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Effect of frozen storage on the quality of Catla (*Catla* *Catla*) fish loaves incorporated with Tofu and Whey protein concentrate

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Abstract

Fish loaf, a minced meat product was prepared from Catla (*Catla catla*) fish meat, tofu, WPC (Whey Protein Concentrate), spices and other additives. Fresh and frozen stored (-18 ± 2 °C) loaves were evaluated for cooking characteristics, microbiological and sensory quality after every 15 days interval. Cooking yield of loaves decreased significantly ($P<0.05$) and the shrinkage increased significantly ($P<0.05$) during the frozen storage period of one month. The total plate counts (TPCs) decreased significantly ($P<0.05$) during the frozen storage. The sensory evaluation scores for appearance, color, flavor, texture, juiciness and overall acceptability decreased significantly ($P<0.05$) during frozen storage. The loaves were found acceptable up to 30 days of frozen storage as per sensory evaluation scores.

Keywords: Catla fish, frozen storage, loaves, tofu and simmering.

1. Introduction

Fish is a good source of animal protein and it has been used from antiquity as a valuable source of food. Around 50 per cent of the world's population derived at least 20 per cent of its animal protein requirements from fish. Fish is also a good source of minerals (Copper, phosphorous, sulphur, iodine and selenium) and rich in calcium, particularly small fish that is eaten with bones. Polyunsaturated fatty acids (especially ω -3 fatty acids) in fish lipids have anti-atherosclerotic and anti-thrombotic properties. In India, annual fish production in 2011-12 was 8.7 million tons (MT) consisting 3.4 MT from marine and 5.3 MT from inland fishery sources [5]. India is the 3rd largest producer of fish in the world and ranks second in aquaculture. Inland fish production which is growing at a rate of 6% contributes 55% in the total fish production. The potential for growth is immense and the country is on the threshold of massive development in fisheries and aquaculture [6].

In Punjab, aquaculture is gaining popularity among the farmers as it is becoming more profitable than the cultivation of other regular crops. It has now established itself as a profit making venture and as a means of diversification from agriculture (wheat-paddy rotation). Four major rivers, several rivulets, reservoirs and lakes support a vast irrigation system, and water is also extracted from a rich ground water resource [4]. The commercial success of carp culture in some of the other states like Andhra Pradesh has also encouraged some of the farmers to venture into carp farming in Punjab. Freshwater aquaculture contributes over 95 per cent share of the total aquaculture production in terms of quantity with carps being the principal group. Although fish production has increased significantly, but the carps raised are not very popular among the local population due to the presence of numerous intramuscular bones [9]. Therefore for maintaining growth in this sector, value added convenience fish products as per the local requirements to enhance consumer acceptability need to be developed and popularized.

Scientists [2] studied the effect of frozen storage on quality changes of five fish species and found that fish and fishery products can undergo undesirable changes during frozen storage due to lipid oxidation and enzymatic hydrolysis of lipid and protein. The damage indices such as peroxide value, thiobarbituric acid (TBA), free fatty acid (FFA) and total volatile nitrogen (TVN) during frozen storage revealed significant ($P \leq 0.01$) correlation with the storage time. The changes in lipid oxidation indices (PV and TBA) and enzymatic

deterioration indices (FFA and TVN) depicted that all the fish species are susceptible to significant change during the frozen storage, especially if the storage time is long and can be stored for five months in a frozen state with low undesirable changes of lipid and protein quality. Scientists [3] studied the impact of freezing on nutritional composition of fishes like *Liza dussumieri*, *Sparidae*, *Sciaenidae* and *Platycephalidae*. The results indicated that protein decreases with increasing duration of frozen storage; with the fresh samples (not frozen) having the highest protein content ($13.02 \pm 0.09\%$) while the least (10.13 ± 0.06) was recorded for fish samples that were frozen for sixty days. Similar results were obtained for the fat content where the highest fat content ($0.25 \pm 0.20\%$) was recorded for the fresh samples and the least value was recorded for those stored for sixty days. Ash content and moisture content also decreased during storage. There was change in approximate composition and microbial count by low temperature preservation in fish muscle of *Labeo rohita* (Ham-Buch) [8]. It was found that the protein, lipid, moisture and ash contents decreased significantly ($P < 0.05$) during the entire storage period. The microbial count increased gradually during the period of storage. The Total Plate Count in fresh fish muscle on day zero was rather low i.e. 2.44 ± 0.2 log cfu/g as compared to the values found on the 21st day i.e. 5.10 ± 0.02 log cfu/g. The Coliform count (CC) also followed the same trend during the storage. On day zero, a few colonies were found and the CC was 1.50 ± 0.15 log cfu/g where as it increased up to 3.08 ± 0.07 log cfu/g on last day of storage. Similarly Psychrophilic count increased from 2.15 ± 0.2 log cfu/g on day zero to 5.06 ± 0.05 log cfu/g on 21st day. With this in view, the present study was undertaken to study the cooking characteristics as well as microbiological quality of fish loaves and to conduct storage studies thereby arriving at the optimum conditions for storage of fish loaves.

2. Materials and Methods

2.1 Raw material

Catla fish was procured from the local market. The fish were cleaned and dressed in the Department of Food Science and Technology, PAU, Ludhiana as per standard hygienic procedures. They were deboned manually; the deboned meat was packed in double layer of low density polyethylene (LDPE) bags and frozen to -18 ± 2 °C in the commercial freezer. The deboned meat was thawed at $5-10$ °C by placing it in a refrigerator 10-12 hrs before use and then minced in a food processor (Inalsa, Maxi Plus) before use. A standard commercial brand of tofu was procured from the local market. The tofu slab was shredded, mixed with 5 per cent water and was ground in food processor for 5 min to form paste, before its incorporation into the batter for loaves. The WPC was obtained from M/s Mahaan Proteins Ltd., New Delhi.

2.2 Standardization of product formulation

The recipe of fish loaves was standardized according to local taste requirements through preliminary trials. The opinion of taste panel members was analyzed in standardizing the recipe of the product. The standard recipe is given in Table 1. The trials were conducted using different levels of salt, spices and different ratios of green curry stuff for optimizing the recipe. The trial preparation also helped in training the taste panel members. After optimization, the trials were conducted using combination of tofu and WPC (tofu + whey protein concentrate = TW) in the ratio 5:1 to replace 0, 5, 10, 15, 20% minced fish. For further reference the combination of tofu and

WPC is designated as TW.

2.3 Preparation of Loaves

The deboned and ground Catla fish and salt were mixed and chopped for 15 min. in food processor to form a fine paste (batter). Ground tofu and WPC along with other ingredients as per formulation were added to the batter and chopped for 15 min in the food processor to form a uniform emulsion. The emulsified batter was filled compactly into metallic moulds (2" x 2") to form loaves approximately 110 ± 5 g in weight. The filled moulds were covered tightly with lids, and then partially cooked (simmered and baked) for half an hour, removed from the moulds after chilling and packed in double layer (LDPE) bags. The loaves were frozen stored at -18 ± 2 °C and kept at this temperature till the end of frozen period (30 days). The loaves were placed in moulds and were cooked by two methods:-

- i) Simmering ($90-92$ °C) in water for two and half hours (till the core temperature of loaves was 71 °C).
- ii) Baking in hot air oven at 150 °C for 2 hours. A container of water was placed inside the oven to maintain high humidity throughout the baking process, for conducting sensory evaluation.

Table 1: Standardized Recipe for the Preparation of Fish Loaves

Ingredients	Quantity (g)
Minced Fish	1000.0
Refined Groundnut oil	100.0
Salt	18.0
Dry spices (White pepper – 7 g, cardamom – 2.5 g)	9.5
Green curry stuff (GCS) (25 g onion + 12.5 g ginger + 12.5 g garlic)	50.0
Maida	35.0
Chilled water	40 ml*
Sodium tripolyphosphate	4.0
Sodium alginate	10.0
Sodium nitrate	0.30
Sodium nitrite	0.15
Calcium Carbonate	3.0

*Additional 2 per cent water was added in the formulation for baked loaves.

Tofu - WPC was incorporated at 0-20 per cent level.

2.4 Physico-Chemical Analysis

The cooking yield, per cent shrinkage and microbiological counts were calculated by following methods.

2.4.1 Cooking yield

Per cent cooking yield was determined by calculating weight differences of the loaves before and after cooking.

$$\% \text{ cooking yield} = \frac{\text{Cooked loaf weight}}{\text{Raw loaf weight}} \times 100$$

2.4.2 Shrinkage

Per cent shrinkage was determined by calculating the volume differences of the loaves before and after cooking.

$$\% \text{ Shrinkage} = \frac{\text{Volume of raw loaf} - \text{Volume of cooked loaf}}{\text{Volume of raw loaf}} \times 100$$

2.4.3 Sensory evaluation

This was done by semi-trained panel comprising ten members of the PAU faculty and post-graduate students, using a nine point Hedonic scale.

2.4.4 Microbiological analysis

Microbiological analysis of raw and cooked loaves was conducted by standard pour plate method [1] and total plate count (TPC/g) was determined by:

$$TPC/g = \text{Mean colony count} \times \text{dilution factor}$$

2.5 Statistical analysis

The data on the proximate composition, cooking characteristics, microbial quality and organoleptic scores of fresh and stored frozen fish products were statistically analyzed and subjected to analysis of variance using completely randomized design (CRD) [10].

2.6 Product economics

It was calculated on relative cost given by Koutsoyiannis [7].

3. Results and Discussion

The data presented in Table 2 shows that cooking yield decreased significantly ($P < 0.05$) from 88.71 per cent in fresh fish-TW loaves to 87.07 percent after 30 days of frozen storage. Shrinkage increased significantly ($P < 0.05$) from 2.10 percent in fresh fish-TW loaves to 2.96 percent after 30 days of frozen storage in case of simmered loaves. Similar trends were observed in the cooking characteristics of baked loaves after 30 days of frozen storage.

Table 2: Effect of frozen storage ($-18 \pm 2 \text{ }^\circ\text{C}$) on the cooking characteristics of Fish-TW loaves (n=3)

	At 0 day	After 15 days	After 30 days	CD (0.05)
Simmered				
Cooking yield (%)	88.71	88.53	87.07	0.25
Shrinkage (%)	2.10	2.38	2.96	0.09
Baked				
Cooking yield (%)	82.73	81.93	80.98	0.22
Shrinkage (%)	4.58	5.27	6.62	0.14

Table 3: Effect of frozen storage ($-18 \pm 2 \text{ }^\circ\text{C}$) on the cooking characteristics of Fish-TW loaves (n=3)

Loaves	At 0 day	After 15 days	After 30 days	CD (0.05)
Raw	3.38×10^5	2.76×10^3	1.81×10^2	1.70
Simmered	2.48×10^3	3.47×10^2	4.56×10^1	1.47
Baked	2.29×10^3	3.39×10^2	4.48×10^1	1.39

The sensory scores (Table 4) for simmered loaves decreased significantly ($P < 0.05$) from 0 to 30 days of frozen storage period. The sensory scores ranged from 8.60 to 6.75 for

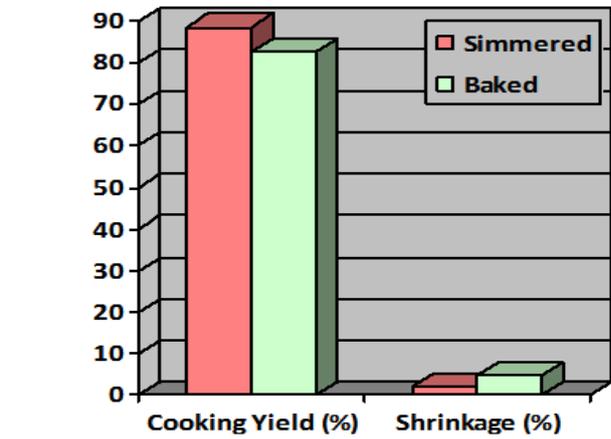


Fig 1: Cooking Yield and Shrinkage Characteristics of Simmered and Baked loaves at 0 day of storage.

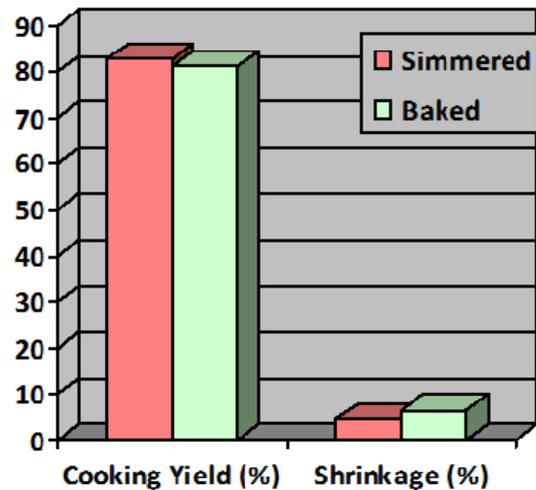


Fig 2: Cooking Yield and Shrinkage Characteristics of Simmered and Baked loaves at 30 days of storage.

The microbiological counts (Table 3) decreased significantly ($P < 0.05$) from $3.38 \times 10^5/g$ in fresh fish-TW loaves to $1.81 \times 10^2/g$ after 30 days of frozen storage period, from $2.48 \times 10^3/g$ in fresh simmered fish-TW loaves to $4.56 \times 10^1/g$ after 30 days of frozen storage period and from $2.29 \times 10^3/g$ in fresh baked fish-TW loaves to $4.48 \times 10^1/g$ after 30 days of frozen storage period.

appearance, from 8.75 to 7.30 for colour, from 8.80 to 6.10 for flavor, from 8.85 to 6.25 for texture, from 8.75 to 7.00 for juiciness and 8.75 to 6.68 for overall acceptability.

Table 4: Effect of Frozen Storage ($-18 \pm 2 \text{ }^\circ\text{C}$) on the Organoleptic Scores of Simmered Fish -TW Loaves (n=10)

Storage period (days)	Appearance	Color	Flavor	Texture	Juiciness	Overall Acceptability
0	8.60	8.75	8.80	8.85	8.75	8.75
15	7.75	7.45	7.25	7.20	7.35	7.40
30	6.75	7.30	6.10	6.25	7.00	6.68
CD (0.05)	0.35	0.54	0.40	0.40	0.35	0.26

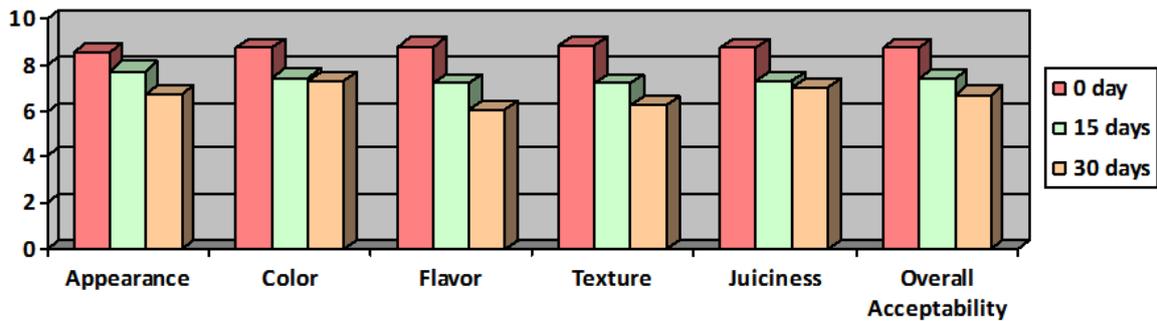


Fig 3: Effect of Frozen Storage on the Organoleptic Scores of Simmered Fish - TW Loaves

The sensory scores (Table 5) for baked loaves also decreased significantly ($P < 0.05$) from 0 day to 30 days of frozen storage. They ranged from 8.5 to 6.65 for appearance, 8.3 to 6.7 for

colour, 8.45 to 6.00 for flavor, 8.15 to 6.6 for texture, 7.75 to 6.0 for juiciness, 8.23 to 6.39 for overall acceptability.

Table 5: Effect of Frozen Storage (-18 ± 2 °C) on the Organoleptic Scores of Baked Fish – TW Loaves (n=10)

Storage period (days)	Appearance	Color	Flavor	Texture	Juiciness	Overall Acceptability
0	8.50	8.30	8.45	8.15	7.75	8.23
15	7.40	7.35	7.15	7.05	7.15	7.22
30	6.65	6.70	6.00	6.60	6.00	6.39
CD (0.05)	0.36	0.54	0.43	0.42	0.36	0.26

n = number of replications, CD = critical difference

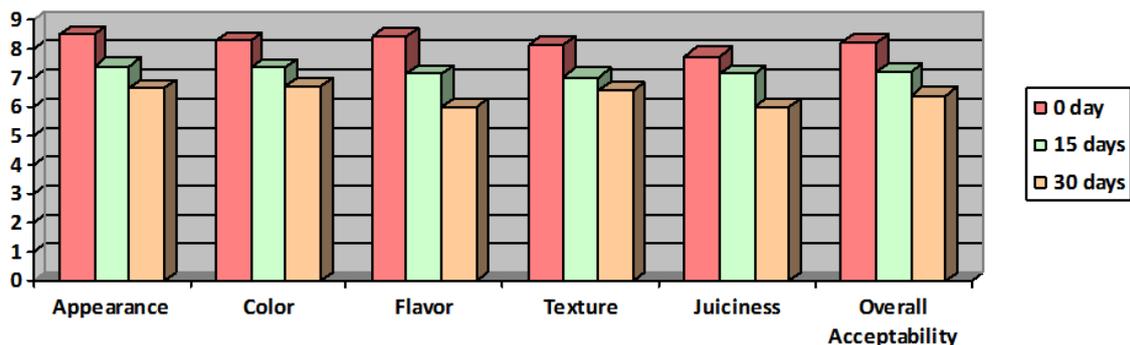


Fig 4: Effect of Frozen Storage on the Organoleptic Scores of Baked Fish - TW Loaves

The scores for overall acceptability of simmered and baked fish loaves at 10 per cent TW levels were found to be the most acceptable and were therefore kept for frozen storage studies.

4. Conclusions

It was thus concluded that tofu and WPC, in the ratio 5:1 can be successfully incorporated into fish loaves at 10 percent level and this combination helped to improve texture, juiciness and stability of frozen stored loaves. The frozen storage resulted in significant ($P < 0.05$) reduction of microbiological load. The loaves were found acceptable up to one month of frozen storage period on the basis of sensory evaluation.

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