Rheological properties of washed and unwashed tilapia (*Oreochromis mossambicus*) fish meat: effect of sucrose and sorbitol

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Abstract In the present study, the dynamic viscoelastic behavior (DVB) and flow behavior of fresh tilapia (*Oreochromis mossambicus*) meat containing cryoprotectants were evaluated with and without water washing. The DVB profile of washed meat with 4% sucrose and sorbitol indicated the maximum structure buildup reaction up to 56.8 °C; thereafter, hydrophobic interactions leading to decreased gelation were suppressed. In both the samples, there was no clear indication of the sol–gel transition temperature. In flow-profile measurements, the presence of cryoprotectants gave rise to the minimum thixotropic area, indicating a low level of impairment in structure. The shear-stress sweep of water-washed tilapia proteins added with cryoprotectants did not reveal significant changes at 28 and 40 °C. In texture-profile analysis, the hardness values were lower in fresh meat than cooked meat. The findings of this study will be helpful in the formulation and design of various mince-based products and in determining the appropriate use of cryoprotectants and water washing in the processing of minced meat.

Keywords Tilapia · Cryoprotectants · Water washing · Flow profile · Dynamic viscoelastic behavior

Introduction

The functional properties of muscle proteins are closely associated with the integrity of proteins. Proteins give food formulations an acceptable and stabilized textural form through their functional properties [1]. Denaturation and aggregation proteins and autolysis of myofibrillar proteins during storage lead to the loss of gel-forming ability [2]. The gel-forming ability of proteins in surimi and surimi seafood as well as their texture are of prime importance in order to get good economic returns. Gel-strength reduction depends on several factors such as species of fish [3]; freshness of fish; condition before, during, and after rigor [4, 5]; and the season of capture [6]. Gelation is a controlled process in which the final network displays some degree of order; this process is controlled by a number of factors, such as heat [7], nature of proteins, concentrations, pH, and ionic environment [8]. The gel-forming ability of surimi proteins varies depending on fish species, habitat, handling during catching, processing methods, and storage [9]. Therefore, a precise measurement of the rheological properties of surimi under the specified conditions of use and in combinations with intended cryoprotectants (CPs) is critical to control product quality and new product development. Production of acceptable gels is very important in the manufacturing of surimi-based products such as sausage, ham, kamaboko, and seafood analogs. Sucrose and sorbitol are widely used, permitted, and commercially important additives in surimi production [10]. Owing to their importance in fish-processing industries, these were selected as CPs in this study. Fish protein gelation [11] imparts functionality, which in turn is responsible for the formation of the desired texture and flavor.

Rheology is an important factor in understanding the mechanism of gelation. It plays an important role in the

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designing, evaluation, and modeling of processes; moreover, it acts as an indicator of the quality of the product [12]. The flow behavior of proteins and their dynamic viscoelastic behavior elucidate the molecular arrangements of the proteins being tested; this makes the assessment of the rheological behavior of fish meat an important parameter.

Tilapia (*Oreochromis mossambicus*), a freshwater fish can be an ideal alternative candidate for the production of mince- and surimi-based products and is becoming a substitute for white-flesh varieties. The worldwide consumption of tilapia in 2014 was 5.4 million metric tons [13]. Tilapia exhibits properties similar to those of other freshwater fish species in terms of their suitability for surimi production and for the preparation of value-added products [14]. Owing to its suitable functional and physicochemical properties for surimi production, which are similar to those of other freshwater fish species, tilapia has potential applications in the production of surimi- and mince-based products for fetching high economic returns from inland waters. For producing mince-based products from tilapia, it is essential to gain a thorough understanding of the properties of proteins so as to improve the textural properties. The functional properties of proteins in food determine the technological value of any fish meat.

The tilapia used in the present study was wild-caught. Marine catch has been showing a dwindling trend since the last decade, and low-cost white-meat fish are not available for surimi production. There are several reports present on cultured species and commercially important fish species; however, we would like to focus on the texture and functional characteristics of wild-caught tilapia. The effect of CPs on fish mince in relation to its rheological properties was investigated. In commercial surimi production, water washing is considered as an essential step for removing water-soluble proteins, total lipids, free amino acids, pigments, and other extractives. This study aims to evaluate the effect of CPs in different ways, e.g., for washed meat with CP and washed meat without CP, for wild-caught tilapia as an alternative species for surimi production. Knowledge of the viscoelastic properties of its meat could help in the effective utilization of meat for the production of high-quality processed fishery products.

Materials and methods

Raw material

Mozambique tilapia (*Oreochromis mossambicus*) fish were freshly caught from a natural inland water resource near Mysore, Karnataka, India. The length of the fish ranged from 210 to 285 mm, while their weight ranged from 150

to 340 g. Soon after catching, the fish were washed and iced (1:1::fish:ice); then, they were brought to the laboratory for further analyses. Next, they were beheaded, gutted manually, and washed again with chilled water (3 °C) before further analysis. All the chemicals used in the present research were of analytical reagent (AR) grade or food grade.

Preparation of surimi

Using minced meat, surimi was prepared using the method of Nishiya et al. [15] and mixed with cryoprotectants (w/w) such as sodium tripolyphosphate (0.2%), sorbitol (4%), and sucrose (4%). The fish meat packed in polyethylene bags was frozen in an air blast freezer at −35 °C. The total time for freezing varied between 4 and 6 h. Whole fish were frozen in a similar fashion. All three frozen samples, namely whole fish, washed meat, and washed meat with cryoprotectants were stored in a deep freezer (Vest frost, Holland) at -20 ± 2 °C during the period of study. Samples were drawn on a monthly basis.

Proximate composition

Proximate composition analysis of the meat (moisture, crude protein, fat, and ash content) was conducted after maceration of the samples using mortar and pestle, as per the method described in Association of Official Analytical Chemists (AOAC) [16].

Measurement of the flow profile (shear-stress sweep)

For measuring the flow profile, an extraction buffer (EB) was used to extract total proteins from fish meat. Precisely, 2 g of meat was added to 18 ml of the EB and homogenized. The sample was then centrifuged at $9000 \times g$ for 15 min. The supernatant solution obtained after centrifugation was used for flow-profile measurements. The total protein concentration was in the range of 4–12 mg/ml. A Carri-Med controlled-stress rheometer was used to measure the flow behavior of the samples at two different temperatures (28 and 40 °C) under varying stress. The equilibration time for the sample before the shearing experiment was 5 min. A cone and plate measuring geometry (4 cm with 59μ truncation) was used for measurements, wherein varied stress from 2 to 6 Pa was applied based on the angular velocity values obtained in the preshear experiments. The peak hold time was 1 min, whereas the ascend and descend times were 2 min each. Measurement of the shear-stress sweep at different temperatures was performed in triplicate, and the graphs for log viscosity against log shear stress were plotted for the average values in order to obtain flow curves.

Dynamic viscoelastic behavior (DVB)

A Carri-Med controlled-stress rheometer (CSR-500, Carri-Med, Surrey, UK) was used to measure the dynamic viscoelastic behavior (DVB) of fish meat in the temperature range of 30–90 °C. Measurements were made in the oscillatory mode with a 4-cm parallel plate measuring geometry. Fish meat without connective tissues, scales, and fins was used for DVB analysis after further preparation. Accurately, 4 g of the fish meat sample was weighted and macerated with 2.5% sodium chloride (w/w) in order to convert it into a fine paste, which formed the sample for DVB analysis.

During rheological measurements, dehydration of the sample was prevented by applying mineral oil to the edges of the sample, with great care taken to prevent the penetration of oil into the sample. The gap between the measuring geometry and the Peltier plate was manually set to 2000 μm at 80 °C using the micrometer attached to the instrument, which allowed accurate gap setting. The gap was set manually but using the micrometer provided at the base of the rheometer during all the sets of the experiment. Several authors have used manual gap setting in viscoelastic parameter calculations [1, 17]. A stress of 500 Pa, which was within the viscoelastic region, was applied. Determination of the linear viscoelastic region was performed through a torque sweep with a frequency of 1 Hz. Measurements were made by applying a small amplitude oscillation (0.005 rad) with a frequency of 1 Hz. The heating rate was 1 °C per min, which was obtained through the Peltier plate of the instrument. The applied stress was compared with the resultant strain. The results of such measurements were expressed as the storage modulus (G') and loss modulus (G''). On an average, three replicates for DVB were used to plot the results.

Texture-profile analysis

A texture-profile analyzer was used for texture-profile analysis. A two-cycle compression test was performed with 40% compression at initial, test, and retract speeds of 1, 1, and 10 mm/s, respectively; the data rate was 200 pps. A cylindrical probe with a 20-mm diameter and equipped with a 50-N load cell was used in the study to assess the texture profile of uniformly cut samples. To perform texture-profile analysis, fish were cut into fillets and further into pieces with a length, width, and height of 2 cm. Force time plot from each two-cycle compression test was used to calculate the mean values of the texture-profile analysis parameters. Texture profile analyzer generates a curve that shows load resulting from deformation. Hardness (N), cohesiveness, springiness (mm), chewiness ($\text{kg}_f\cdot\text{mm}$), and gumminess (kg_f) of the fish muscles were calculated as

defined in the texture-analyzer user manual. Tilapia were washed, beheaded, gutted, and cut into steaks. Tilapia in raw form and steamed form were subjected to texture-profile analysis. Steaming of tilapia was performed using a steamer for 15 min with 2% salt as an additive. In total, two batches (raw and steamed) comprising three samples each were analyzed for texture-profile measurements.

Statistical analysis

The experimental data for the texture profile of raw and cooked tilapia meat was analyzed using SPSS version 16.0. The analysis was performed in triplicate, and results are expressed as the mean $\pm$ standard deviation (SD). Further, Student's t test was used to compare the means at a significance level of 5%.

Results and discussion

Composition and dressing yield of fish

As the fish used in the present study was wild-caught tilapia, the physical and raw-material characteristics of the fish were recorded. The sizes and weights of the fish used in this study were comparatively greater than the mean values reported for tilapia, which are given in Table 1. The yield of fish after beheading and gutting was 64.3%, and the yield of meat from the whole fish was 44% (Table 1). A higher yield may have resulted from the relatively bigger size of the fish. The size of the fish is always a problematic factor in trade-related issues and for obtaining the commercial yield ratio. Moreover, big fish are not suitable for surimi production because of the belt movement in the deboning/meat-picking machine. In general, the yield of meat picked from freshwater fish varies from 40 to 47% [18, 19]. The composition of the meat indicated high moisture content and less fat content (Table 2). There are several reports wherein the fat content of tilapia was reported to be below 3% [20, 21]. The moisture content obtained herein was slightly higher than the reported values

Table 1 Physical characteristics of fresh tilapia (*Oreochromis mossambicus*) meat

Average length (cm)	23.76 $\pm$ 2.23
Standard length (cm)	18.9 $\pm$ 1.9
Average weight (g)	252.6 $\pm$ 56.9
Dressed yield (%) ^a	64.3
Separated meat yield (%) ^b	44.44

Values are given as mean $\pm$ SD, n = 25

^a After removal of head, entrails, and scales, ^b from whole fish to separated meat

Table 2 Composition and properties of total proteins from fresh tilapia (*Oreochromis mossambicus*) meat

Parameters	Fresh fish
Moisture (%)	81.34 ± 1.7
Total protein (%)	16.72 ± 0.55
Total fat (%)	0.88 ± 0.02
Total ash (%)	0.99 ± 0.07

Values are given as mean ± SD, n = 3

[22, 23]. In addition, comparable results were observed for crude protein content in the fish meat [18, 20, 22, 23].

Rheological properties of unwashed and washed tilapia meat

The nature of the deterioration reaction in surimi mainly arises from random aggregation leading to the formation of inextractable aggregates. This reaction has a serious impact on the gelling ability and quality of the final product [24]. To minimize the aggregation process during the frozen storage, additives such as polyols, sugars, amino acids, citrates, and phosphates are usually added in different concentrations. The combination of 4% sucrose, 4% sorbitol, and 0.3% polyphosphate has been found to be an ideal and effective cryoprotectant [16]. In the present investigation, 4% (w/w) sucrose and sorbitol and 0.2% (w/w) polyphosphate were used as cryoprotectants. The effect of added cryoprotectants on the protein properties of unwashed and washed tilapia meat was assessed, with special emphasis on the gelation properties.

Flow profiles of unwashed and washed meat

Resistance to shearing, which is an indication of structural impairment, is generally indicated by flow-profile measurements of fish myofibrillar proteins at a specific temperature [25]. The shear-stress sweeps of total proteins from unwashed meat added with 4% cryoprotectants at 28 and 40 °C are illustrated in Fig. 1(A), (B). The initial viscosity at 28 °C is log −0.57 mPa at a shear stress of log −4.33 kPa. The down curve data indicate a higher viscosity value at a shear stress below log −3 and log −4 kPa at 40 °C (Fig. 1).

The shear-stress sweeps of total proteins from washed meat added with 4% cryoprotectants at 28 and 40 °C are illustrated in Fig. 2(A), (B). The up-curve profile at 28 °C indicates the usual behavior, i.e., reduction in viscosity with an increase in shear stress. The down-curve data indicate at certain values of shear stress that the values recorded were higher at the corresponding stress values in the up-curve. At a shear stress of log −4.2 kPa, the viscosity value was log −1.7 mPa. The shear-stress sweep at

Fig. 1 Flow profile of total proteins from unwashed meat with 4% cryoprotectants obtained using EB as the solvent; (A) flow profile at 28 °C and (B) flow profile at 40 °C

Fig. 2 Flow profile of total proteins from washed meat with 4% cryoprotectants obtained using EB as the solvent; (A) flow profile at 28 °C and (B) flow profile at 40 °C

40 °C indicates a low viscosity profile throughout the range of the experiment. At certain shear-stress value, the viscosity values are higher than their corresponding values in the up-curve.

The presence of cryoprotectants gives rise to the minimum thixotropic area, indicating a low level of impairment in the structure. Thixotropy is a time-dependent shear-thinning property. In many fluids, viscosity is mostly independent of time and is the function of shear rate or shear stress, concentration, and temperature. The viscosity of concentrated dispersions does not reach a steady value for some time upon the application of shear stress or shear rate. This steady state is dependent on the stabilization of the internal network structures. The flow profile of the fish-protein solution used in the present study is measured and presented as an up-curve (increase in the shear rate) and down-curve (decrease in the shear rate, ultimately reverting to the initial shear rate [26].

The presence of cryoprotectants gives rise to the shear-thickening phenomenon, as revealed by the down-curve. It is also evident that the presence of cryoprotectants increased the resistance of the meat to both shearing and temperature. It should be noted that the shear-stress sweep at 40 °C might have been influenced by coagulation of proteins. The viscosity of thixotropic materials does not follow the same path of structural breakdown and recovery when the shear rate decreases to the original value. In most cases, when the shear rate is reduced, the stress path forms a hysteresis loop, which then returns to a point lower than the initial critical shear stress (this is the stress that makes the fluid flow). The area within the hysteresis loop represents the energy consumed in the structural breakdown [26]. As the fish-protein solution used in the study comprises proteins like myosin, actin, troponin, tropomyosin, and other minor proteins, the flow behavior/viscosity profile of the protein solution is also affected by proteins' composition and structures.

The shear-stress sweep of total proteins from washed tilapia meat with cryoprotectants does not reveal any significant change at 28 and 40 °C. The profile observed at 40 °C with cryoprotectants shows a large thixotropic area possibly due to coagulation. A similar profile was observed at 28 °C; this indicates that the added cryoprotectants had no significant effect in improving the resistance to shearing. It is not clear whether sucrose and sorbitol could extend the protective behavior of unwashed meat in comparison with washed meat owing to shearing and temperature.

Dynamic viscoelastic behavior of unwashed and washed meat

Changes in G' can be used to monitor the gelation behavior of proteins [27]. The dynamic viscoelastic behavior of unwashed meat with 4% cryoprotectants in the temperature

range of 30–90 °C is given in Fig. 3A. Increased temperatures result in an increased value of G' , and the maximum rate of increase is observed between 63.3 and 70.1 °C, indicating structure buildup or network formation. The G'' values increase with temperature; however, they are considerably lower than the G' values. The initial $\tan\delta$ value is 0.4318. The decrease in $\tan\delta$ values with increasing temperature was recorded during the experiment. The dynamic viscoelastic behavior profile does not indicate the sol–gel transition range. However, at 70.1 and 76.7 °C, there is a slight increase in the $\tan\delta$ values. The increases in the G' values indicate the formation of an elastic gel network and rigidity of the sample [28].

The dynamic viscoelastic behavior of washed meat with 4% cryoprotectants in the temperature range of 30–90 °C is shown in Fig. 3(B). The G' values increase with temperature, and the rate of increase is found to be the maximum between 63.3 and 70.1 °C. The G'' values also increase with temperature; however, the absolute values of G'' are lower than that of G' values, indicating the formation of a viscoelastic network. The presence of cryoprotectants in washed meat lead to higher viscous nature of the meat as indicated by the higher $\tan\delta$ value. In addition to the suppression of the hydrophobic interaction, the higher initial

Fig. 3 Dynamic viscoelastic behavior (DVB) of tilapia meat; (A) unwashed meat with 4% cryoprotectants and (B) washed meat with 4% cryoprotectants

Table 3 Texture-profile analysis of raw and cooked tilapia meat

Tilapia meat	Raw	Cooked
Hardness (N)	23.98 ± 4.36 ^a	1.94 ± 0.23 ^b
Chewiness (kgf.mm)	9.84 ± 1.75 ^a	0.58 ± 0.09 ^b
Gumminess (kgf)	14.56 ± 2.35 ^a	0.92 ± 0.11 ^b
Springiness (mm)	0.67 ± 0.05 ^a	0.64 ± 0.04 ^a
Cohesiveness	0.61 ± 0.03 ^a	0.47 ± 0.06 ^b

Value are expressed as mean ± SD; n = 3

Different lower-case letters indicate significant difference between the samples

moisture content could be another factor for the lower G' value. The dynamic viscoelastic behavior of washed tilapia meat revealed a more pronounced structure build-up reaction compared with the unwashed meat [29]; similar observations have been reported by Karthikeyan et al. [30]. Protein gelation depends on several factors such as nature of protein, ionic strength, pH, heating rate, and binding agents [31].

Texture profiles of raw and cooked tilapia

The texture profiles of raw fillets and cooked fillets (steamed with 2% salt for 15 min) was analyzed (Table 3). Texture-profile analysis indicated a decrease in the hardness of tilapia meat upon cooking for 15 min with 2% NaCl. Hardness is defined as the resistance (N) at the maximum compression during the first compression of the sample [32]. In the present study, the hardness of raw fish was found to be significantly higher than that of cooked fish ($p < 0.05$). The results are in accordance with the previous report on tilapia steaks by Dhanapal et al. [33]. Cohesiveness represents the ratio of the positive force during the second compression to that during the first compression, whereas springiness represents the force at maximum compression during the second compression cycle. No significant difference was observed in the springiness of the raw and cooked samples of tilapia meat ($p > 0.05$). Chewiness measures the energy required to chew a solid sample to a steady swallowing phase [32]. The values of chewiness, gumminess, and cohesiveness of raw tilapia were significantly higher than that of the cooked samples ($p < 0.05$). Similar observations for tilapia steaks have been reported by Dhanapal et al. [33]. Decreased values of hardness, chewiness, gumminess, and cohesiveness upon cooking may be a result of protein denaturation. Decreased hardness, chewiness, and gumminess of the fish muscles may be due to the uncoiling of polypeptide chains. During cooking, fish muscles usually become soft as a result of a heat-induced conversion of connective tissues into gelatin. Decreased hardness values upon cooking have also been reported by Murthy et al. [34] for *Litopenaeus vannamei*.

The findings of this study will be useful in strategizing the use of cryoprotectants and water-washing steps for achieving the desired gelation properties of fish proteins.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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