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Prospects of Development of Emulsion Sausages from Mince of Indian Major Carps

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ABSTRACT

The investigation was carried out during the year 2019 at Central Institute of Fisheries Education, Mumbai, India for the development of fish sausages . The objective of the research was to study the characteristics of fish muscle of Indian major carps (*Labeo catla*, *Labeo rohita*, *Cirrhinus mrigala*) for development of fish products such as sausages. The prepared sausages were heat processed at 40°C for 30 m followed by at 90°C for 20 m. Presence of sarcoplasmic proteins in the mince impaired quality of sausages made from Indian major carps. The highest gel strength (146.05 g.cm) and water holding capacity (89.68%) was obtained in sausages made from mince of mrigal fish. Higher hardness was observed in mrigal sausages which were in concomitant with the highest gel obtained in the present study. Higher cohesiveness was recorded for catla sausages whereas, higher adhesiveness was noticed in rohu sausages. Significant variation ($p>0.05$) in textural parameter were not observed among sausages made from mince of Indian major carps. Ingredients such as corn starch, sunflower oil used during preparation of sausages significantly affected colour properties of resultant sausages. Sausages made from mrigal fish were more white and lighter in colour than others. Among Indian major carps, sausages made from mince of mrigal fish showed better quality in terms of gel strength, hardness and were more yellowish in colour. Overall, freshness of fish greatly affected the quality characteristics of sausages made from mince of Indian major carps.

KEYWORDS: Carps, mince, sausages, rheological properties, texture, colour

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1. INTRODUCTION

India is bestowed with abundant fisheries resources with the total production of fish resources reaching 16.248 mt in 2021–22 (Anonymous, 2023). Recently, due to health concerns, there is growing interest in consumption of fish and fishery products. Fish offers easily digestible proteins, poly unsaturated fatty acids, particularly EPA and DHA, vitamins, mineral and serves healthy diet to millions of people in the world. Nowadays, there is a processing trend of developing fish products instead of consumption of fish in fresh form to attract young consumers' and to fetch high profits. One such avenue in fish food industry lies in the production of fish sausages, a healthier alternative to meat sausages.

Fish sausages, particularly emulsion sausages are not very popular among Indian consumer compared to meat sausages. Emulsion fish sausages are those prepared from mince or surimi of fish mixed with ingredients such as seasoning and spices and heat processed (Feng et al., 2020). Fish mince and or surimi (washed fish mince) have recently been utilized for development of fish sausages (Yousefi and Moosavi-Nasab, 2014). Fish mince can also be used to manufacture patties, balls, wafers, loaves, burgers, fish fingers, fish fritters, and pickled products in developing countries. The characteristics of fish muscle protein are very important component for development of gel-based fish products such as sausages. Gelling capacity of fish muscle protein dictates functional and textural properties of fish products (Xiong, 2018). The quality of produced sausages is generally affected by characteristics of fish muscle, and processing conditions employed during production process leads to decline in fish product quality in the market. Hydrolysis of proteins due to leakage of digestive enzymes after harvesting during transportation, lipid oxidation during storage causing unpleasant flavours, denaturation of proteins, etc. affects gel forming ability of muscle proteins (Benjakul et al., 2008). All these factors collectively contribute to degradation of physicochemical and textural properties of resultant product.

The development of sausages from freshwater fish also reported by Chattopadhyay et al. (2019) for sutchi catfish (*Pangasianodon hypophthalmus*); Oliveira et al. (2014) for salmon (*Oncorhynchus gorbuscha*), etc. using different additives. However, very few researchers (Panpipat and Yongsawatdigul, 2008, Sini et al., 2008) have successfully developed fish sausages from Indian major carps and studied their rheological and textural quality.

Indian major carps (*Labeo catla*, *Labeo rohita*, *Cirrhinus mrigala*), an abundantly cultured freshwater fish around the world and fulfills most of the nutritional needs of consumers of inland areas. These species are mostly fully utilized for consumption in fresh form with relatively good value in spite of their relatively good gel-forming ability (Sankar and Ramachandran, 1998). Compared to marine fish, which is mostly available to the people of coastline and being exported to earn foreign exchange, Indian major carps are distributed in ice to interior areas of the states and finds delicacy in the diet of Indian people. In the era of globalization, there is a demand of ready-to-eat or ready-to-serve products, and therefore, huge potential has been recognized for development of mince based fish products worldwide. Good textural properties and sensory characteristics of fish sausage can be obtained using Indian major carps as a raw material. Therefore, an objective of the present study was to investigate characteristics of fish muscle of Indian major carps (*Labeo catla*, *Labeo rohita*, *Cirrhinus mrigala*) for development of fish products such as sausages.

2. MATERIALS AND METHODS

2.1. Material

The study was performed in the laboratory of Post-Harvest Technology, Central Institute of Fisheries Education, Mumbai, Maharashtra, India during 2019. Fresh Indian major carps (*Labeo catla*, *Labeo rohita*, *Cirrhinus mrigala*) were procured from a local fish market (Dadar, Mumbai, Maharashtra) and brought in (1:1) iced condition to the laboratory of Post-Harvest Technology, Central Institute of Fisheries Education, Mumbai, Maharashtra, India. On arrival in laboratory, the fish were immediately processed for deheading, gutting, descaling and manually filleted. The mince of catla, rohu and mrigal was prepared from processed flesh using a deboning machine (Baader 694, Lubeck, Germany) with a counter-rotating belt and drum mechanism. Dressed fishes were fed into the drum sieve having 5 mm diameter holes and the mince was prepared. The temperature was maintained below 10°C throughout the process. The obtained mince was sealed in polythene pouches (LDPE) separately and kept in a refrigerator (4°C) until the preparation of sausages.

2.2. Preparation of sausages

The sausages were prepared by grinding the 65% (w/w) mince of each species with 19.15% (w/w) ice water containing 1.6% (w/w) sodium chloride (NaCl), 0.25% (w/w) sodium tri-polyphosphate (STPP) and 1% (w/w) sugar for 3 m in a pre-cooled silent cutter. The temperature was maintained below 10°C throughout the processing. The ingredients mixed mince of each species again comminuted by slow addition of 8% (w/w) sunflower oil using silent cutter for 3 m and finally corn starch at the level of 5% (w/w) was mixed and blended further for 2 m. The finally blended mince was filled manually using hand stuffer into krehalon casing of 2.5 cm diameter. The care was taken to eliminate the trapped air as much as possible during manual filling of mince in sausage casings. The ends of the tubes were tied and pre-incubated at 40°C for 30 m followed by heating in a temperature-controlled water bath at 90°C for 20 m. The obtained sausages were immediately cooled in iced water and stored in a refrigerator (4°C) overnight before further analysis.

2.3. Determination of gel strength

The gel strength of prepared sausage gel samples was measured using Rheo Tex (Type SD-700, Sun Scientific Co. Ltd, 4-Chome, Kamiyoga, Setagaya-KU, Japan). After approximately 2 h of equilibration at room temperature (25°C), sausage gel samples were subjected to the puncture test using a Rheo Tex equipped with a 5 mm-diameter round-ended metal probe (5 mm diameter, 60 mm m^{-1}) with load cell of 2 kg. The load (g as breaking force) and the depth of depression (mm as deformation) when the gel sample lost its strength and ruptured were recorded. The gel strength of the sample was expressed in g.cm.

Gel strength (g.cm)=Breaking force (g)×Deformation (cm) …….(1)

2.4. Determination of water holding capacity (WHC)

Water holding capacity (%) of the sausage gel samples was determined according to the method of Verbeken et al. (2005). Briefly, 10 g of sausage sample was centrifuged at 12,000 × g for 30 m at 4°C. WHC was calculated as a percentage of retained water, using the following equation

WHC (%)=W_2/W_1×100 ………(2)

Where, W_2=Weight (g) of sausage sample after centrifugation

W_1=Weight (g) of sausage sample prior to centrifugation

2.5. Folding test (FT)

Folding test was performed according to the method of Lanier (1992). Sausage samples were cut into a 3 mm thick slice. The slices were held between the thumb and the forefinger and folded to observe the way that they broke. The grade used was as follows: (5) no cracks showing after folding, (4) cracks immediately when folded in half, (3) cracks gradually when folded in half, and (2) cracks occur immediately after folding in half, and (1) breaks by finger pressure

2.6. Texture profile analysis (TPA)

TPA of sausage gel sample for each species was performed using the Texture Analyzer TX-700 (Lamy Rheology, France). The samples were equilibrated at room temperature before measurement. The load cell used was a cylindrical probe of 50 mm diameter equipped with a sensor of 50 kg. Prior to the test, the sausage samples were equilibrated to room temperature for 30 m and sectioned into a 2.5 cm diameter and 3 cm height. The samples were compressed twice by setting the following parameters: height of 40% at a deformation rate of 1 mm s^{-1} with the down speed of 1 mm s^{-1}, force to start: 0.5 N, wait position: 5 mm; delay: 5 s and up speed 1 mm s^{-1}. A force-time curve was recorded and peak force, time difference and area of peaks were calculated from the recorded data and the following mechanical parameters were determined: hardness, cohesiveness, adhesiveness and elasticity.

2.7. Determination of instrumental colour

Colour of sausage samples was measured using a colorimeter (Hunter LabScan XE, Hunter Associates Laboratory, USA) provided with a 9.9 mm port size, under illuminant D65 (ASTM) and 10° of observer angle, which gives acceptable level of sausage sample based on L^*, a^* and b^* values. The colorimeter was calibrated with the standard (No. LX17376) using white (X=79.81, Y=84.71, Z=90.95) and black (X=16.11, Y=21.94, Z=16.66) tiles under "C" illuminate condition according to the CIE (Commission International de I'' eclairage). The L^* variable represents lightness (L^*= 0 for black, L^*=100 for white), redness (+a^*) or greenness (-a^*) and yellowness (+b^*) or blueness (-b^*) were the colour parameters evaluated for the samples. The sausage samples were equilibrated to room temperature for 30 m prior to the colour measurement using colorimeter. The sausage samples were cut in 2.5 cm diameter and 3 cm height to determine colour properties. L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were measured and whiteness was calculated as described by Park (1994) as follows:

Whiteness index=100-√(100-L^*)^2+(a^*)2+(b^*)^2 …………(3)

3. RESULTS AND DISCUSSION

3.1. Rheological characteristics

Rheological properties such as gel strength, water holding capacity and folding score of sausages made from Indian major carps (catla, rhou and mrigal) are presented in Table 1. The gel strength and water holding capacity (WHC) of the prepared sausages is graphically represented in Figure 1.

3.1.1. Gel strength

The significant effect (p<0.05) of mince of different species of Indian major carps was observed on gel strength in the present study. The highest gel strength (146.05 g.cm) was recorded for mrigal emulsion sausages followed by catla (110.06 g.cm) and rohu (88.45 g.cm) sausages. The partially unfolded proteins with uncoiled polypeptide chain that interact at specific points forming a three-dimensional cross-linked network to form a gel during heat treatment. The sarcoplasmic proteins in mince will coagulate during heat setting of salt added sol and does not participate in the formation of gel network resulted in lower gel strength. This could be the reason for lower gel strength in

Table 1: Rheological characteristics of emulsion sausages prepared from Indian major carps			
Fish	*Labeo catla*	*Labeo rohita*	*Cirrhinus mrigala*
Gel strength (g.cm)	110.06±6.35^{b}	88.45±2.43^{a}	146.05±15.25^{c}
WHC (%)	89.41±1.56^{a}	88.27±1.10^{a}	89.68±1.70^{a}
Folding score	2.00±0.00^{a}	2.00±0.00^{a}	2.00±0.00^{a}

Data are expressed as the mean±SD (n=3). Different superscripts in the same row signify statistical difference (p<0.05)

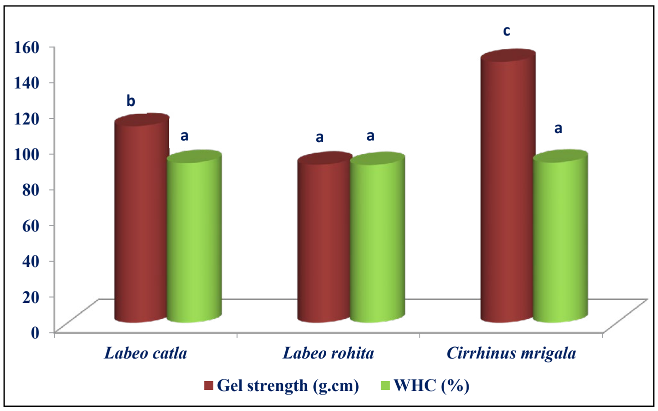

Figure 1: Gel strength (g.cm) and WHC (%) of emulsion sausages prepared from Indian major carps

emulsion sausages made from mince of Indian major carps. The different setting temperature for formation of gel in species of Indian major carps may also be implicated for lower gel strength and good gel did not set at 40°C in the present study (Gore et al., 2021). The present results are in agreement with Sankar and Ramachandran (2002) who reported the gels of catla, rohu and mrigal were not set at 25°C, 30°C and 40°C as actomyosins from these fishes do not unfold to the extent required to bring about changes for the development of gel network. Similarly, the freshwater carp did not follow the same pattern of gel formation mechanism as the marine fish (Nowsad et al., 1999). The autolytic activity in mince causes textural degradation of myofibrillar proteins including myosin heavy chain, actin, and tropomyosin (Yongsawatdigul et al., 2013, Cao et al., 2005). To some extent, ingredients used during preparation may be responsible for lower gel strength in mince sausages made from Indian major carps. Though, mince of carp showed good elastic characteristics and gelling properties which is comparable with that of the washed flesh of marine fish (Sankar and Ramachandran, 1998), the lower gel strength was obtained for emulsion sausages in the present study. The freshness of fish affects the gel strength of mince. The functional properties of fish proteins also affected by changes in physicochemical properties when stored in ice (Yathavamoorthi et al., 2012). Contrary to this, the salt solubilisation of myofibrillar protein concentrate of washed mince or surimi with an adequate amount of water results in the formation of sol, which finally turns into an elastic gel upon heating (Lee, 1992).

3.1.2. Water holding capacity (WHC)

The capacity to hold moisture is one of the important qualities of sausage and other emulsified products. WHC determines how well the moisture was retained in the cooked product. The results of the present study substantiated water holding capacity of a gel is directly related to the gel network formation. The non-significant (p>0.05) higher WHC was observed for mrigal sausages (89.68%) whereas, lower was obtained for rohu sausages (88.27%). The low expressible moisture indicates firmness of the gel (Kim et al., 1987) and coincidental with the increased breaking force (Luo et al., 2010) which was found in the present study. The alteration in physicochemical properties affects the three-dimensional structure of protein, affecting the water holding capacity of proteins (Yathavamoorthi et al., 2012). The WHC of sausages also affected by accelerated decomposition, aggregation and cross-linking of myofibrillar protein due to mincing of fish, presence of lipids (Chaijan et al., 2010). Several other factors like pH and protein denaturation affects the water binding capacity of gels (Rahmanifarah et al., 2015).

3.1.3. Folding test (FT)

Folding test analyzes the elasticity of the prepared sausage gels. All carp species studied obtained folding test score of 2 for the mince gel sausages showing lowest elasticity, which could be attributed to presence of heat stable alkaline proteases enzyme interfering the gelation in mince or different setting temperature for carp species (Sankar and Ramachandran, 2002) and was in commensurate with gel strength. In contrast to the present study, higher folding score as 'AA' (soft, firm, chewable, more elastic) in mince of catla and mrigal was reported in the study of Sankar and Ramachandran (1998). Yathavamoorthi et al. (2012) recorded similar results for common carp as the present study. Consequently, scores of folding test is unable to differentiate quality of emulsion sausages made from different species of Indian major carps.

3.2. Textural characteristics

The results of texture profile analysis (TPA) of emulsion sausages prepared from mince of Indian major carps are presented in Table 2.

The textural characteristics of the mince are mainly related to the quality of myosin in the myofibrillar protein (Chan et al., 1995). The significant differences (p>0.05) among

Table 2: Textural characteristics of emulsion sausages prepared from Indian major carps.

Fish	*Labeo catla*	*Labeo rohita*	*Cirrhinus mrigala*
Hardness (N)	4.05±0.31[a]	3.80±0.57[a]	4.09±0.80[a]
Cohesiveness	0.96±0.06[a]	0.94±0.10[a]	0.95±0.07[a]
Adhesiveness (J/m^3)	0.20±0.20[a]	0.37±0.31[a]	0.30±0.17[a]
Elasticity (mm)	1.13±0.03[a]	1.12±0.04[a]	1.11±0.03[a]

Data are expressed as the mean±SD (n=3); Different superscripts in the same row signify statistical difference (p<0.05)

TPA parameters were not observed for textural quality in emulsion sausages made from mince of Indian major carps. This could be due to myofibrillar protein degrades during storage of fish in ice and fish muscle generally becomes softer during chilled storage. Therefore, exact mechanism of textural changes is not known (Verrez-Bagnis, 1997). Hardness is defined as the resistance (N) at the maximum compression during the first compression of the sample (Huidobro et al., 2005). The hardness in emulsion sausages of Indian major carps were ranged from 3.80 N to 4.99 N. The lower hardness was observed in rohu sausages and directly related to lower gel strength and water holding capacity. The characteristics of flesh, processing method employed, addition of corn starch, oil, salt activation, coagulation of muscle proteins affected textural parameters of prepared sausages (Dincer et al., 2017, Troy et al., 1999). The lower values of adhesiveness could be implicated in lower hardness in this study. The starch content also influences the hardness of the product (Huda et al., 2013, Rahman et al., 2007). Murthy et al. (2017) observed higher hardness value for raw tilapia fillets than the cooked fillets. The hardness comparable to the present study was reported by Ninan et al. (2004) for tilapia fish.

In TPA analysis, cohesiveness gives an indication of how well the samples withstand the deformation and it was found around 0.73‒0.80 for all samples irrespective of species. The low cohesiveness values in the present study indicates rupture of the sample gels. The sarcoplasmic proteins are thought to be retarding gel network formation by interfering with the actomyosin crosslinking process might have contributed to the poor textural properties (Okada, 1964, Shimizu and Nishioka, 1974). Adhesiveness is work required to overcome the sticky forces between the sample and the probe. Adhesiveness values in the present study was ranged between 0.20‒0.37 J m^{-3}. From the results, it can be seen that adhesiveness of emulsion sausages are inversely proportional to the gel strength. In TPA analysis, springiness or elasticity represents the force at maximum compression during the second compression cycle. The values of elasticity of emulsion sausages under study were ranged in between 1.11‒1.13 (p>0.05). The higher elasticity values in the cooked sausages were due to pre-emulsion of mince with higher percentage of oil (8%). The lower values of hardness, cohesiveness, adhesiveness and elasticity upon cooking may be a result of protein denaturation due to the uncoiling of polypeptide chains. During cooking, fish muscles usually become soft. The observations similar to the present study were made by Murthy et al. (2017) for cooked tilapia fillets and tilapia steaks except springiness (Dhanapal et al., 2012). However, Ninan et al. (2004) noticed lower cohesiveness and adhesiveness values and higher springiness values for tilapia mince stored in ice for 2 days than the present study. In addition to this, Rahmanifarah et al. (2013) also reported lower hardness, cohesiveness and elasticity and higher adhesiveness in washed silver carp (*Hypophthalmichthys molitrix*) sausages than the unwashed mince group, which was similar to only adhesiveness in the present study. The decrease in hardness, elasticity and cohesiveness values was reported by Yathavamoorthi et al. (2012) for common carp surimi during ice storage.

3.3. *Instrumental colour characteristics*

Color is one of the important sensory attributes that determines the consumer acceptability of fish sausage (Sachindra and Mahendrakar, 2010). The Hunter L^*, a^*, b^* and whiteness values of emulsion sausages prepared from mince of Indian major carps are shown in Table 3.

In the present study, significant differences (p<0.05) in colour characteristics were observed in emulsion sausages prepared from mince of Indian major carps, which could be due to the presence of heme pigments, blood, sarcoplasmic proteins, enzymes, etc. and ingredients used during preparation. The myoglobin plays an essential role in the whiteness and whiteness is one of most important factors in the quality of sausages. The higher whiteness (70.10) was observed for mrigal sausages than catla (69.40) and rohu (69.20) sausages due to white flesh of mrigal. The ingredients did not affect colour of sausages after cooking to

Table 3: Instrumental colour characteristics of emulsion sausages prepared from Indian major carps

Fish	*Labeo catla*	*Labeo rohita*	*Cirrhinus mrigala*
Whiteness	69.40±0.32[ab]	69.20±0.61[a]	70.10±0.75[b]
L^*	72.21±0.41[a]	72.33±0.79[ab]	73.16±0.69[b]
a^*	-0.54±0.09[a]	0.24±0.15[b]	-0.58±0.62[a]
b^*	12.80±0.43[a]	13.51±0.41[b]	13.16±0.52[ab]

Data are expressed as the mean±SD (n=3). Different superscripts in the same row signify statistical difference (p<0.05) a large extent, which can be seen from results of the present study. Lightness was suggested as the major contributing factor to whiteness (Chen et al., 1997) and the same was evident from the present results of the study. The trend similar as whiteness was also observed for lightness during the study. The light scattering effect of oil droplets can be seen from the values of lightness in the present study. Partially, slightly off-white colour of corn starch might have offset the red colour of the mince during preparation of emulsion sausages.

The positive redness (a^*) values in rohu (0.24) emulsion sausages indicated mince containing red pigments and or red muscle. The color remaining after preparation was the result of insoluble pigments (Chen et al., 1997), since same ingredients were used for preparation of emulsion sausages from Indian major carps. The more negative values of redness (a^*) in catla (-0.54) and mrigal (-0.58) emulsion sausages showed ingredients like starch could have masked the red colour of the pigments in the meat and white flesh and turned sausages into green colour. The enhanced yellowness (b^*) in the present study were directly related to the use of yellow coloured oil during preparation of emulsion sausages. In contrast to the present results, redness (a^*) value decreased due to leaching of blood adhering to mince during washing and decrease in yellowness (b^*) was due to lighter colour of tilapia fillet in Nile tilapia sausages (de Oliveira Filho et al., 2012). The disparate changes in L^*, a^* and b^* was reported by Rahmanifarah et al. (2013) in mince sausages made from silver carp. Tanuja et al. (2014) recorded values of L^*, a^* and b^* of 50.81, 4.31 and 10.96 for mince of *Pangasianodon hypophthalmus*. The present results are in agreement with the Yongsawatdigul et al. (2013) who reported that mince gels of small-scale mud crab (SC) and common carp (CC) showed relatively low L^* values of 70.44–78.38 and higher b^* values of 12.59–14.93, resulting in a brownish appearance. They found that mince from CC yielded a more brownish appearance than SC mince.

4. CONCLUSION

Fish sausages could be made from mince of Indian major carps. However, sarcoplasmic proteins and additional ingredients used in making of emulsion sausages affected functional, textural and colour characteristics of produced sausages. Impairment in gel quality and texture of minced sausages was mainly due to inability of muscle proteins of carps to set at 40°C. The colour characteristics of produced sausages were affected by addition of ingredients.

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