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Physicochemical and Functional Properties of Frozen Stored Minced Meat of Nile Tilapia (*Oreochromis niloticus*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The physicochemical and functional properties of frozen stored Tilapia (*Oreochromis niloticus*) minced meat were analysed. The organoleptic attributes of mincemeat has been analysed as well as the physicochemical parameters were colour, pH, proximate composition, PV, FFA, TMA-N, TVB-N, TBA were measured. The microbiological and functional parameters analysed were TPC, water holding capacity, cook loss and emulsion stability. The storage period was for 45 days and each time analysis had been conducted in a week time interval. The frozen storage has a major effect on the mincemeat and there was significant difference in all the parameters ($p<0.05$). The organoleptic quality of the mincemeat was also acceptable till the end of frozen storage period.

Keywords: Mincemeat; frozen storage; tilapia; value added products.

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1. INTRODUCTION

The total world aquaculture production in 2018 is around 114.5 MT and India ranks second with annual fish production of 9.06 MMT [1]. Tilapia as a raw material for mince preparation has shown better results. The species is easy to grow, hardy and white-fleshed. It has given a better taste to consumers. Nile tilapia was introduced at Thailand in 1965. Because of their palatability and rapid growth, tilapia is considered as one of the most important and widely grown groups of farmed fish. A low fat and high protein content fish gives a good quality of gel emulsion and analog products [2]**.** It also has a wide availability throughout India and minced meat is highly for the consumption as well as research purpose.

The minced meat technology has opened a wide way in modernizing fish processing equipment and new technologies. It increased the utilization of underutilized and low value fishes [3]. Generally we know minced meat is a flesh taken out skin and body of fish by removing scales, bones and fins of fish. It has given a new idea for utilization of fish protein in human food [4]**.** The mince can be utilized for the production of various products like cutlets, fish balls and chilly fish are simple and a cost effective means of converting tilapia to value-added convenience products [5]. The minced meat technology has reduced the fish wastes, using existing resources, production of new nutritious foods that gives an ultimate advantage to both producer and consumer also. It is also used widely as a raw material for preparation of huge number of value-added products such as, cakes, patties, balls, pastes, texturized products, fish sausage, cutlets etc.

Several authors have worked on the frozen storage studies of tilapia has also been reviewed. The mince from Nile tilapia and red tilapia were found to be more physically stable and in good condition after 180 days in frozen storage [6]. Ninan et al. [5] also studied the frozen storage activity of mince based products from tilapia whereas Subbaiah et al. [7] measured the protein degradation and instrumental texture changes in Nile tilapia during frozen storage. The main objective of this study is to measure the physicochemical, microbiological and functional changes in the mincemeat of Nile tilapia during the 45 days of frozen storage period taken for analysis. The organoleptic attributes have also been discussed to observe the shelf life of the mincemeat of the species.

2. MATERIALS AND METHODS

2.1 Fish Sample Collection

Tilapia (*Oreochromis niloticus*) brought from Cochin local market and transported to Laboratory in iced condition and the minced meat of the fish was obtained.

2.2 Preparation and Processing of Fish Mince

The fish mince has been taken out by beheading and eviscerating in deboning machine. The mince was stored in freezer in several airtight pouches at -20 degree Celsius which was further taken for analysis purpose. The analysis was conducted for 45 days at a week time interval. Every time, the labelled pouches of different days were taken out and analysed.

2.3 Proximate Composition of Fish Paste

The proximate composition of fish meat includes moisture, fat, protein and ash content were measured according to AOAC, 2005.

2.4 Physical Analysis

2.4.1 Physical sensory quality of mincemeat of tilapia during frozen storage

2.4.1.1 Colour analysis

The colour of fish meat was determined by colorimeter (Hunter Lab Scan XE USA). The L* (lightness), a*(redness/greenness) and b* (yellowness/blueness) were measured and whiteness was calculated as described by Park [8] as follows:

$$\text{Whiteness} = 100-[(100-L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

2.4.1.2 Sensory parameters

The sensory parameters include texture, appearance, odour, colour, flavour and overall acceptability of frozen stored tilapia mincemeat. It has been measured by a panel of ten experts on the basis of 10-point hedonic scale. The attributes were analysed on a week interval for a period of 45 days.

2.5 Biochemical Analysis

2.5.1 Protein degradation products during the frozen storage

2.5.1.1 pH measurement

The pH of fish minced meat will be measured by Chattopadhyay et al. [9] throughout the frozen storage period. 10g of fish paste will be mixed with 50ml distilled water (1:5 w/v) in a homogenizer for 45 sec at 11000 rpm and the pH value of the homogenate will be measured with a pre standardized pH meter.

2.5.1.2 Determination of TMA-N & TVB-N of mincemeat during frozen storage

The TMA-N content of fish samples during frozen storage was determined according to Beatty and Gibbons [10]. The TVB-N content of fish samples during frozen storage was determined according to Beatty and Gibbons [10].

2.5.2 Lipid Oxidation products during the frozen storage

2.5.2.1 Determination of Free Fatty Acids (FFA)

The FFA content of fish samples during frozen storage was determined according to the titrimetric method of Takagi et al. [11].

2.5.2.2 Determination of Peroxide Value (PV)

Oxidative stability of frozen stored fish mince was measured using titrametric determination of the amount of peroxide or hydro-peroxide groups, the initial product of lipid oxidation (peroxide value). The peroxide value was expressed as milli equivalent of O_2/kg fat [12].

2.5.2.3 Determination of Thiobarbituric acid (TBA)

The Thiobarbituric acid value will be determined colorimetrically by the method described by Kirk & Sawyer [13].

2.5.3 Protein and Lipid functional properties during the frozen storage

2.5.3.1 Water holding capacity (WHC)

The water holding capacity of the samples will be measured according to the method given by Verbeken et al. [14].

$$WHC = (W_2/W_1) \times 100$$

Where, W_2 = weight of sample after centrifugation
W_1 = weight of sample before centrifugation

2.5.3.2 Cook loss

It is calculated by the method given by Chattopadhyay et al. [9].

$$\text{Cook Loss} = \frac{\text{initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

2.5.3.3 Emulsion stability

The emulsion stability of fish meat will be measured according to the method given by Huang et al. [15]. 30g of fish sample will be taken and centrifuged for 30 min at 4^0C. After centrifugation supernatant containing exudates will be drained and weighed again.

$$\text{Emulsion stability} = (W_2 / W_1) \times 100$$

Where, W_1 = Weight of the sample before centrifugation
W_2 = Weight of the sample after centrifugation

2.6 Microbiological Quality Analysis during Frozen Storage

2.6.1 Total plate count (TPC)

Total plate count (TPC) of the mincemeat of frozen muscle was determined using tryptone glucose beef extract agar (TGA). The plates were incubated at 28 + 2°C (RT) for 48 h and counts taken.

2.7 Statistical Analysis

The parameters were analysed in triplicate using ANNOVA in the statistical software SPSS Version 16.0 and SNK test for post-hoc analyses having the value of α=0.05 (95% level of significance).

3. RESULTS AND DISCUSSION

3.1 Proximate Composition

The proximate composition of tilapia minced meat includes moisture, fat, ash and protein content. The moisture content of mince was 73.083±0.188 %. The fat, ash and protein content was analysed on dry weight basis as 8.1±0.141 %, 3.85±0.212 %, 54.417±1.341 % respectively. Vidya Sagar Reddy and Srikar [16] also found a similar result on the frozen stored pink perch mince. The fat, ash and protein content were also analysed on the wet weight basis as 2.193 %, 1.036 % and 14.804% respectively.

3.1.1 Changes in physical sensory properties of frozen mincemeat

3.1.1.1 Changes in the colour of minced meat (Instrumental colour analysis)

The instrumental colour profile of frozen stored minced meat includes lightness (L*), redness or blueness (a*) and yellowness or greenness (b*). The changes in colour parameters (L*, a* and b*) and whiteness index are shown in Fig. 1, 2, 3 and 4 respectively. There was a significant increase in L* and b* values where a decreased pattern was shown by a* values. By calculating the whiteness index, there was a considerable increase in the values as the storage period progressed.

The L*, a* and b* values of fish meat were 50.35, 2.93 and 16.90 respectively. Murthy et al. [17] also did not found any significant changes in the colour of whole and gutted ice stored milkfish with respect to the storage period. Arannilewa et al. [18] studied on the colour changes of frozen tilapia and found that there were slight changes in colour of the fish samples as there was a temperature fluctuation effect on the frozen foods [19] which is in the agreement with present study.

3.1.1.2 Sensory parameters

The changes in sensory parameters include texture, appearance, odour, colour, flavor, overall acceptability of frozen stored tilapia meat are shown in Table 3 respectively. The sensory scores significantly decreased as the storage period progressed ($p<0.05$) and measured on the basis of 9-point hedonic scale. The mince was acceptable upto 5 point limit on hedonic scale and it was analysed by a panel of 10 experts. Siddaiah et al. [20] found a significant decrease in the sensory attributes like appearance, odour and texture of raw silver carp mince during storage ($p<0.05$). They stated that the texture of meat was tough which might be due to the decrease of protein solubility because of aggregation [16]. Other authors like Vidya Sagar Reddy and Srikar [16] also found a similar result of significant decrease in sensory parameters especially texture had some prominent effects on protein denaturation ($p<0.05$) which relates with the present study.

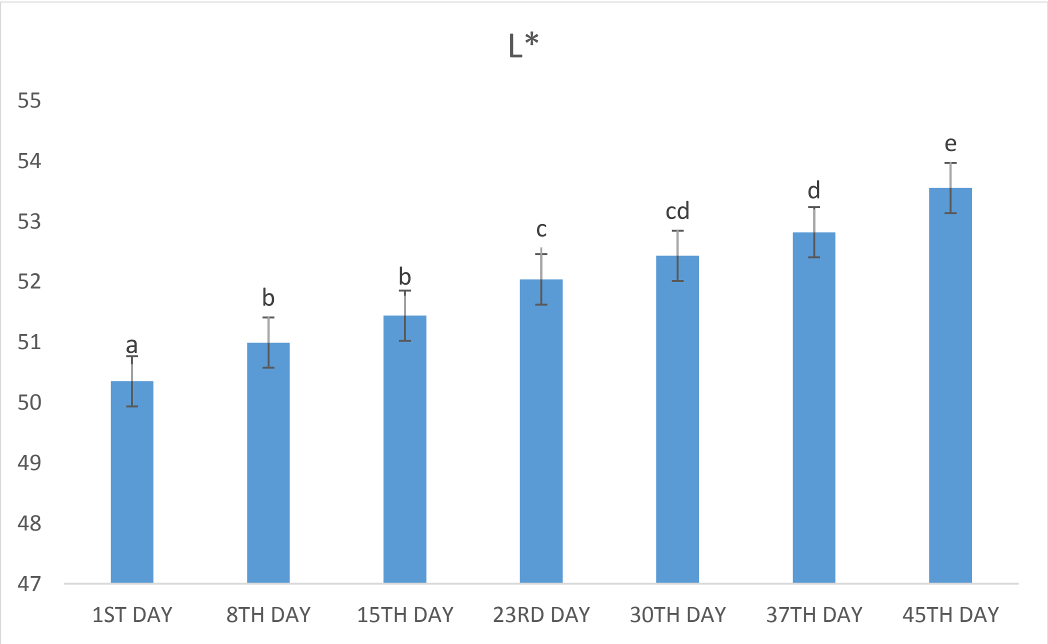

Fig. 1. Changes in L* values of tilapia mince w.r.t. days of frozen storage

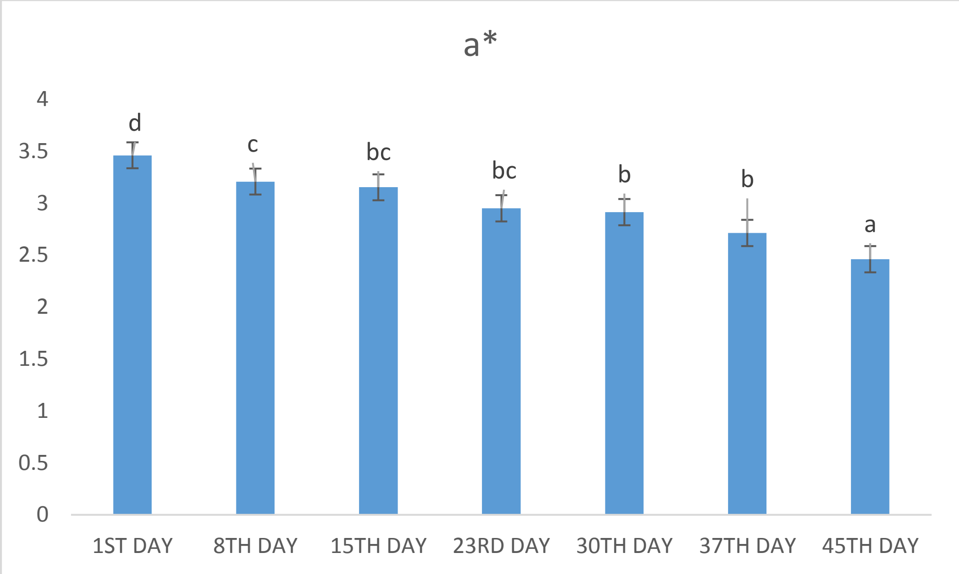

Fig. 2. Changes in a* values of tilapia mince w.r.t. days of frozen storage

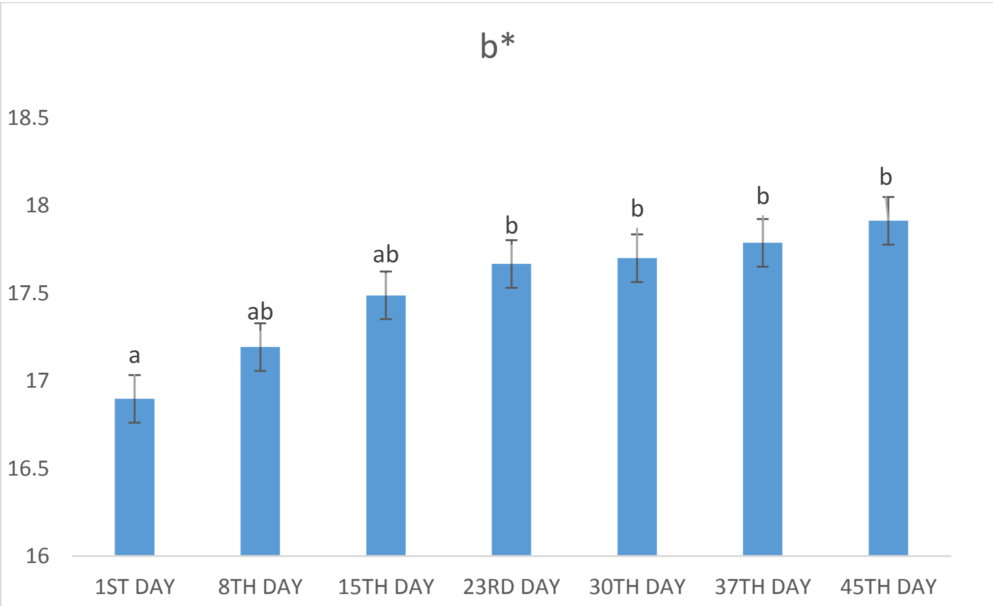

Fig. 3. Changes in b* values of tilapia mince w.r.t. days of frozen storage

Table 1. Changes in the sensory parameters of frozen stored tilapia minced meat

Days	Texture	Appearance	Odour	Colour	Flavour	Overall acceptability
1	8.80±1.00^{e}	8.63±0.15^{e}	8.66±0.15^{f}	8.80±0.10^{g}	8.56±0.15^{g}	8.73±0.15^{e}
8	8.60±1.00^{e}	8.00±0.20^{d}	8.36±0.15^{f}	8.36±0.15^{f}	8.20±0.10^{f}	8.03±0.15^{d}
15	8.60±0.20^{e}	7.60±0.20^{c,d}	7.90±0.20^{e}	7.73±0.20^{e}	7.80±0.10^{e}	7.76±0.66^{d}
23	7.86±0.30^{d}	7.16±0.55^{c}	7.43±0.20^{d}	7.16±0.25^{d}	7.30±0.10^{d}	6.76±0.15^{c}
30	7.06±0.15^{c}	6.46±0.45^{b}	6.86±0.30^{c}	6.70±0.20^{c}	6.80±0.10^{c}	6.23±0.15^{b}
37	6.03±0.32^{b}	5.80±0.36^{a}	6.30±0.17^{b}	5.90±0.10^{b}	6.20±0.10^{b}	5.73±0.20^{b}
45	5.16±0.15^{a}	5.26±0.37^{a}	5.43±0.40^{a}	5.10±0.10^{a}	5.40±0.36^{a}	5.16±0.15^{a}

Different superscripts given in the values (a, b, c, d, e, f, g) within column indicate significant differences between them ($p<0.05$).

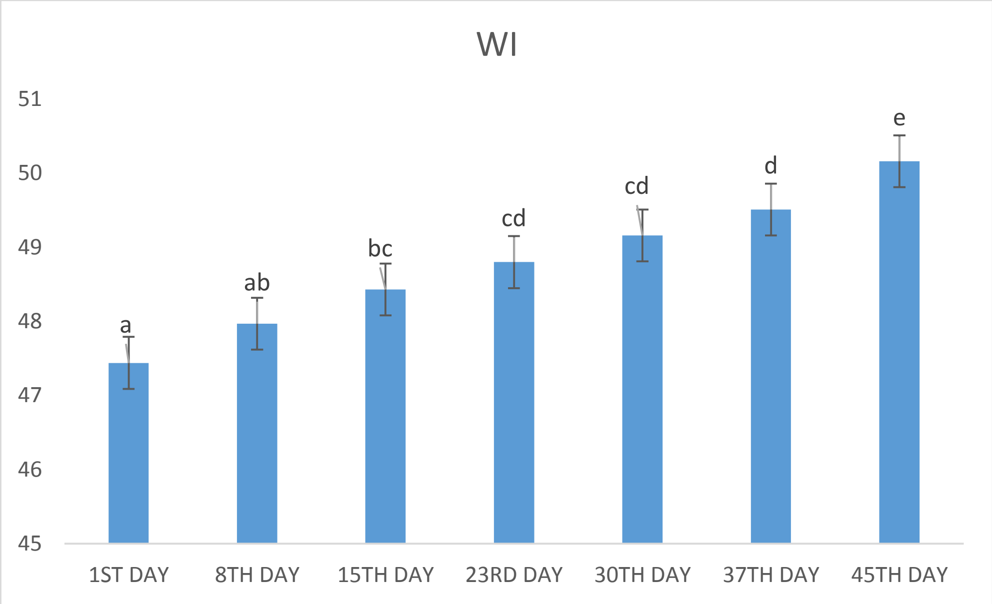

Fig. 4. Changes in whiteness index values of tilapia mince w.r.t. days of frozen storage

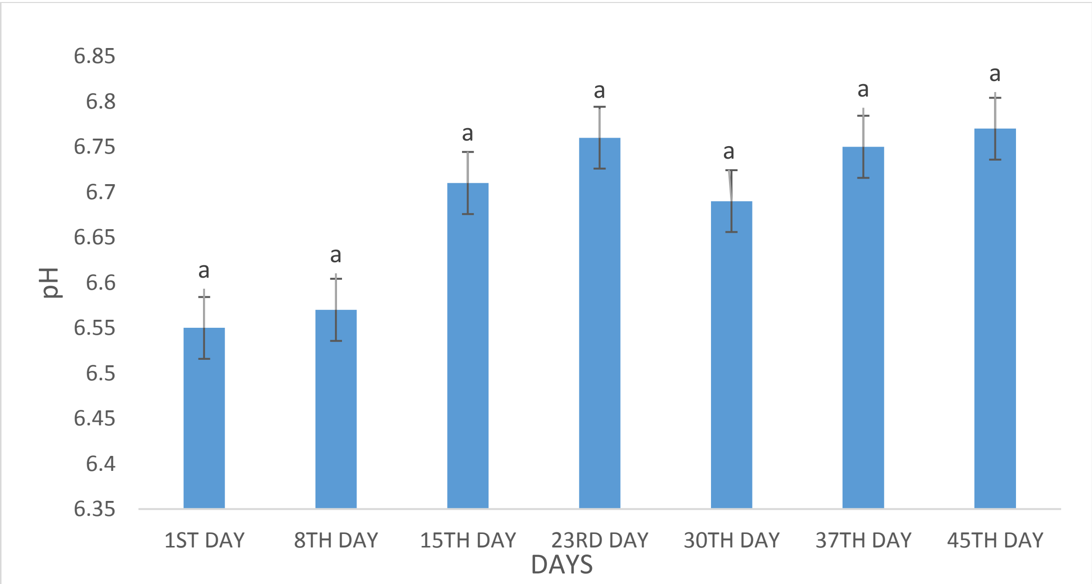

Fig. 5. Changes in pH values of tilapia mince w.r.t. days of frozen storage

3.2 Changes in Protein Degradation and Lipid Oxidation Products during Frozen Storage

The pH values of the frozen stored meat were measured by using a pre standardized pH meter. The initial values of the meat samples were 6.49, 6.62, and 6.54 on triplicate whereas the values increased to 6.92, 6.64 and 6.76 on 45th day (Fig. 5). Liu et al. [21] measured pH in tray-packed tilapia fillets stored at 0^{0}C and found that there was a significant increase in the pH values with respect to the storage time. This relates with the present study which might be due to the autolytic accumulation of alkaline compounds or microbial metabolism [22]. There was an increase in values up to 23rd day and a slight decrease has been observed in 30th day and again increased as the storage period progressed. Although there was no significant difference between the values but there is a considerable increase throughout the storage period.

The changes in all the physicochemical parameters like FFA, PV, TMA-N, TVBN, and TBA are shown in Table 1. When we observe each parameter individually, there was a significant increase in all the parameters as the storage period progressed. The initial values of FFA were 0.073 % of lipid as oleic acid and 1.106 % of lipid as oleic acid in 1st day and 8th day respectively. Then it increased significantly to 1.821% of lipid as oleic acid on 45th day of storage period. Many factors are responsible for this significant difference in the lipid oxidation which includes hydrophobic effect of FFA on major proteins [23], interaction of lipids with cysteine, lysine and several other amino acids of fish proteins that influence the sarcoplasmic and myofibrillar protein solubility during frozen storage. There was a similar observations found by Vidya Sagar Reddy and Srikar [24] in pink perch storage period of 180 days where the FFA increased significantly that directly relates with the present study.

The changes in the PV values were significantly different ($p<0.05$) and the values were observed increasing throughout the storage period. The values on the 1st day was 2.42 meq of O_2/kg of fat whereas it increased to 8.93 meq of O_2/kg of fat on 45th day. Siddaiah et al. [20] also found similar changes in the PV values as the storage period progressed which might indicates the oxidative deterioration [25]. The separation of fat from skin and tissue during deboning and mincing increases the oxidative changes in the fish mince effectively [26]. Therefore, we can say that both PV and FFA significantly influence the fish mince throughout the storage period ($p<0.05$).

There were significantly different ($p<0.05$) changes in the TMA-N values as the storage period progressed. The values on 1st day and 45th day were 2.15 mg/ 100 g of meat and 10.14 mg/100 g of meat respectively. There was significant increase throughout the storage period. Leelapongwattana et al. [27] observed the changes in TMAO activity of lizardfish (*Saurida micropectoralis*) and found there was increase in TMAO activity in the initial 6 weeks of frozen storage period which relates with the present study which might be due to disruption of membranes of fish muscle in reference to frozen storage. They concluded that frozen storage may release the mitochondrial and lysosomal enzymes into the sarcoplasm [28] which ultimately leads to increase of TMAO activity as the storage period progressed during initial weeks.

The changes in the TVBN content of frozen stored minced meat has been observed with a significant increase ($p<0.05$) as the storage period progressed from 7.18 mg/ 100 g of meat on first day to 27.82 mg/ 100 g of meat on 45th day. Other authors such as Chakrabarti [29] and Vidya Sagar Reddy et al. [30] observed the similar trend in frozen stored IMC and pink perch mince respectively. This might be produced due to microbial degradation of nitrogenous tissue compounds that increased the TVBN content significantly ($p<0.05$) [20].

The initial TBA content on the 1st day was 0.06 mg of malonaldehyde/kg of meat and on the final day was 1.64 mg of malonaldehyde/kg of meat. The values were observed to be increasing throughout the storage period and significantly different as well ($p<0.05$). Sarma et al. [31] stated that the values were low which might be due to the interaction of malonaldehydes with the muscle proteins that stops the extraction and increased the TBA content to a larger extent [32]. Similar results were also found out by some

Table 2. Changes in the physicochemical parameters of frozen stored tilapia minced meat

Days	TMA-N[1]	TVBN[1]	PV[2]	FFA[3]	TBA[4]
1	2.1560±0.056[a]	7.1867±0.427[a]	2.4207±1.947[a]	0.0737±0.013[a]	0.0621±0.031[a]
8	2.3867±0.030[a]	8.4800±0.442[b]	3.6013±1.260[b]	1.1067±0.074[b]	0.1643±0.046[a]
15	3.8067±0.231[b]	9.8600±0.746[c]	5.2050±0.364[c]	1.1883±0.007[c]	0.5843±0.019[b]
23	4.4800±0.314[c]	13.6667±0.350[d]	6.7707±0.160[d]	1.2390±0.023[c]	0.8827±0.066[c]
30	5.3400±0.341[d]	17.4000±0.385[e]	7.3283±0.275[e]	1.5630±0.024[d]	1.2457±0.036[d]
37	7.4933±0.453[e]	22.2533±0.742[f]	7.6357±0.260[e]	1.6350±0.062[e]	1.2777±0.232[d]
45	10.1467±0.422[f]	27.8267±1.252[g]	8.3903±0.227[f]	1.8210±0.030[f]	1.6407±0.051[e]

Where, [1] = mg of N / 100 g of meat
[2] = meq of O_2/kg of meat
[3] = % of lipid as oleic acid
[4] = mg of malonaldehyde/ kg of meat
Different superscripts given in the values (a, b, c, d, e, f, g) within column indicate significant differences between them ($p<0.05$).

authors in frozen stored oil sardine [33], marine catfish [25] and pink perch [24] during frozen storage. The oxidized products of lipids affect the solubility of proteins by interaction with their amino acids and binding with their functional groups such as lysine NH_2 group, cysteine- SH group etc. and these interactions increases the hydrophobicity and aggregation with the soluble proteins [31,34].

3.3 Changes in Protein and Lipid Functional Properties during Frozen Storage

The changes in all the functional parameters such as cook loss, emulsion stability and water holding capacity (WHC) with respect to storage days are shown in Table 2 respectively. The initial values of cook loss on 1st day was 30.4710 % whereas the values significantly increased to 37.5967 % on the final day of analysis ($p<0.05$). Cooking loss generally refers to the loss of water and fat [35]. The products with lower cooking loss possess better quality [36]. Chattopadhyay et al. [9] found that the chitosan treated sausage samples have higher cooking loss than control as chitosan involves in reduction of water holding capacity with higher acidity level and other loss of weight during thermal processing. Vidya Sagar Reddy and Srikar [37] observed an increase in the cook loss of frozen stored pink perch mince as the storage period progressed that relates with the present study which indicates delay in processing of fish prior to freezing as well as poor water retention capacity due to denaturation of proteins during frozen storage.

The emulsion stability of frozen stored tilapia mince has been recorded as 79.9077 % on the initial day whereas 70.0153 % on the final day. The values decreased significantly throughout storage period ($p<0.05$). Santana et al. [38] reported that the treated sausage samples have higher emulsion stability than the control and the values decreased significantly which might be due to the amount of fluid that has been expressed by the use of treatment. The lower values of emulsion stability and WHC values cause the protein denaturation in treated samples. Similar results found out by Chattopadhyay et al. [9] on the treated and control sausages where the control samples have lower emulsion stability and the decrease in emulsion stability caused by protein-protein aggregation of fish protein.

Water holding capacity (WHC) is the major parameter that determines the shelf life of the final product. The initial values on the 1st day was 69.85 % and 57.79 % on the final day respectively. The values decreased significantly as the storage period progressed ($p<0.05$). Suvanich et al. [39] found out that WHC is correlated with myofibrillar protein content [40]. On the other hand, WHC is also related with the gel product quality [41]. Cheng et al. [42] observed the loss in WHC of tissues during frozen storage was been directly correlates with a decrease in myofibrilliar protein solubility. A decrease in WHC leads to an increase in expressible moisture as well as protein denaturation [37] which relates with the present study.

3.4 Microbiological Quality Analysis during Frozen Storage

3.4.1Total plate count (TPC)

The total aerobic bacterial plate count of tilapia mincemeat during frozen storage has been initially decreased to a certain extent and then started increasing in frozen storage period. This may be due to the initial death of microbes after freezing and then the psychrophilic bacterial count increases after one month of storage period [43]. Perigreen et al. [44] also found a similar result with the present study in the ice storage characteristics of common murrel.

Table 3. Changes in the functional parameters of frozen stored tilapia minced meat

Storage days	Cook loss (%)	Emulsifying stability (%)	WHC (%)
1	30.4710±0.22^{a}	79.9077±0.73^{f}	69.8500±1.29^{f}
8	32.1777±0.12^{b}	78.6773±0.33^{e,f}	67.8933±1.17^{e}
15	33.4483±0.23^{c}	77.9263±0.52^{e}	65.5267±0.29^{d}
23	34.6800±0.15^{d}	76.3327±1.61^{d}	63.9300±1.61^{d}
30	36.4647±0.20^{f}	74.7070±0.41^{c}	62.1167±0.48^{c}
37	35.6217±0.17^{e}	72.3673±0.15^{b}	60.2567±0.79^{b}
45	37.5967±0.28^{g}	70.0153±0.73^{a}	57.7900±0.84^{a}

Different superscripts given in the values (a, b, c, d, e, f, g) within column indicate significant differences between them ($p<0.05$).

Table 4. Changes in TPC of frozen stored tilapia mince meat

Days	1	8	15	23	30	37	45
TPC (cfu/g)	6.2×10^4	4.3×10^4	3.6×10^4	2.1×10^4	3.4×10^4	6.3×10^4	8.4×10^4

4. CONCLUSION

The bacteriological, physicochemical, functional and sensory parameters of frozen stored tilapia mincemeat have been analysed. The analysis had been done in a week interval for 45 days of storage period. There were significant differences within the parameters and the frozen storage have major influence on tilapia minced meat ($p<0.05$). The fish was in acceptable condition upto 45 days of frozen storage organoleptically which can be used by food industry as well as consumers. This study has somehow fulfilled the research gap but many more experiments should be done in the near future to illustrate the major utilization and findings with importance and benefits of mincemeat from different species of tilapia and other fishes as well.

GENERAL APPLICATIONS

This topic creates a breakthrough among researchers and industrialists to engage themselves in the new technology of mincing of high value fishes. The same profit and yield put them into consideration for utilizing tilapia mince for several other purposes like product development etc. From the mince, various intermediate products can be developed like fish pastes, surimi which will directly help the processing industries to gain the profit. For all these reasons, I choose this topic to put more light on it and grab attention of the researchers and industry people.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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