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EFFECT OF NATURAL PRESERVATIVES ON PROXIMATE COMPOSITION OF PRESERVED SEA FISH, PROCESSED UNDER TRADITIONAL COOKING METHODS

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Fish contains high quality of protein with essential amino acids which is important for growth and development and thus are commonly consumed. This study aims to compare the proximate composition of two easily available sea fish, i.e., pomfret (*Pampus argenteus*) and hilsa (*Hilsa ilisha*) preserved with the natural preservative, i.e., combination of salt and turmeric and without preservative both up to 15 days. These preserved fishes were subjected to traditional cooking methods such as open pan dry roasting, boiling, shallow frying and deep frying and macro nutrient contents were analyzed after immediate (24 hours) and long term (15 days) preservation. The nutrient contents significantly decreased in the cooked fishes than those in the raw condition. The changes of the nutrient content occurred due to cooking as it involves the application of heat, oil, water. Boiling caused more reduction of nutrients, whereas it is better restored in deep fat frying method. The study conclude that the elimination of nutrients occurred due to both the preservation as well as the cooking conditions but these losses can be diminished by the use of the combination of salt and turmeric. It was also found that among the two sea fish, restoration was better in pomfret.

Keywords: Sea fish, Macro nutrients, Cooking methods, Natural preservatives, Deep fat frying

INTRODUCTION

It is well known that fish contain adequate macro and micro nutrients especially it is consumed as a rich source of protein. But it is needed to be cooked for consumption and sometimes raw fish is preserved for further use. Some previous studies showed that the preservation and cooking can change the nutrient contents. A study was carried out in Nigeria, where commonly available three species of marine fish were cooked by the methods of boiling, frying and roasting and the effects of these cooking methods on the fish were observed, which indicated that the protein contents were reduced for all the species (Goldman, 1997). Another research was carried out in Turkey on the determination of amino acid and proximate compositions in six easily available raw and cooked marine fish. The amino acid and proximate contents were significantly changed for all the cooking methods in all the studied species of fish (Erkan *et al.*, 2010). Another research showed that cooking methods were applied for vegetable samples which could also be relevant for this present study. Three cooking methods, such as boiling, steaming and stir-frying were used to determine the effect on nutrient components of bamboo shoots, resulting in decrease in protein, soluble sugar and ash content. It was noted that boiling caused a significant loss in the total free amino acids content. All cooking procedures were carried out for 10 minutes (Mkandawire and Masamba, 2014). In yet another research, the effects of different cooking methods, namely boiling, baking, frying and grilling were

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investigated on the macro and micro nutrient composition of snakehead fish. The changes of protein and fat content were significantly higher in fried and grilled fish (Marimuthu *et al.*, 2012). Another study was designed to observe the effect of natural preservatives, i.e., turmeric and salt on *tengra* (Mystus vittatus) for several days, stored with preservatives of 2% of the sample weight, resulting in nutrient restoration (Akter *et al.*, 2013). In a study of Bangladesh, the protective effect of salt and turmeric was determined on the biochemical and quality analysis of sun dried salted and turmeric treated sun dried salted *tengra* (Mystus tengra). The result showed that the fish was highly accepted in salt-turmeric preservation and the quality was also maintained (Farid *et al.*, 2015). The significance of the present study lies in the fact that the macro nutrient contents of sea fish are estimated post preservation for few days and then these were subjected to different conventional cooking methods. Preservation can reduce the nutrient content; however, the amount of macro nutrients can be restored by using a combination of salt and turmeric for 15 days of preservation.

- This study aims to estimate the macro nutrient contents of preserved sea fish in both the raw and the cooked conditions post preservation for 24 hours and 15 days with and without preservative.
- This study also aims to compare the nutrient contents of preserved and cooked sea fish with and without preservative.
- To find out the deterioration level of preserved fishes in the perspective of the nutrient contents.
- The most important aspect of this study is to identify the best fish and the best cooking method in respect of nutrient restoration.

MATERIALS AND METHODS

Sample Preparation and Cooking

Pomfret (*Pampus argenteus*) with a length of 12 cm and weight of 250-300 g and *Hilsa (Hilsa ilisha)* with a length of 25-30 cm and weight of 1 kg were bought from the local fish market in Kolkata. They were stored in a plastic container, transported to the laboratory (University of Calcutta, Laboratory of Food Science) and washed with tap water several times to remove the surface dirt, blood, mucous and scales. At first the fish sample was filleted into three sections and each section was divided into five groups. In the first section one group was left uncooked while the other four were boiled, dry roasted in open pan, shallow fried and deep fried. Boiling was performed at 99-101 °C (water temperature) for 10 minutes. Open pan dry roasting of fillets was carried out in a pan at 180 °C for 10 minutes. The frying of fillets was done in a domestic frying pan of 2 L capacity at a temperature of approximately 180 °C for 10 minutes. Mustard oil was used as the medium for frying. In case of shallow frying, 10 ml oil was used and for deep frying 20 ml. The other two sections were allowed to preserve up to15 days. Among these two sections, one was preserved in the refrigerator at -20 °C without preservative and the other section was preserved at deep freezer with the combination of salt and turmeric. The amount of combination of salt and turmeric was used as 2% of the weight of the fish sample preserved in 1:1 ratio. The raw, fresh and preserved samples were then subjected to analysis post cooking on the 1st and 15th days of preservation (Jana and Chakrabarti, 2016).

Proximate Composition Analysis

Both raw and cooked fresh as well as preserved fish fillets were homogenized properly. After that they were put forward for triplicate analyses of proximate composition, i.e., carbohydrate, protein and fat contents.

Estimation of carbohydrate by Anthrone Method (Ludwig and Goldberg, 1956)

In a test tube 100 mg of the sample was taken and it was placed in a boiling water bath for three hours with 5 mL of 2.5 NHCl and allowed to be hydrolyzed. Then it was cooled to room temperature. Following cooling it was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifugation was done at 3000 RPM for 15 minutes. 1 ml was used from the collected supernatant, for analysis. 4 ml anthrone was added to this solution and it was heated for eight minutes in a boiling water bath and then cooled rapidly when a green to dark green colour appeared. The reading was then measured at 630 nm in a spectrophotometer (Perkin Elmer Lambda 25).

Estimation of Protein by Lowry Method (Lowry et al., 1951)

20 ml of buffer composed with sodium dihydrogen phosphate and disodium hydrogen phosphate was added to 200 mg of sample. It was then homogenized finely and kept overnight. Following this, cold centrifugation was carried out at 5000 RPM for 20 minutes. Then for analysis, 1 ml was used from the collected supernatant. The

supernatant was allowed to incubate for 10 minutes after adding 5 ml of the Lowry reagent in it. After that 0.5 ml of Folinciocaltue reagent was added and incubated again for 30 minutes until a dark blue colour appeared. The reading was measured at 660 nm in a spectrophotometer (Perkin Elmer Lambda 25).

Estimation of Fat by Soxhlet Extraction Method (Manirakiza et al., 2001)

The thimble of the apparatus was packed up with 5gm of dried sample. Then the distillation flask was filled up with the extraction solvent petroleum ether of 60-80 boiling range and it was placed on the heating mantel. The solvent was heated to reflux when the solvent vapour travelled up a distillation arm and flooded into the chamber housing the thimble of solid. The condenser ensured that the solvent vapour cooled and dripped back down into the chamber housing containing the solid material and slowly filled with warm solvent. The solvent which returned to the distillation flask helped to dissolve the fat present in the sample. This cycle was allowed to repeat for 12 hours. After completion of this procedure when fat was completely extracted in the solvent, it was poured into a weighed petridish and the solvent was allowed to be evaporated and the final weight of the petridish containing fat was taken. From this the amount of the fat was calculated.

Statistical Analysis

Mean and Standard Deviations were used to analyze the effect of different cooking methods on macro-nutrient contents of fresh and preserved sea fish. The nutrient contents in respect to cooking methods among preserved fishes with and without preservative were compared by using One way ANOVA. Differences were considered to be significant when P value was <0.05. Data were analyzed by using SPSS package (Version 17).

DISCUSSION

Tables 1, 2 and 3 exhibited the carbohydrate, protein and fat contents of fresh and preserved pomfret with and without preservative up to 15 days after subjecting it to different cooking methods. The raw and cooked values were displayed here. The nutrient contents were reduced due to the application of different cooking methods. The highest loss occurred in boiling and it was found to be restored in deep fat frying method. The result also showed that the

Table 1: Carbohydrate Content of Fresh and Preserved Pomfret with and Without Preservative (gm/100 gm)

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
Fresh	2.17± 0.17	1.42±0.07	1.74± 0.11	1.90± 0.10	2.14± 0.19
24 hours	1.58±0.18	0.95±0.12	1.17±0.06	1.41±0.03	1.85±0.11
15 days	2.63±0.11	1.33±0.12	2.59±0.09	2.60±0.07	4.33±0.10
24 hours (S+T)	6.01±0.07	2.90±0.05	1.58±0.07	2.73±0.07	3.07±0.07
15 days (S+T)	4.19±0.11	2.08±0.08	2.08±0.07	2.76±0.04	3.20±0.05

Note: Restoration; Loss.

Table 2: Protein Content of Fresh and Preserved Pomfret with and Without Preservative (gm/100 gm)

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
Fresh	16.36±0.31	4.22±0.20	6.24±0.04	6.82±0.02	7.93±0.12
24 hours	15.06± 0.05	3.36± 0.05	5.74± 0.10	5.97± 0.02	7.42± 0.19
15 days	7.50±0.25	6.66±0.33	11.04±0.52	10.43±0.43	10.00±0.55
24 hours (S+T)	5.58 ±0.38	3.33± 0.33	7.05± 0.27	6.95± 0.43	8.10± 0.29
15 days (S+T)	7.50±0.25	3.33±0.33	8.40±0.55	6.95±0.43	7.75±0.25

Note: Restoration; Loss.

macro nutrient content was increased in pomfret, preserved with salt-turmeric, which indicated that nutrient losses can be prevented by the use of natural preservatives.

Tables 4, 5 and 6 showed the carbohydrate, protein and fat contents of *hilsa* preserved with and without preservative for 15 days. Results indicated that there was significant difference present between the raw and cooked values of the macronutrients. Carbohydrate content reduced mainly in open pan dry roasting, whereas protein content mainly lowered in boiling. The fat content was diminished in deep fat frying method. Application of salt and turmeric for preservation of *Hilsa* was not very effective as the nutrient content decreased after long term preservation though it was preserved with salt and turmeric.

Tables 7, 8 and 9 exhibited the comparison of carbohydrate, protein and fat contents of pomfret and *hilsa*, preserved with and without preservative. Result showed that there was a significant difference present between the two sea fish in respect of different cooking methods along with both short and long term preservation.

Table 3: Fat Content of Fresh and Preserved Pomfret with and Without Preservative (gm/100 gm)

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
Fresh	1.71±0.09	2.78±0.07	5.78±0.08	5.43±0.04	4.47±0.04
24 hours	5.59± 0.08	6.25± 0.05	2.42±0.03	8.43± 0.04	5.15± 0.05
15 days	13.07±0.03	8.83±0.04	11.76±0.07	12.59±0.03	7.38±0.04
24 hours (S+T)	19.40±0.18	21.26±0.05	14.40±0.18	16.92±0.03	18.82±0.03
15 days (S+T)	21.95±0.04	9.69±0.07	13.88±0.08	15.65±0.05	9.06±0.05

Note: Restoration; Loss.

Table 4: Carbohydrate Content of Fresh and Preserved *Hilsa* with and Without Preservative (gm/100 gm)

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
Fresh	3.59±0.14	2.37±0.15	1.72±0.14	2.30±0.10	3.25±0.10
24 hours	1.52± 0.13	1.03± 0.10	0.99± 0.17	1.22± 0.14	1.17± 0.07
15 days	1.72±0.08	2.40±0.10	1.27±0.08	2.05±0.05	2.50±0.08
24 hours (S+T)	2.74±0.09	1.45±0.05	0.58±0.07	1.25±0.05	2.00±0.05
15 days (S+T)	3.25±0.05	0.65±0.05	1.65±0.05	1.75±0.05	2.62±0.13

Note: Restoration; Loss.

Table 5: Protein Content of Fresh and Preserved *Hilsa* with and Without Preservative (gm/100 gm)

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
Fresh	24.07±0.11	6.58±0.20	9.64±0.18	10.73±0.17	11.92±0.11
24 hours	20.53± 0.04	3.92± 0.09	6.10± 0.02	6.56± 0.12	7.90± 0.12
15 days	11.42±0.87	4.84±0.41	4.09±0.19	2.96±0.37	3.85±0.30
24 hours (S+T)	11.42±0.52	3.06±0.83	2.79±0.49	3.24±0.25	5.50±0.50
15 days (S+T)	5.25±0.25	2.80±0.40	2.98±0.44	3.36±0.40	3.50±0.50

Note: Restoration; Loss.

Table 6: Fat Content of Fresh and Preserved *Hilsa* with and Without Preservative (gm/100 gm)

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
Fresh	13.21±0.17	13.12±0.10	6.28±0.03	6.14±0.04	3.62±0.04
24 hours	26.71± 0.30	13.20± 0.16	6.43± 0.04	8.16± 0.13	5.37± 0.03
15 days	29.10±0.10	20.52±0.03	14.89±0.09	13.18±0.03	9.64±0.06
24 hours (S+T)	23.45±0.05	22.72±0.08	9.24±0.01	18.60±0.10	11.20±0.12
15 days (S+T)	21.98±0.04	20.37±0.06	14.62±0.10	16.10±0.10	10.81±0.10

Note: Restoration; Loss.

Table 7: Comparison of Carbohydrate Content of Preserved Pomfret and *Hilsa* with and Without Preservative

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
24 hours	0.638(NS)	0.468(NS)	0.150(NS)	0.038(S)	0.001(S)
15 days	0.000(S)	0.000 (S)	0.000(S)	0.000(S)	0.000(S)
24 hours (S+T)	0.000(S)	0.000(S)	0.000(S)	0.000(S)	0.000(S)
15 days (S+T)	0.000(S)	0.000(S)	0.001(S)	0.000(S)	0.002(S)

Note: P-value = <0.05 = significantly different; S = significant, NS = Non significant.

Table 8: Comparison of Protein Content of Preserved Pomfret and *Hilsa* with and Without Preservative

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
24 hours	0.000(S)	0.001(S)	0.004(S)	0.001(S)	0.002(S)
15 days	0.002(S)	0.004(S)	0.000(S)	0.000(S)	0.000(S)
24 hours (S+T)	0.000(NS)	0.637(NS)	0.000(S)	0.000(S)	0.001(S)
15 days (S+T)	0.000(S)	0.151(NS)	0.000(S)	0.000(S)	0.000(S)

Note: P-value = <0.05 = significantly different; S = significant, NS = Non significant.

Table 9: Comparison of Fat Content of Preserved Pomfret and *Hilsa* with and Without Preservative

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
24 hours	0.000(S)	0.000(S)	0.000(S)	0.022(S)	0.002(S)
15 days	0.000(S)	0.000 (S)	0.000(S)	0.000(S)	0.000(S)
24 hours (S+T)	0.000(S)	0.000(S)	0.000(S)	0.000(S)	0.000(S)
15 days (S+T)	0.544(NS)	0.000(S)	0.001(S)	0.002(S)	0.000(S)

Note: P-value = <0.05 = significantly different; S = significant, NS = Non significant.

Tables 10, 11 and 12 showed the percentage of loss of carbohydrate, protein and fat contents after cooking of the preserved pomfret with and without preservative in respect to the fresh values. Result indicted that the deterioration of macronutrient content occurred mainly in 24 hours of preservation without preservative, but it can be reduced by

Table 10: Percentage of Loss of Carbohydrate in Preserved Pomfret Without Preservative in Comparison to Fresh

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
24 hours	27.18	33.09	32.75	25.79	13.55
15 days	-21.2	6.33	-48.85	-36.84	-102.34
24 hours (S+T)	-176.95	104.23	9.2	-43.68	-43.46
15 days (S+T)	-93.08	-46.47	-19.54	-42.26	-49.53

Note: Restoration.

Table 11: Percentage of Loss of Protein in Preserved Pomfret with Preservative in Comparison to Fresh

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
24 hours	7.94	20.38	8.01	12.46	6.43
15 days	54.15	-57.82	-76.92	-52.93	-26.1
24 hours (S+T)	65.89	21.09	-12.98	-1.91	-2.14
15 days (S+T)	54.15	21.09	-34.62	-191	2.26

Note: Restoration.

Table 12: Percentage of Loss of Fat in Preserved Pomfret with Preservative in Comparison to Fresh

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
24 hours	-226.9	-124.82	58.13	-55.25	-15.21
15 days	-664.63	-217.63	-103.46	131.86	-65.1
24 hours (S+T)	-1034.5	-664.75	-149.13	-211.6	-321.03
15 days (S+T)	-1183.63	-248.56	-140.14	-188.21	-102.68

Note: Restoration.

Table 13: Percentage of Loss of Carbohydrate in Preserved *Hilsa* with Preservative in Comparison to Fresh

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
24 hours	57.66	56.54	42.44	46.95	64
15 days	52.09	-1.27	26.16	10.87	23.08
24 hours (S+T)	23.67	38.81	66.27	45.65	38.46
15 days (S+T)	9.47	72.57	4.07	23.91	19.38

Note: Restoration; Reduced percentage of loss.

the application of salt and turmeric up to 15th day of preservation.

Tables 13, 14 and 15 exhibited the deterioration percentage of carbohydrate, protein and fat contents post cooking of the preserved *hilsa* with and without preservative in respect to fresh values. Result showed that carbohydrate losses mainly occurred in 24 hours of preservation without preservative, whereas fat and protein loss occurred in mainly

Table 14: Percentage of Loss of Protein in Preserved *Hilsa* with Preservative in Comparison to Fresh

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
24 hours	14.7	40.43	35.52	38.86	33.72
15 days	52.55	26.44	57.57	72.41	67.7
24 hours (S+T)	52.55	53.5	71.06	69.8	53.86
15 days (S+T)	78.19	57.45	69.09	68.69	70.63

Note: Reduced percentage of loss.

Table 15: Percentage of Loss of Fat in Preserved *Hilsa* with Preservative in Comparison to Fresh

Duration	Raw	Boiling	Dry Roasting	Shallow Frying	Deep Frying
24 hours	-102.2	-0.61	-2.39	-32.9	48.34
15 days	-120.29	-56.4	-137.1	-114.66	-166.3
24 hours (S+T)	-77.52	-73.17	-47.13	-202.93	-209.39
15 days (S+T)	-66.39	-55.26	-132.8	-162.21	-226.24

Note: Restoration.

15 days of preservation with salt and turmeric. Nutrient content reduced in both short and long term preservation but the use of preservative was not significantly effective for the restoration of nutrient contents of preserved *Hilsa*.

Figure 1 showed the loss percentage of carbohydrate contents following cooking of the preserved and cooked sea fish in respect to the raw values. It was estimated that boiling caused more losses in Pomfret, whereas less in deep fat frying. On the other hand Open pan dry roasting caused more losses in *Hilsa*, whereas less in deep frying. The data indicated that losses of macronutrients were more in *hilsa* rather than *in* pomfret. In case of pomfret the nutrient loss were restored by the use of natural preservative salt and turmeric but this was not very much effective for *hilsa*.

Figure 2 exhibited the percentage of loss of protein contents post cooking of the sea fish cooked and preserved with and without preservative. Data indicated that boiling caused most of the losses and it was better restored in deep

Figure 1: Percentage of Loss of Carbohydrate in Cooked Pomfret and *Hilsa* Preserved with and Without Preservative

Figure 2: Percentage of Loss of Protein in Cooked Pomfret and *Hilsa* Preserved with and Without Preservative

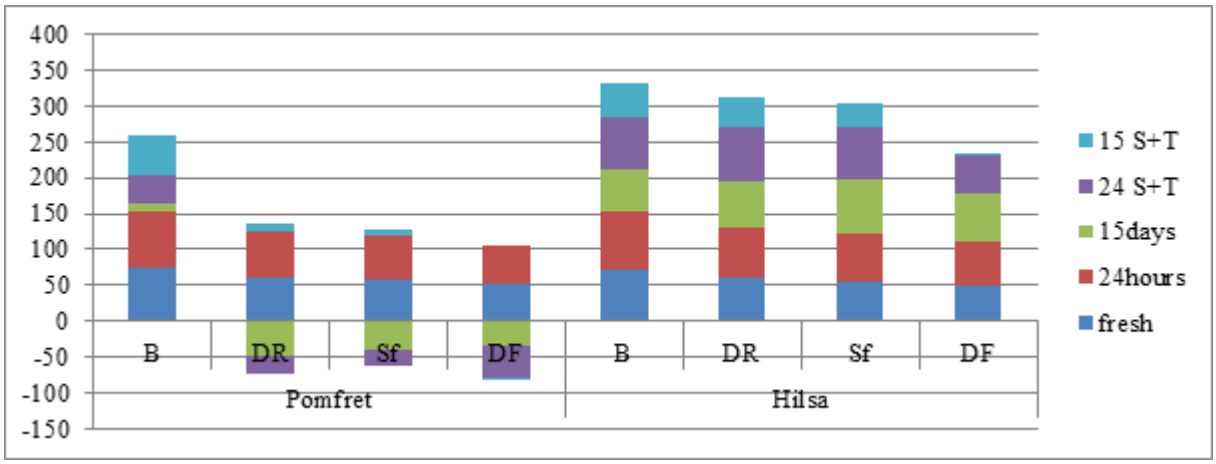
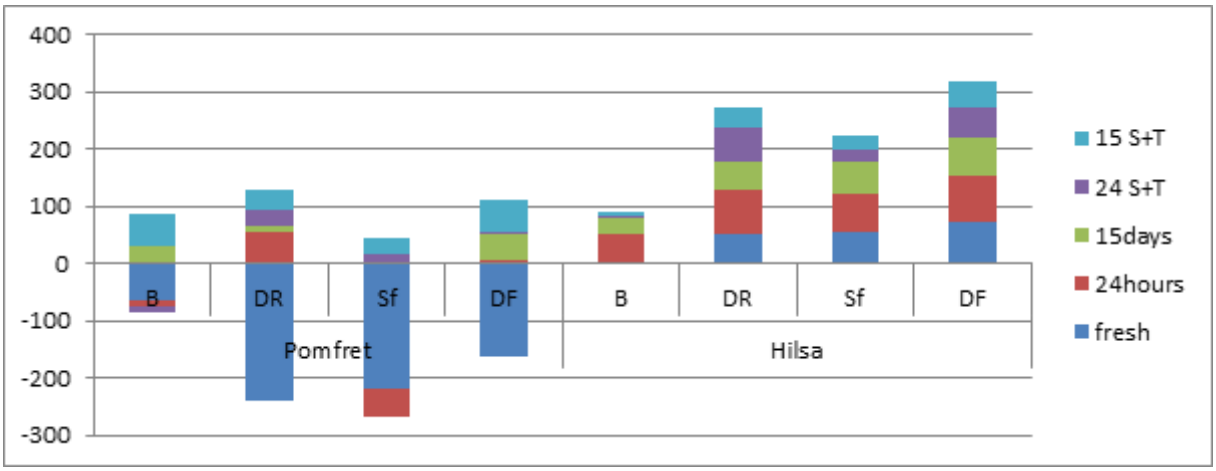


Figure 3: Percentage of Loss of Fat in Cooked Pomfret and *Hilsa* Preserved with and Without Preservative



fat frying for both the fish. But the comparison showed that losses were more in *hilsa* than in pomfret. The protein loss can be restored by the addition salt and turmeric for pomfret but the preservatives were not very effective for *hilsa*.

Figure 3 exhibited the loss percentage of fat contents after cooking of the sea fish preserved with and without preservative and cooked under different conventional methods. Result showed that very less amount of fat was lost, rather fat content increased due to the addition of cooking oil and loss of the water from the fish due to the application of heat during cooking. In case of Pomfret the fat content increased in all the cooking methods except boiling, whereas it was reduced mainly in deep fat frying method in case of *Hilsa*. The fat content was restored more in pomfret than in *hilsa*.

CONCLUSION

All cooking methods can reduce the nutrient contents of the sea fish. The maximum restoration of the macronutrient content was found to be occurring mostly in deep fat frying method while the loss occurred in boiling method. After preservation of the fishes up to 15th days, the deterioration of nutrient content occurred in 15th days but this deterioration could be reduced to some extent with the use of natural preservative, i.e., combination of salt and turmeric, which help to restore nutrient contents up to 15th days of

preservation. Maximum restoration occurred in 24 hours of salt-turmeric preservation for both the fishes. The study concludes that, that deep fat frying is the best method for the restoration of all the macro nutrients among the other cooking methods and the use of salt and turmeric can delay this deterioration post cooking for both the fish. But long term preservation caused more loss of protein in *hilsa*, which could not be reduced by the use of preservative, thus natural preservatives were not very much effective for preserving *hilsa* while these were effective for pomfret. The study also found that among the two sea fish, pomfret is better than *hilsa* in respect of nutrient restoration, both post preservation and cooking.

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