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Research Article

# EFFECT OF REFRIGERATING KINETIC PARAMETERS (RELATIVE HUMIDITY AND AIR VELOCITY) ON *PANGASIUS* FILLET

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**The present study on the effect of different refrigerating kinetic parameters (relative humidity and air velocity) on *pangasius* fillet has been carried out to understand the refrigeration engineering aspects and their effects on fish quality. It has been revealed from the results that 90% RH and an air velocity of 2m/sec inside the chiller mostly retained the water holding capacity of fish fillets up to 14th day of refrigerated storage, whereas higher air velocity (90%) is required to retain the total collagen content intact. Fiber diameter and sarcomere length are most susceptible to high humidity and faster air speed inside the chiller.**

**Key words:** Refrigerating kinetic, *Pangasius* fillet

Marine species provide valuable components to human nutrition but are known to deteriorate rapidly post-mortem due to the effects of a variety of degradation mechanisms. Consequently, the actual increasing consumer's demand for high quality marine food has led to the development of advanced technologies that are able to present attractive, nutritional and safe products (Ashie *et al*., 1996 and Oms-Oliu *et al*., 2010). Refrigeration slows down the chemical and biological processes in foods and the accompanying deterioration and the loss of quality. The freezing of fish is an effective way of long term preservation and it has been shown that fish stored for up to three months under ideal conditions cannot be distinguished from fresh fish regarding colour, taste and texture (Cappeln *et al*., 1999 and Nielsen and

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Jessen, 2007).

During thermal processing operation, protein denaturation is considered to be one of the main reasons for quality changes in fish muscle. The denaturation of the proteins also leads to reduced water holding capacity and shrunken muscle fibers, subsequently leading to a harder and more compact tissue texture (Savitri, 2011).This study aimed to reveal the optimum application of combination of refrigeration parameters on *Pangasius* fish fillets. *Pangasius pangasius* is a catfish species of the family *Pangasiidae* under the order *Siluriformes*. It forms a good fishery of considerable value and is used to fetch high market price as a food fish due to its good taste and deliciousness with high protein, mineral and fat content in its flesh. It is also popular as a game fish. *Pangasius pangasius* is very hardy in nature; has high tolerance for temperature, salinity and turbidity; but due to over exploitation, habitat degradation, water pollution, destruction of the breeding grounds etc. natural populations of this fish species are facing the threat of extinction and its now high time to take proper measures on serious note to conserve its natural population (Gupta, 2016).

Several scientists and workers have studied on the effect of low temperature and time on fish muscle architecture and the shelf-stability of fish, but a combination of optimum refrigeration parameters viz. temperature, relative humidity, rate of cooling, air velocity inside the refrigerator and stocking density of the fish inside the cooler is yet to be standardized. Hence the present study has been designed to evaluate the changes in micro-structural level of *Pangasius* fish fillets after 0, 3, 7, and 10$^{th}$ days of refrigeration period.

## MATERIALS AND METHODS

The experiment was conducted in the Department of Fish Engineering, West Bengal University of Animal and Fishery Sciences, Chakgaria Campus, Kolkata, India from June 2017 to August 2017. *Pangasius* spp (*Pangasius bocourti*) of fish (local name – Basa fish) were procured live from local fish market of Kolkata, and then brought to the department. The fish were 1350±100 g in weight, 370±10 mm in length and 120±10 mm in width. The fishes were gutted, washed and then filleted by standard procedure under laboratory condition and fillets measuring approx. 2cm × 2 cm size were separated out, washed with water and categorized into different groups for subsequent trial. Low density polyethylene pouches were purchased from local market and UV sterilized before use. Approx. 1 kg of fillet sample was taken for each trial and a total of six nos. of such replications were carried out and each analysis was conducted on duplicate. All the chemicals and media used in the study were of analytical grade and procured from Hi-media laboratories, Pvt. Ltd., Mumbai. The experiment has been conducted to evaluate the effect of different parameters viz. relative humidity and air velocity on fish muscle architectures at chilling temperature

(0-4°C). Samples were analyzed on 0th, 3rd, 7th and 15th day of refrigerated storage. Humidity inside the chiller has been adjusted by humidifier system at 70%, 80% and 90% for different samples named C70, C80 & C90 respectively. On the other hand, the chiller unit is equipped with the controlled air velocity inside the chiller at 2m/sec, 5m/sec and 7.5 m/sec respectively for the samples termed as C2, C5 and C7.5 respectively.

All the 6 nos. of samples (C70, C80, C90 C2, C5 and C7.5) were exposed to the chilling temperature (0-4°C) at standard pre-defined conditions without altering the other parameters for which the particular sample was set for. The representative samples were kept under controlled atmosphere inside the chiller for comparing the effect of refrigeration parameters on *Pangasius* fish fillets architectures.

**Preparation of sample for physico-chemical analysis :** All the samples (6) have been minced separately in a Meat Mincer (M/S Stadler Corp.Ltd., Mumbai ) with 5 mm plate followed by common size 3 mm plate. The minced samples were kept in refrigerator (0-4°C) for subsequent analyses on the same day.

**Determination of physico-chemical characteristics of raw fish fillets:**

**pH:** pH of the minced fish samples were estimated by immersing the electrode of a calibrated (with known buffers of pH 7 and 4.01 before use every time) pH meter into aliquot of the sample as per the procedure described by Egbert *et al*. (1992).

**Expressible moisture (%):** Expressible water of the minced fish was measured using the method of Ramirez *et al*. (2002).

**Total collagen estimation:** The total collagen content was determined by acid hydrolysis as described by Palka (1999).

**Fibre diameter:** Fibre diameter of given fish muscle sample was measured as per the method outlined by Jeremiah and Martin (1977).

**Sarcomere length:** Sarcomere length was determined as per the method outlined by Cross *et al*. (1981) with certain modifications.

**Statistical analysis:** Statistical analysis of the data obtained was carried out using ANOVA technique according to the method described by Snedecor and Cochran (1989) by completely randomized design (CRD) for all parameters except storage stability study parameters for which Randomized Block Design was followed. To compare the means, Tukey's HSD test was adopted by using SPSS-16 software package. Six replications of the study were carried out and measurements for all the parameters were taken in duplicate each.

## RESULTS

Parameter wise results were tabulated in Table 1.

**pH :** pH of samples were found to be in the range of 6.16±0.085 for C70 sample to

6.25±0.067 for C5 sample in 3rd day chiller storage, whereas from 6.26±0.021 for C7.5 sample to 6.58±0.083 for C90 sample in 14th day. Analysis of variance indicated that pH value increased significantly ($p<0.05$) with the advancement of time throughout the storage period except in C7.5. Das *et al.* (2006) noticed an increasing trend in pH of ground buffalo meat pre-blended with different levels of carnosine. pH values of the carnosine-treated ground buffalo meat samples were significantly ($p<0.05$) higher than those of the control sample. The pH showed inconsistency as the carnosine level differed.

**Expressible moisture (%):** Water holding capacity affects both the economic and sensory attributes of meat (Van Oeckel *et al.*, 1999). It has been revealed from the results that C90 sample always holds higher expressible moisture content in fish muscle in chiller storage suggesting that high humidity (90% RH) could be a beneficial measure to retain the juiciness and other organoleptic parameters in prolonged storage.

**Total collagen content (mg/g):** It has been revealed from the data that total collagen content of fish fillets shown a diminishing tendency as the storage period progresses both in chiller storage. It might be due to cross linking of collagen and transforming into a more complex cellular matrix, leading to 'shrinkage' of muscle fiber. At 90% relative humidity (C90 sample) and at an air velocity of 7.5 m/sec (C7.5 sample), there were no significant effect on total collagen although arithmetic value of the data declined non-significantly ($p>0.05$).

**Fibre diameter (µm):** The fibre diameter of the samples found to be in the range of 42.51±0.159 µm on 0 day in refrigerated storage and decreased up to an extent of 37.28±0.146 µm on 14th day of refrigerated storage for C90 sample. It has been revealed from the results obtained that fibre diameter of the fish white muscle samples decreased significantly ($P<0.05$) with the advancement of storage period. C90 sample showed highly significant ($P<0.01$) variation in the fibre diameter. However,

**Table 1. Showing changes in *Pangasius* fillet architecture in refrigerated storages**

| Sample | pH | | | | |
|---|---|---|---|---|---|
| | Day 0 | Day 3 | Day 7 | Day 14 | Sig. |
| $C_{70}$ | | $6.16^{AP}$±0.085 | $6.30^{AQ}$±0.057 | $6.50^{BCR}$±0.077 | P<0.05 |
| $C_{80}$ | | $6.17^{APQ}$±0.057 | $6.23^{AQ}$±0.081 | $6.36^{AR}$±0.036 | P<0.05 |
| $C_{90}$ | $6.09^{AP}$ ± 0.098 | $6.21^{ABQ}$±0.059 | $6.35^{ABR}$±0.049 | $6.58^{CS}$±0.083 | P<0.01 |
| $C_{2}$ | | $6.22^{ABQ}$±0.061 | $6.39^{ABR}$±0.075 | $6.51^{BCS}$±0.022 | P<0.05 |
| $C_{5}$ | | $6.25^{BQ}$±0.067 | $6.41^{BR}$±0.082 | $6.47^{BR}$±0.024 | P<0.05 |
| $C_{7.5}$ | | $6.21^{Q}$ ±0.083 | $6.24^{Q}$ ± 0.053 | $6.26^{R}$ ±0.021 | NS |

**Cont. Table 1. Showing changes in *Pangasius* fillet architecture in refrigerated storages**

| Sample | Expressible moisture (%) | | | | |
|---|---|---|---|---|---|
| | Day 0 | Day 3 | Day 7 | Day 14 | Sig. |
| $C_{70}$ | $26.62^{AX} \pm 0.634$ | $25.21^{AX}\pm0.236$ | $23.24^{AXY}\pm0.142$ | $20.12^{AY}\pm0.163$ | P<0.05 |
| $C_{80}$ | | $25.16^{AX}\pm0.541$ | $23.41^{AXY}\pm0.281$ | $21.26^{AY}\pm0.212$ | P<0.05 |
| $C_{90}$ | | $25.85^{X}\pm0.263$ | $23.17^{X}\pm0.267$ | $23.78^{X}\pm0.241$ | NS |
| $C_{2}$ | | $25.16^{X}\pm0.432$ | $23.51^{X}\pm0.281$ | $22.11^{X}\pm0.252$ | NS |
| $C_{5}$ | | $24.15^{AX}\pm0.163$ | $22.71^{AXY}\pm0.312$ | $20.15^{AY}\pm0.314$ | P<0.05 |
| $C_{7.5}$ | | $26.01^{AX}\pm0.282$ | $20.15^{AY}\pm0.553$ | $17.01^{AZ}\pm0.285$ | P<0.01 |
| **Sample** | **Total collagen content (mg/g)** | | | | |
| | **Day 0** | **Day 3** | **Day 7** | **Day 14** | **Sig.** |
| $C_{70}$ | $3.32^{AP} \pm 0.046$ | $3.01^{BQ}\pm0.085$ | $2.94^{BQ}\pm0.157$ | $2.78^{CR}\pm0.105$ | P<0.05 |
| $C_{80}$ | | $3.05^{BQ}\pm0.085$ | $2.98^{BQ}\pm0.157$ | $2.84^{CR}\pm0.065$ | P<0.05 |
| $C_{90}$ | | $3.11^{Q}\pm0.086$ | $3.07^{Q}\pm0.064$ | $3.03^{Q}\pm0.081$ | NS |
| $C_{2}$ | | $3.06^{BQ}\pm0.053$ | $3.05^{BQ}\pm0.091$ | $2.91^{BCR}\pm0.075$ | P<0.05 |
| $C_{5}$ | | $3.12^{ABQ}\pm0.10$ | $3.06^{BQ}\pm0.023$ | $2.89^{CR}\pm0.045$ | P<0.05 |
| $C_{7.5}$ | | $3.21^{Q}\pm0.93$ | $3.11^{Q}\pm0.065$ | $3.02^{QR}\pm0.033$ | NS |
| **Sample** | **Fibre diameter (µm)** | | | | |
| | **Day 0** | **Day 3** | **Day 7** | **Day 14** | **Sig.** |
| $C_{70}$ | $42.51^{AP}\pm 0.159$ | $41.25^{AP}\pm0.176$ | $40.75^{AP}\pm0.198$ | $39.45^{BQ}\pm 0.145$ | P<0.05 |
| $C_{80}$ | | $41.38^{AP}\pm0.204$ | $41.04^{AP}\pm0.117$ | $38.81^{BCQR}\pm0.234$ | P<0.05 |
| $C_{90}$ | | $40.86^{AP}\pm0.256$ | $38.21^{BQ}\pm0.139$ | $37.28^{CR}\pm0.146$ | P<0.01 |
| $C_{2}$ | | $41.53^{AP}\pm0.194$ | $41.24^{AP}\pm0.241$ | $40.17^{BQ}\pm0.295$ | P<0.05 |
| $C_{5}$ | | $40.28^{ABP}\pm0.614$ | $40.14^{ABPQ}\pm0.281$ | $39.15^{BQ}\pm0.430$ | P<0.05 |
| $C_{7.5}$ | | $41.214^{AP}\pm0.342$ | $40.51^{ABPQ}\pm0.165$ | $39.59^{BQ}\pm0.241$ | P<0.05 |
| **Sample** | **Sarcomere length (µm)** | | | | |
| | **Day 0** | **Day 3** | **Day 7** | **Day 14** | **Sig.** |
| $C_{70}$ | $2.21 \pm 0.054^{AP}$ | $2.16^{P}\pm0.062$ | $2.05^{PQ}\pm0.081$ | $1.91^{Q}\pm0.092$ | NS |
| $C_{80}$ | | $2.19^{P}\pm0.092$ | $2.10^{PQ}\pm0.076$ | $1.89^{QR}\pm0.092$ | NS |
| $C_{90}$ | | $2.15^{AP}\pm0.085$ | $2.02^{ABQ}\pm0.059$ | $1.85^{CR}\pm0.076$ | P<0.05 |
| $C_{2}$ | | $2.16^{P}\pm0.091$ | $2.01^{QR}\pm0.064$ | $1.95^{Q}\pm0.097$ | NS |
| $C_{5}$ | | $2.10^{BQ}\pm0.063$ | $2.00^{BQ}\pm0.058$ | $1.91^{CR}\pm0.083$ | P<0.05 |
| $C_{7.5}$ | | $2.18^{ABP}\pm0.058$ | $2.08^{ABQ}\pm0.086$ | $1.94^{CR}\pm0.077$ | P<0.05 |

*Values sharing common superscripts between and within rows do not differ significantly

when compared in a specific storage day, data obtained from different samples group were not significantly different (P>0.05).

**Sarcomere length (µm):** The sarcomere length of the samples found to be in the range of 2.21±0.054 µm on 0 day in refrigerated storage and decreased up to an extent of 1.85±0.076 µm on 14th day of refrigerated storage for C90 sample. The decrease in sarcomere length in refrigerated storage may be attributed to the muscular fragmentation index supported by several earlier studies on fish or other species meat.

## DISCUSSION

There were significant effects (p<0.05) of carnosine treatment and treatment × storage interaction on pH of meat. Kelleher *et al.* (2004) postulated that, the flesh of six white-muscled fish had pH's at the time of processing above pH 6.6 and greater than 80% of their myofibrillar/cytoskeletal proteins were soluble in water. The flesh of three pelagic species and a shark had pH values when processed below 6.6 and the water solubility of their myofibrillar and cytoskeletal proteins was less than 40%.

Although the values for expressible moisture content of C90 sample declined gradually with the advancement of storage period but the changes were non-significant (p<0.05). On the other hand high air speed inside the chiller (7.5 m/sec) resulted into a highly significant (p<0.01) loss in expressible moisture of fish muscle. A moderate speed of 5 m/sec also revealed significant (p<0.05) loss, but low air velocity inside the chiller revealed no significant decline in the results.

The decrease in muscle fibre diameter of fish fillets may be attributed to the reason that muscle fibre shrinkage. The reduction of muscle fibre CSA observed during the refrigeration results from a lateral shrinkage of myofibrils whose amplitude depends on the slaughter stress of animals and of the stunning technology (Guignot *et al.*, 1993). A significant decrease in the diameter of type IIB fibres was found 96h compared to 24h post-mortem that muscle fibre diameter decreases during storage of meat is evidenced by studies of Hegarty (1970) and Swatland and Belfry (1985). Diesbourg *et al.*(1988) suggested that muscle fibre diameter decreases during post-mortem as a result of shrinking lateral connections of myofibrils with the cell membrane. Likewise, Huff-Lonergan and Lonergan (2005) hold that post-mortem muscle fibre size depends on the extent of degradation of cyto -skeletal proteins which were connect myofibrils to the sarcoplasm.

The aging phase is characterized by various ultra-structural changes and results in the fragmentation of muscle fibers. The action of different proteolytic systems results in characteristic myofibrillar ruptures along the Z lines (Listrat *et al.*, 2016). Mitochondria are deformed and their membranes altered (Abbott *et al.*, 1977 and Ouali *et al.*, 2013). As a consequence of the degradation of costameres, that is, the junction of cyto-skeletal proteins to the

sarcolemma, the sarcolemma separates from peripheral myofibrils (Taylor *et al*., 1995). Muscle shrinkage reduces meat tenderness rapidly because sarcomere length is shortened by the overlapping of actin filaments in the center of the I-band and myosin filaments close to the Z-disk (Marsh and Carse, 1974). Lampila and Brown (1986) also observed some distortion of the sarcomere during thermal processing evidenced by greater magnification and high resolution. The sarcomere has been extremely shortened and large spaces have developed between each structural unit. This may thus indicate the combined effects of freezing and thermal processing on the muscle structure.

A better understanding of changes in micro-structural deformation along with micro-elements of muscle cells would be possible on different cooling temperature exposures and different parameters. Besides, study on different species muscle would support and enrich the findings of the present study.

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