 days after which it increased sharply up to 90 days. It increased from 4.58 % of TL as oleic acid in 0 day to 5.91% of TL as oleic acid in 50 days and to 18.27% of TL as oleic acid in 90 days.

The microbial characteristics of fried fish steaks during frozen storage were estimated in terms of TPC. TPC was decreased over the period of storage from an initial level of 4.52×10^5 cfu / g of meat in 0 day to 5.61×10^3 cfu / g of meat in 90 days (Figure 2). The mean sensory characteristics of fried fish steaks during frozen storage, all the attributes such as appearance, color, odor, taste, flavor, texture and overall acceptability steadily decreased throughout the period of the storage. There is negligible difference in appearance, color, odor, taste, flavor, texture and overall acceptability.

Quantitative and qualitative changes of fish steak curry during frozen storage

The moisture content of the products did not show much variation during the frozen storage period. The lowest and highest values of moisture were 70.34% and 72.09%. The values of total protein content are fluctuated between 21.65% and 22.89%. The total lipid content showed negligible fluctuation and the minimum and maximum values were 4.71% and 5.84%. The values of ash content fluctuated between 1.09% and 1.86% (Table 3). Similar observations have been reported in frozen storage of fish steak curry from sardine and mackerel (Rao, 1989).

The TVBN values showed an overall increasing trend and are 30.10 mg/100g at the end of 90 days. Total volatile bases are a mixture of ammonia, TMA, DMA, MMA, etc. These are the products formed by progressive hydrolysis and putrefactive processes under the influence of microbial enzymes. These volatile bases show an exponential increase leading to a deterioration of odor and flavor in fish and shellfish (Kolakowski, 1986). TVBN content is one of the most common indices of quality used universally for deciding the state of freshness of fishery products and seafood. In the present study, all the samples had less TVBN value than all these limits throughout the period of frozen storage. This may be attributed to the absence of microbial activity during frozen storage. This corroborates well with the microbial load, which showed a declining trend in all the products throughout period of storage. However, the slight increase in TVBN values up to three months may be attributed to the relative decrease in moisture content throughout the frozen storage period. Lakshmisha *et*

al. (2008) has also noted similar changes in mackerel (*Rastrelliger kanagurta*) during frozen storage. Total volatile base nitrogen (TVBN) of surimi prepared from *Priacanthus hamrur* increased gradually during frozen storage (Sing *et al.*, 2004). Vanitha *et al.* (2013) reported increase in TVBN values during the frozen storage of fish cutlet prepared from catla minced meat.

NPN values showed similar trend of fish steaks. There was a marginal increase in the values over the period of frozen storage. It increased from 56.55 mg/100g in 0 day to 59.40 mg/100g in 90 days in 90 days of frozen storage period. The NPN substances are produced by the action of enzymes, both bacterial and endogenous, on the fish tissue, which hydrolyze the proteins producing free amino acids thereby increasing the NPN. The absence of any remarkable increase of NPN, may be attributed to the absence of microbial activity which was completely seized at the frozen storage temperature of -20°C . According to Almas (1981), most bacteria cease to grow if the temperature is reduced to -5°C . In practice, there will be no bacterial activity at -10°C . In addition, the activity of the endogenous enzymes was also absent as the enzymes got denatured during frying of the fish steaks.

Initially, the PV shows the below of acceptability (i.e., 10-20 milli moles of oxygen/kg fat). However, during frozen storage for 90 days, the PV showed increasing trend from 12.40 milli moles of oxygen/kg fat (day 0) to 21.98 milli moles of oxygen/kg fat (day 90). This may be attributed to the autoxidation of fish tissue lipids by the oxygen present inside the packs of the products. Though the products were packed in oxygen impermeable packages and sealed airtight the oxygen present inside the packs were not removed, as it requires vacuum packaging system. Similar observations were observed in frozen mackerel flakes curry and mackerel steaks curry during frozen storage (Rao, 1989). Piskarev *et al.* (1960) also observed an increase in the PV value of minced herring muscle to about 45 milli moles of oxygen/kg fat over a 70-day frozen storage period at -10°C . Awad *et al.* (1969) also observed an increase of PV from 13 to 35 milli moles of oxygen/kg fat in six weeks frozen storage of white fish muscle at -10°C . Similar trend was observed in frozen minced meat of silver carps (Bijoy, 2008). Joseph *et al.* (1986) also observed that the PV increased from 3.35 to 8.30 milli moles of oxygen/kg fat in minced meat of cat fish.

In order to evaluate the lipid quality, FFA value plays a vital parameter. The lower value always represents the higher quality of the product and as well as the lower oxidation. The acceptable limit for

FFA content is reported to be 7% as oleic acids to the total lipids (Bimbo, 1998). FFA values showed an overall increase, though sharply towards the later part of the storage period. It increased from 3.90% of TL as oleic acid in 0th day to 6.80% of TL as oleic acid in 90 days. Similar observation made by Rao (1989), in curry prepared from sardine and mackerel during frozen storage. Hydrolysis of lipids in fish and fishery products to free fatty acids and glycerol is a common feature. Lipid hydrolysis is brought about by the endogenous tissue lipases or the lipases produced by lipolytic bacteria. Increase in FFA indicates lipolysis. Lipases and phospholipases which are present in the animal tissue and microorganisms are known to be responsible for liberation of FFA. Similar observations were recorded in minced meat of silver carps during frozen storage (Bijoy, 2008). Reddy and Bhandary (2014) recorded increase in the free fatty acids during frozen storage of reef cod (*Epinephelus chlorostigma*). The increase in FFA may be due to the highly heat stable lipases which might be present in the fish tissue and might have been deactivated by the process of frying. Usually most of the enzymes get denatured at the high temperature of the frying (Lall et al., 1975), but the increasing FFA as obtained in the present investigation envisages the presence of highly stable lipases in the fish tissues of the three carps. Similar observations have been reported by Ninan et al. (2008) in mince based products from tilapia meat which was heat processed before frozen storage at -20°C.

TPC also showed the similar trend with fried fish steaks. It decreased from 5.50 X 10⁴ cfu / g. of meat in 0 day to 6.41 X 10² cfu / g of meat in 90 day (Figure 2). Initial TPC was higher in fish steak curry as compared to that in fried fish steaks. This may be attributed to the high load of spore forming bacteria in the spice and other ingredients used to prepare the curry. According to Hersom et al. (1956) sugars and spices harbor high load of bacteria, most of which are thermophilic in nature. In both fried fish steaks and fish steak curry the total plate count decreased throughout the period of the storage. This may be attributed to injury caused to the bacterial cells by the process of freezing. The number of bacteria in seafood's is markedly reduced by freezing, probably because the flora contains such a large proportion of gram-negative bacteria, which generally are quite sensitive to the freezing process (Lakshmanan, 2000; Reddy et al., 2011). Similar results were observed in frozen storage studies of mince based products like fish cutlets, fish balls and chilly fish developed from tilapia (Ninan et al., 2008). Jeyasekaran et al. (2002) observed that freezing results in reduction

of bacterial count and the number will continue, in most cases, to fall during frozen storage. Decrease in microbial load may be due to the exposure to low temperature for long duration. This is in agreement with report of Siddaiah et al. (2001) and Anand et al. (2002) for frozen mince of sand front tilapia, pink perch and silver carp.

The organoleptic characteristics of frozen fish steak curry, showed steady decreased throughout the period of storage. There were negligible differences observed among all the attributes of fish steak curry. The changes in the organoleptic qualities of the frozen fried fish steaks and fish steak curry products in the present study were evaluated on a 5-point hedonic scale based on the attributes like appearance, color, odor, taste, flavor, texture and overall acceptability. The quality of fried fish steaks and fish steak curry on 0 day was found to be nearly excellent with respect to all the attributes. This corroborates well with the quality parameters like TVBN, NPN, PV and FFA, which were much below the limit of acceptance. In both the products the sensory scores steadily decreased throughout the period of the storage. This may be attributed to the development of rancid flavor in the products, resulting from the autoxidation of lipids present in the products, as indicated by the increase of PV in all the products. According to Khuntia (2013), in fishery products, particularly in frozen fish, in which microbial and enzymatic activities are almost completely inhibited, autoxidation continues unabated during cold storage, though at slower rate, and dominates the loss of quality of frozen fish. Therefore, autoxidation is the dominant deterioration agent in frozen fish. Similar report of decrease in the organoleptic scores of fish cutlets, fish balls and chilly fish prepared from tilapia during storage at -20°C have been made by Ninan et al. (2008). Rao (1989), also reported decrease in the organoleptic scores of mackerel and sardine fish curry during frozen storage at -18°C.

Between the two types of products, fish steak curry showed better organoleptic qualities than their fried fish steak counter parts. In fried fish steaks all the organoleptic characteristics such as appearance, color, odor, taste, flavor, texture and overall acceptability decreased steeply. In contrast, the organoleptic scores for all these attributes in case of fish steak curry showed gradual decline over the entire period of storage. As a result, fish steak curry had better quality and longer shelf life than their fried counter parts. Frozen fried fish steaks were found to have shelf life of 51 days. In contrast, frozen fish steak curry had shelf life of 86 days. The superior quality and longer shelf life of the frozen fish steak

curry over frozen fried fish steaks may be attributed to the protective effect of the gravy present in the curry products. The gravy covered the steaks and became hard during freezing protecting the steaks from contact with the atmospheric oxygen. As a result, its lipids could not undergo remarkable autoxidation and showed lower rancidity level. In contrast, the fried fish steaks came in direct contact with the atmospheric oxygen present in the packs, as the packs were not vacuum packaged. This led to rapid rate of lipid autoxidation and development of rancid flavor. This corroborates well with the peroxide value (PV), which showed a fast increase in fried fish steaks from 12.4 to 56.2 milli moles of oxygen/kg fat in comparison to the gradual increase of PV in frozen steak curry from 12.4 to 21.98 milli moles of oxygen/kg fat. According to Khuntia (2013), enzymatic and microbial deterioration are almost completely inhibited in frozen products allowing lipid autoxidation and concomitant development of rancid flavor to be the main cause of quality deterioration in frozen foods.

Statistical analyses

Regression analysis of overall acceptability on storage period was used to find out the shelf life of the products using the hedonic value "3" as the limit below which the products were not marketable as value added products of prime quality. Fried fish steaks were found to have a shelf life of 51 days each. Frozen fish steak curry showed the shelf life of 86 days.

Conclusion

Results clearly show that the fish steak curry had better quality and longer shelf life than their fried fish steaks. Frozen fried fish steaks were found to have shelf life of 51 days while frozen fish steak curry had shelf life of 86 days.

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