ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 2(6): 243-247

www.fisheriesjournal.com
Received: 21-05-2015
Accepted: 23-06-2015

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Quality evaluation and shelf life assessment of raw and value added fish product (fish cutlet) of *Wallago attu* during frozen storage conditions (-12 °C)

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Abstract
An attempt was undertaken to evaluate and compare the shelf life of frozen raw and value added fish product (fish cutlets) of *Wallago attu*. The fish samples were subjected to biochemical and microbial analysis at an interval of ten days during thirty days of storage period. Results of biochemical and microbial assessment indicates a decreasing trend for protein, lipid, ash and moisture and an increasing trend for free fatty acid (FFA), thiobarbituric acid (TBA) and pH for both raw and fish cutlets. However, the fish cutlets depicted a lower percental decrease ($P<0.05$) for protein, lipid, ash and moisture as compared to the raw fish muscle. It was 39.2%, 60.94%, 13.72%, 9.38% in raw samples and 11.44%, 16.87%, 5.68 %, 3.31% in fish cutlets for protein, lipid, ash and moisture respectively at the end of storage period. Similarly, the microbial quality of fish cutlet was 2.8 log cfu/g against 8.55 log cfu/g in case of raw fish for Total plate count (TPC) at the end of thirty days. Thus, the biochemical and microbial analysis revealed that frozen value added fish product (fish cutlets) are healthy for human consumption upto the end of storage period while the raw fish muscles crossed the permissible limits for the same on 10thday of frozen storage.

Keywords: shelf life, biochemical, microbial, fish cutlets and thiobarbituric acid.

1. Introduction
A mass contribution to the survival and health of a significant portion of world's population, particularly to the developing nations has been done by the fish. Fish constitutes the major source of proteins, poly-unsaturated fatty acids, vitamins like vitamin A, vitamin B2, vitamin B6, micronutrients and minerals viz. iron, calcium, iodine, potassium etc. Since, fish is composed of 70-84 percent water, therefore such high moisture content makes it extremely perishable. Immediately a fish dies, a number of physiological and microbial deterioration set in and thereby degrade the fish (Davies and Davies, 2009). Also, the seasonality of the fish food puts a constraint over the availability of fish throughout the year, especially during the lean season. Hence, Processing and value addition is the need of the hour. Value addition means 'any addition activity that in one way or the other change the nature of a product thus adding to its value at the time of sale. Moreover, with the move towards busy life style, people are developing interest towards ready-to-eat precooked products like fish balls, cutlets, nuggets etc. Thus, the aim of present study is to assess the biochemical and microbial changes in frozen raw and value added fish product (fish cutlet) of *Wallago attu* during 30 days of storage period.

2. Materials and Methods
2.1 Collection of fish samples
Fresh samples of *Wallago attu* 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 and the fish was washed with large amount of water and filleted. These fillets were divided into two groups. Group A (Gp. A) samples were kept raw, used as control sample packed in aluminum foil and kept in freezer at -12±2 ˚C. The group B (Gp. B) samples were deboned

after steaming; the flesh was minced and then made into fish cutlets after mixing with some spices.

2.2 Preparation of fish cutlets

The fish cutlets were made according to the recipe proposed by Pawar *et al.,* 2012 and A.S. Talab (2013) with some modifications. It involved the mixing of mince (75%) with refined oil (6%), starch (8%), onion (1.5%), ginger (2%), garlic (2.5%), black pepper (0.6%), red pepper (0.5%) salt (3%), cumin (0.4%) and coriander (0.5%). It was then made into various shapes and covered with bread crumbs. The cutlets were then flash fried in vegetable oil. They were packed in aluminum foil, packed in air- tight containers and stored in refrigerator at -12 ±2 ^{o}C. Analytical procedures for biochemical and microbiological changes were done on 0, 10th, 20th and 30th day of storage.

2.3 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 statistical programme.

2.4 Statistical Analysis

Means and standard errors were calculated for different parameters. The data analyses were performed using SPSS software (12.0 for Windows). Differences between treatments were analyzed using independent-measures one-way ANOVA. Post-hoc comparisons were conducted using Duncan's test. The values were expressed as mean ± SE. values <0.05 were considered as significant and p values <0.001were considered as highly significant *p*.

3. Results and Discussions

3.1 Proximate composition

3.2 Protein content: During the present course of investigation, the initial values for the protein on day 0 was observed to be 15.45±0.05% and 19.83±0.05% in frozen raw muscles and fish cutlets of *Wallago attu.* These values showed a decreasing trend reaching upto 9.38±0.% and 17.56±0.% on 30th day of storage. However, the total percental decrease on the last day of storage in fish cutlet is11.44 % which is very low when compared to that of raw fish. I.e. 39.20%. The higher protein content in fish cutlets is attributed to the moisture loss during frying. Similar results are proposed by Garcia-Arias *et al.,* (2003) in cooked sardine Hakimeh *et al.,* (2010) in processed Silver carp, Devi and Sarojnalini (2012) in cooked *Amblypharyngodon*, Pawar *et al.,* (2013) in *Catla catla* fish cutlets who proposed that water losses during frying results in higher protein content in fried fish as compared to fresh fish. Also, this decrease in protein contents of both raw and fish cutlet during the whole storage period could be connected with denaturation of fish proteins during frozen storage. These results are supported by the studies of Gopakumar (2002), Siddique *et al.,* (2011) in *Puntius* and Gandotra *et al.,* (2012) in *Labeo rohita* and Rathod and Pagarkar (2013) in fish cutlets made from *Pangasius* fish, who suggested that loss of protein might be due to denaturation of proteins during frozen storage and the leaching effect of amino acids with melting ice.

Table 1: Proximate composition of raw muscle of *Wallago attu* stored under frozen conditions at -1 ˚C.

DAYS	PROTEIN (%)	LIPID (%)	ASH (%)	MOISTURE (%)
0 day	15.45±0.05	4.15±0.02	1.02±0.02	81.66±0.4
10th day	14.02±0.01	3.11±0.5	0.98±0.01	79.2±0.07
20th day	12.22±0.3	2.05±0.05	0.91±0.05	76.94±0.04
30th day	9.38±0.01	1.10±0.07	0.88±0.05	74.00±0.02

Table 1(1): Percent decrease in proximate composition of raw muscle of *Wallago attu* stored under frozen conditions at -12±2 ˚C.

DAYS	PROTEIN (%)	LIPID (%)	ASH (%)	MOISTURE (%)
0-10th day	9.25±0.05	25.06±0.01	3.92±0.1	3.01±0.05
0-20th day	20.90±0.03	50.60±0.02	10.78±0.03	5.78±0.02
0-30th day	39.2±0.01	60.94±0.03	13.72±0.02	9.38±0.01

Table 2: Proximate composition of fish cutlets of *Wallago attu* stored under frozen conditions at -12±2 ˚C.

DAYS	*PROTEIN* (%)	*LIPID* (%)	*ASH* (%)	*MOISTURE* (%)
0 day	19.83±0.05	8.12±0.02	4.22±0.02	54.66±0.4
10th day	19.22±0.01	7.66±0.5	4.14±0.01	54.10±0.07
20th day	18.05±0.3	7.06±0.05	4.08±0.05	53.82±0.04
30th day	17.56±0.01	6.75±0.07	3.98±0.05	52.85±0.02

Table 2(1): Percent decrease in proximate composition of fish cutlets of *Wallago attu* stored under frozen conditions at -12±2 ˚C.

DAYS	PROTEIN (%)	LIPID (%)	ASH (%)	MOISTURE (%)
0-10thday	3.07±0.05	5.66±0.01	1.89±0.1	1.02±0.05
0-20thday	8.97±0.03	13.05±0.02	3.48±0.03	2.67±0.02
0-30thday	11.44±0.01	16.87±0.03	5.68.56±0.02	3.31±0.01

3.3 Lipid content

As observed from Table-1 and 1(a), the lipid content in both raw muscles and fish cutlets revealed a decreasing trend. It decreased from 4.15% at day 0 to 1.10% at day 30 in raw fish and from 8.12% at day 0 to 6.75% at day 30 in fish cutlets. The higher lipid content in fish cutlets on day 0 is related to the oil absorption after partial evaporation of water during frying (Ninan *et al.*, 2008, Rathod and Pagarkar, 2013, Talab, 2013 and Khanipour *et al.*, 2014).

Similarly, the decreasing lipid trend has been reported by Siddique *et al.,* (2011) in *Puntius sps*., Aberoumand (2013) while studying the nutritional status of some less known Iranian fishes and Gandotra *et al.*. (2014) in ice glazed Labeo rohita. They opined that this reduction in percentage fat is associated with oxidation of the fat (McGill *et al.,* 1974; Josephson, 1989).

Also, the fish cutlets reported a lower percental decrease (16.87%) as compared to raw fish muscle (60.94%) on 30th day of storage. This may be attributed to the antioxidant

effects of spices viz. garlic, ginger, onion and cumin etc. added in minced muscles during preparation of fish cutlets which reduced the lipid oxidation process. (Gokoglu *et al.,* 2011 and Frank *et al.,* 2014).

3.4 Ash content
The ash content also followed a decreasing trend in both raw muscles and fish cutlets as observed from Table 1 and 1(a). However, the higher ash content in fish cutlets could be due to the addition of salts and moisture loss (Kocatepe, 2011, Devi and Sarojnalini, 2012 and Talab, 2013).
Also, the decrease in ash content during the storage period could be due to the drip loss resulting in loss of bulk and trace elements (Gandotra *et al.,* 2014).

3.5 Moisture content
The moisture content in day 0 was found to be 81.66% and 54.66% and it decreased upto 74% and 52.85% in raw and fish cutlets respectively. These results are in accordance with the studies of Hakimeh *et al.,* 2010 in Silver carp, Devi and Sarojnalini, 2012in *Amblypharyngodon mola*, Pawar *et al.,*2013 in *Catla*, Talab, 2013 in fish cutlets who also observed a decrease in moisture content of fish after frying. It may be attributed to the heating effect that resulted in moisture loss during cooking.

4. Biochemical composition
4.1 Thiobarbituric acid (TBA)
TBA value is an indicator of degree of lipid oxidation. A steady increase in TBA was observed in both raw muscles and fish cutlets. It increased from 0.17±0.05 to 10.56±0.01 mg MA/kg and from 0.15±0.03 to 2.02±0.02 mg MA/kg in raw and fish cutlets respectively during 30 days of frozen storage period. Similar results have been proposed by 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. 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, Coban, 2013 and Frank *et al.,* 2014).

Table- 3: Change in bio-chemical composition of raw and fish cutlets of *Wallago attu* during frozen storage (-12±2 ˚C).

DAYS	TBA		FFA		pH	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
0 day	0.17±0.05	0.15±0.03	0.88±0.02	0.56±0.05	6.3±0.05	6.4±0.05
10th day	5.95±0.01	0.98±0.02	4.91±0.1	1.12±0.01	6.7±0.05	6.4±0.04
20th day	8.25±0.3	1.34±0.02	9.25±0.05	1.95±0.05	7.1±0.05	6.5±0.02
30th day	10.56±0.01	2.02±0.02	11.02±0.07	2.15±0.02	7.4±0.05	6.6±0.02

4.2 Free fatty acids (FFA)
FFA are formed through chemical or enzyme linked hydrolysis of triacylglycerides and are expressed as oleic acid per 100 gm of oil. It is an important reaction that indicates the post mortem changes occurring in fish lipids (Chaijan *et al.,* 2006). Change in FFA values of raw muscles and fish cutlets of *Wallago attu* during 30 days of frozen storage period is given in Table-2. FFA in raw fish muscle increased from 0.88±0.02% to 11.02±0.07% while in fish cutlets, it depicted a lower increase i.e. from 0.56±0.05% to 2.15±0.02% during the 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 and Vanitha *et al.,* (2013) in minced based products of Catla who proposed that oxidative hydrolysis of lipids during frozen storage result in the formation of FFA, thus deteriorating the quality of meat. However, the lower FFA formation in fish cutlets is due to the antioxidant effect of spices added. In accordance to our results, Gokoglu *et al.,* (2011) observed a lower FFA formation in marinated Anchovy treated with tomato and garlic. Frank *et al.,* (2014) also reported the effectiveness of garlic and ginger as antioxidants in inhibiting the synthesis of FFA.

4.3 pH
The present study depicted an increase in pH of both samples viz. raw and fish cutlets. It increased from 6.3±0.05 to 7.4±0.05 in raw and from 6.4±0.05 to 6.6±0.02 in fish cutlets. Similar trend has been observed by Rathod and Pagarkar (2013) in *Pangasius* fish cutlets, Coban, (2013) in fish fingers (*Sarda sarda*), Talab, (2013) in fish cutlets of Common carp. It may be attributed to the decomposition of nitrogenous components in post mortem period which increases the pH (Bett and Dionigi, 1997). Dhanpal *et al.,* (2012) associated this increased pH to the breakage of hydrogen bond and electrostatic interactions.

4.5 Microbial changes
The changes in TPC of both frozen raw and fish cutlets of *Wallago attu* during 30 days of frozen storage period is given in fig.-1. The results show a lower value for TPC in fish cutlets as compared to the raw fish muscle during the whole frozen storage period. In frozen raw muscles, the values for TPC increased from 3.44 lof cfu/g on day 0 to 8.55 lof cfu/g on 30th day of frozen storage, thus crossing the permissible limits of 6 lof cfu/g after 10th day of storage. However, in case of fish cutlets, the values increased from 1.1 lof cfu/g on day 0 to 2.8 lof cfu/g on day 30 and hence were within the acceptable limits upto the final storage period. Similar results has been reported by Talab, 2013 in fish cutlets of Common carp who attributed it to the antimicrobial properties of food additives. Koch and Lawson (1996) reported that garlic have sulfur-containing compounds (A. Allicin, A. Alliin and allinase enzymes) which are responsible for antimicrobial properties. Idris et al... 2010 reported a lower microbial count in smoked catfish treated with ginger. Similarly, Hassanin and El- Daly (2013) related the lower microbial count in frozen Nile Tilapia (*Oreochromis niloticus*) to the synergistic antimicrobial activity of garlic and propolis. Frank *et al.,* (2014) also observed a lower microbial count in garlic and ginger treated smoked Silver carp and attributed this result to the antimicrobial activity of organ sulphur compounds and other active compounds present in garlic and ginger (Lu *et al.,* 2011).

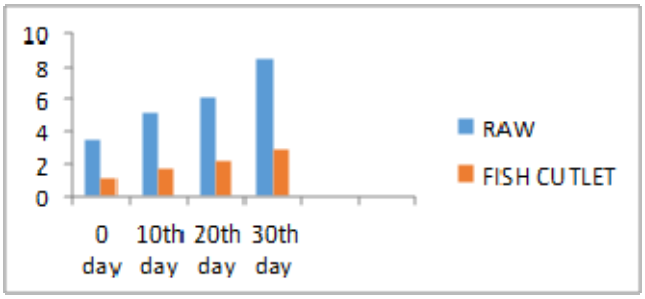

Fig.-1: Comparative changes in Total plate count (TPC) in raw and fish cutlets of *Wallago attu* during frozen storage (-12±2 ˚C).

5. Conclusion

From the present study it is concluded that the value added fish products containing natural spices have longer shelf life as well as provide a healthy ready to eat food to the consumers. The natural spices with their antioxidant and antimicrobial properties reduce the rancidity in fish muscles and hence their use as natural additive in fish products is recommended over the synthetic ones to enhance the shelf life of fish products.

6. Acknowledgement

The authors wish to express their profound gratitude to Dr. Vikas Gupta and the faculty of Deptt. Of Zoology, University of Jammu, Jammu for their full financial support and other facilities during the practical work.

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