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Optimizing Quality and Shelf Life of Pomfret (*Paratromateus argenteus*) Fillets through Ultrasound Processing

Vivek Kumar[1], M.K. Yadav[2*] and Anand Rao Azad[2]

[1]Assistant Technical Manager, Agriculture Technology and Management Agency Khagaria (Bihar), India.
[2]Assistant Professor, Sanjeev Agrawal Global Educational (SAGE) University Bhopal (Madhya Pradesh), India.

(Corresponding author: M.K. Yadav)*
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ABSTRACT: Pomfret *(Paratromateus Argenteus)* is one of the important seafood popular among the people and traded worldwide. It is high value commodity with good demand for fresh, chilled and frozen fish in world sea food market. The packaged samples were treated with frequency of 28 kHz and 40 kHz at time interval of 10 min (10, 20, 30, and 40 min) at room temperature (30°C). Untreated samples and the ultrasound (US) treated samples were analyzed for quality changes in terms of physical (colour, texture), biochemical content (TVB-N), and microbiological (total plate count) attributes. In results it was found that ultrasound processing is effective in enhancing microbiological safety and quality of seafood. Results reveled that US is very effective in reducing microbial loads in pomfret fillets with maximum reduction at 40 kHz treated for 40min. For storage stability and self-life evaluation the changes in different quality attributes of LDPE packed pomfret fillets was assessed at refrigerated storage (4 °C) interval of 5 days for 25 days, in significant relationships were observed between color and texture.

Keywords: TVB-N, Biochemical, Cavitation, Quality and Reduction.

INTRODUCTION

Pomfret (*P. argenteus*) is commercially important fish species having persistent demand in global market due to its distinct taste, flavor, texture and nutritive value. Traditional thermal preservation technique has detrimental effects on organoleptic quality and is therefore unacceptable to many consumers hence to overcome these limitation an alternative methods for pasteurization and sterilization are gaining importance; due to increased consumer demand for new methods of food processing that have a reduced impact on nutritional content and overall food quality. Ultrasound processing or sonication is one of the alternative technologies that have shown promise in the food industry (Barbosa-Canovas *et al.,* 1997; Carcel *et al.,* 2007).

Fish is an important source of nutrients and generally has a relatively low calorific value and good organoleptic properties appreciated by consumers. Fish contains a high percentage of proteins with a high biological value vitamins and regarding lipid content fish oils have high levels of omega 3 fatty acids along with omega 6 fatty acids cannot be synthetized by the body and must be included in the diet (Murray and Burt 2001). Fish a very highly perishable product from the moment of its capture, enzymes, micro-organisms and oxidative processes act quickly causing deterioration in quality. The main cause of spoilage in fish is due to microbial growth and metabolism which results in formation of sulphides, alcohols, amines, aldehydes, organic acid and ketones. These compounds produce odours and off-flavours which are likely to be unacceptable for consumers (Gram and Dalgaard 2002; Briones *et al.,* 2010). Conventional thermal pasteurization and sterilization are the most common techniques currently used to inactivate microorganism in food products, however the demand for new methods that have reduced impact on the nutritional content and overall food quality is increasing. New preservation techniques have been developed that could eliminate microbial activity while significantly reducing or completely eliminating the amount of heat required. These processes are, for the most part, less energy intensive and therefore more cost efficient and environmental friendly than conventional thermal processing. Thermal processing does kill vegetative microorganism and some spores; however its effectiveness is dependent on treatment temperature and time.

There are two different type ultrasound machine used in laboratory one is the ultrasound bath type system and the second is the ultrasound probe type system. Ultrasound bath system are commonly used for solid dispersion into solvent and it is easy to handle & economically advantageous (Chemat *et al.,* 2011 & Chemat *et al*., 2017). It delivered intensity is low & highly attenuated by water contained in the bath. Probe system also known as horn system is much more powerful and because ultrasonic intensityis delivered on small surface compared to bath system. Less attenuation happen because probe is directly immersed in reaction flask. These pressure changes cause cavitation and gas bubble formation in the medium. Two types of cavitation bubbles are known for

ultrasound applications, *viz*., transient and stable cavitation (Zhao *et al.,* 2010).

These cavitation bubbles generate a series of physical and chemical effects which are the basis for the application of ultrasound to decontaminate surfaces on food products (Zhou *et al.,* 2012 & Alliger, 1975). Currently research on high intensity ultrasound is mainly focused on liquid food processing and product surface decontamination however few study using ultrasound for microbial detachment from surfaces have been published, with a small number having investigated the effect of ultrasound on fish and fish product quality attributes.

Pomfret *(P. argenteus*) are the most edible fish in India and fetch the highest price in markets, their popularity stemming from the clean white appearance of the body, firmness of flesh and flavours. They are inshore species, usually found in schools over the muddy water. It is one of the most popular fish species and possesses high economical, nutritive, and edible value. Pomfret rich with unsaturated fatty acids and microelements, such as selenium and magnesium, which protect against hyperlipidemia, high cholesterol, coronary atherosclerosis, and other cardiovascular 4 diseases (Gudmundsson *et al.,* 2002; Zhao *et al.,* 2010). The demand of pomfret has increased during recent years, even with the decline of pomfret production due to overfishing and ecological changes. Keeping in view the above the project has been undertaken with the following objective one to study the effect of ultrasound processing on quality attribute of Pomfret fillets and objective second to study the shelf life of ultrasound treated Pomfret fillets through refrigeration storage study (Erkan and Ozden, 2008 & Erkan and Uretener, 2010).

MATERIAL AND METHODS

The experimental methodology including raw material procurement, sample preparation, instruments, chemicals, and reagents used, experimental design applied to obtain specific targets, analytical methods different analytical and modeling tools used in the study. The entire work was undertaken in two phases. In first phase the effect of ultrasound having frequency 28 kHz and 40 kHz for different time interval of 10 to 40 min was evaluated on physical, chemical and microbiological quality of pomfret fillets. Phase second the study include shelf life extension of all sample under refrigerated storage for 25 days.

A. Ultrasonication Bath System

A low frequency (28 kHz and 40 kHz) ultrasound bath having 3 liters capacity and having ultrasonic power of more than 10 watt per square cm were used in this experiment. The 28 kHz ultrasound machine (Model: UD80-SH-3L; Make: Takashi) having length of 27 cm width of 16.5 cm and height of 22 cm was used for treatment Similarly, a 40 kHz ultrasonication machine (Model: EL-5LH; Make: Electronics industries, Mumbai) having tank size of 250 mm length 150 mm breadth and 150 mm height and tank capacity of 5 liters and ultrasonication power of 150 W was used for treatment (Coakley *et al.,* 1989).

B. Sample Preparation

Freshly Pomfret *(P. argenteus)* fish with an average length of 15 cm breadth 10.5 cm and weight 72 g were procured from local seafood supplier from local seafood market and transported to laboratory and kept under chilled conditions till further processing. The sample were washed with chilled water, beheaded, Shelled and packed in LDPE films. Then the pomfret fish fillets were having average weight of sample was 61.3 gram, average length of 5 cm and width 4 cm. The entire pomfret sample was from the same batch and fillets were made of same dimension. The samples were cut aseptically inside the laminar flow inorder to reduce the contamination. The fillets were packaged in LDPE films prior to treatments. All the equipment was sterilized during the whole study to minimize the contamination.

C. Sample Treatments

Sample is thoroughly washed with distilled water before and after filleting and then it was packed in low density polyethylene (LDPE) with one pomfret fillets per packet were sealed and stored in deep freezer till further processing.

D. Storage conditions of samples

After ultrasound treatment, all samples were stored at refrigeration temperature (4 °C) for shelf life study and the reading of four analysis (colour, texture, TVB-N and microbiological) of all the sample was taken at 5 days interval till 25th day of the storage period. On each analysis day (0, 5th, 10th, 15th, 20th and 25th day), sample taken at random from packaged samples of different treatment time (0, 10, 20, 30 and 40 min) each containing pomfret fillets of equal dimensions were analyzed for changes in total plate count, colour, texture and TVB-N content and compared with those of the raw pomfret fillets (Control sample) stored at refrigeration temperature and also with sample US treated sample for different duration. Two Control samples were kept one at 4 °C and another at 27 °C were analyzed and after 10 days of storages because of their microbial and chemical spoilage these samples are not analyzed.

E. Microbiological analysis

Raw and treated samples were analyzed for total plate count (TPC) as per American Public Health Association, 2001 (APHA, 2001) methods. The microbiological counts were enumerated by serial dilution pour plate methods. A sample of 5 g was aseptically cut from ultrasound treated sample of different duration and macerated with 45 ml distilled water (0.1%) in a sterile glass mortar and was then serially diluted up to 5 dilution (1:5) per sample. Tryptone glucose agar was made by mixing 24 g in 1000 ml of distilled water. One ml of suitable dilutions was pour plated on tryptone glucose agar (TGA) and plates were incubated at 37 °C for 36-38 h. All samples were analyzed in duplicates and results were expressed as log of colony forming unit per gram (CFU/g) of sample. Total mesophilic viable counts psychrophilic viable counts were determined. For analysis five-fold dilution series of sample were prepared.

F. Colour measurements

The CIE color parameters L* (brightness), a* (+a, red; -a, green), and b* (+b, yellow; -b, blue) of sample surface were measured using portable colorimeters (Model: Spectro-guide 45/0 gloss; Make: BYK Gardner, Germany) in reflection mode with D65 illuminant and 10° standard observer angle. The colorimeter was standardized using white ceramic plate (L*= 95.69, a*=0.83 and b*=1.04). The measuring orifice was 8 mm wide. Results were expressed as the mean of the four measurements two from each side of the fillets was measured. After US treatments, fish samples were kept at cold freezers before further treatment. Results are presented as mean values of measurements taken from four different locations on each sample. All experiments were performed in triplicate.

To illustrate the degree in colour change the total colour difference (ΔE*) was computed using equation.

$$\Delta E* = ((\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2)^{0.5}$$

Where $\Delta L*$, $\Delta a*$, and $\Delta b*$ are the difference in the L*, a* and b* values between treated samplesand control.

G. Total volatile basic nitrogen (TVB-N)

TVB-N was determined according to Conway micro-diffusion method as proposed by Beatty and Gibbons. Triplicate analysis was performed on all the samples and results were expressed in mg N/100 g of sample. The sample extract were prepared by homogenizing 10 g of silver pomfret muscle with 10 ml of 20% trichloroacetic acid. The homogenate was filtered using whatman filter paper number 41 and filtrate was used for analysis. One ml of prepared sample extract and one ml of saturated potassium carbonate was pipetted into the outer ring of micro- diffusion unit, the inner ring of which contained 2 ml of boric acid solution prepared by mixing boric acid, a methyl red and bromocresol green indicator. The unit was immediately covered and sealed using silica grease and rotated gently to mix the solution of the outer ring, taking care that solution is not mixed from one ring to the other. The inner ring solution (green colour) after incubation of 90 min at 37 °C was titrated using 0.02 N sulfuric acid until it turned pink. A blanktest was also carried out using 1 ml of 2% trichloroacetic acid (TCA), instead of sample extract. The following formula was used to find TVB-N content.

TVB-N (mg %) = 14 * Normality of H_2SO_4 * Volume of H_2SO_4 * 100

H. Texture profile analysis

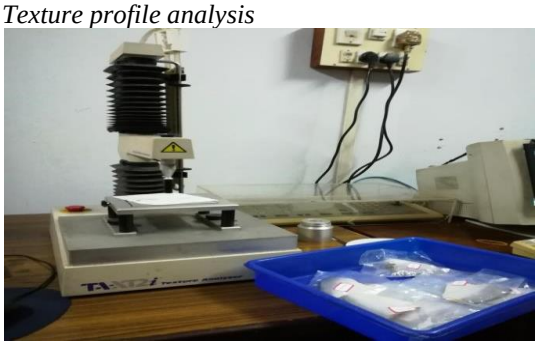

Fig. 1. Texture analyzer (Model: TA.TX-2; Make: Stable micro system, UK).

The texture of pomfret fillets before and after processing were evaluated using texture analyzer (Model: TA.XT-2; Make: Stable Micro Systems, UK) equipped with 25 kg load cell capacity. Samples were equilibrated at room temperature for 1 h before analysis. A double-bite test was conducted where pomfret fillets were compressed using cylindrical probe of diameter 6 mm to 30% of its original height with a test speed of 2 mm/s. Three readings were taken on each fillet and From TPA curve obtained (Fig. 2) textural parameters such as hardness, gumminess, springiness and chewiness was calculated. The hardness value is the peak force of the first compression of the product. The hardness need not occur at the point of deepest compression, although it typically does for most products.

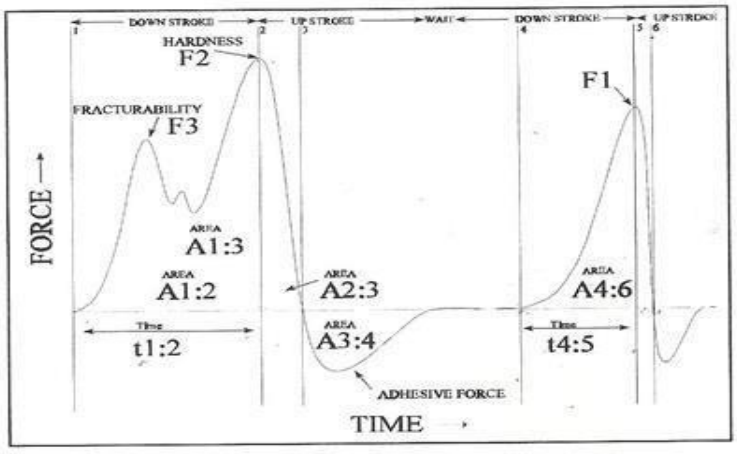

Fig. 2. Texture profile curve generated by texture analyzer (Kaur *et al.*, 2013).

Hardness defined as the force necessary to attain a given deformation. Cohesiveness is how well the product withstands a second deformation relative to how it behaved under the first deformation. It is measured as the area of work during the second compression divided by the area of work during the first compression. (Refer to Area 2/Area 1 in the graph below (Fig. 2)). Cohesiveness defined as the ratio of areas under the second and first curve. Gumminess only applies to semi-solid and is the product of hardness cohesiveness. Gumminess is mutually exclusive with Chewiness since a product of the both a semi-solid and

a solid at the same time. Springiness is how well a product physically springs back after it has been deformed during the first compression. The spring back is measured at the down stroke of the second compression, so the wait time between two strokes can be relatively important.

In some cases an excessively long wait time will allow a product to spring back more than it might under the conditions being researched, springiness defined as a material returns to its original condition after the deformation force is removed and calculated as length 2/length 1 from Fig. 2. Chewiness: gumminess × springiness. The reading was taken on each fillet and analysis was conducted in triplicates and results were expressed as their mean.

I. Statistical Analysis

Two-Analysis of variance (ANOVA) of the data was carried out using SPSS 17 for Windows software package (SPSS Statistical Software, Inc., Chicago, IL, USA). The results were expressed or plotted as the mean values ± standard deviation.

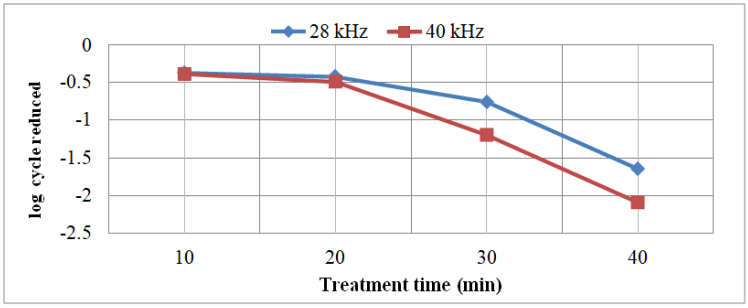

Fig. 3. Mean log reduction of total plate count.

The microbial growth increased significantly ($p<0.05$) during storage and this increase was found to be higher in raw pomfret fillets that is controlled sample which was stored at 27 °C reached the unacceptable limit by day 5 of storages (7.11 log CFU/g) however the controlled sample which was kept at 4 °C reached unacceptable limit by day 10 of storage (7.62 log CFU/g)with much lower count in ultrasound treatment sample (4.02 and 3.88 log cfu/g for 28 kHz and 40 kHz, respectively. Both frequency treated sample increased the shelf life of the pomfrets fillets however sample treated at 40 kHz showed counts below the critical limit throughout the storage period studied (6.59 log CFU/g on day 25). As stated by international commission of Microbiological Standards for Foods (ICMSF, 1978), the maximum acceptable limit of microbial loads in fresh and refrigerated seafood is 7 log CFU/g. The bactericidal effects of ultrasound have been previously demonstrated at laboratory scale (Raso *et al.*, 1998). This technology has also been combined with heat or pressure to enhance microbial inactivation (Sala *et al.*, 1995a). A number of previous studies have assessed the ability of ultrasound to reduced microbial levels on fruits and vegetables. The effectiveness of ultrasound for reducing or inactivating microbial growth has been previously reported, in safety of poultry (Haughton *et al.*, 2010), pork (Morild *et al.*, 2011).

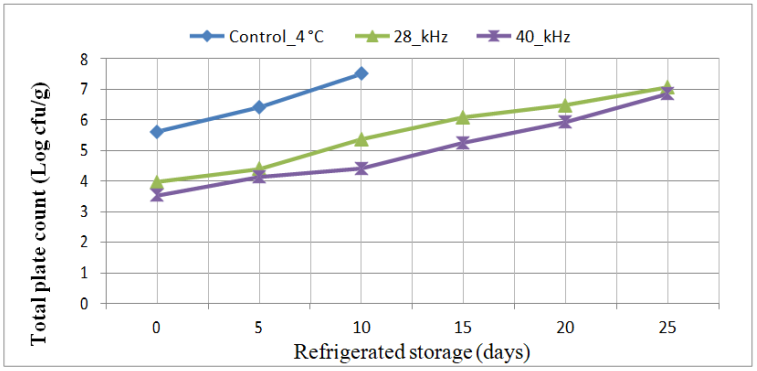

Fig. 4. Total plate count growth curves of pomfret fillets stored at 4 °C.

J. Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N), a parameter that quantifies the compounds composed of ammonia and its derivatives is used as a biochemical index to determine keeping quality and shelf life of fresh fish and seafood. The Fig. 5 show the variation in TVB-N content with different treatment time at different frequency. Initially TVB-N content of fresh silvery pomfret is 6.34 mg N/100 g suggesting good quality. Initial TVB-N content was reduced to 3.64 mg N/100 g which is 42.58 % and 3.36 mg N/100g which is 47.70 % of initial values when treated atfrequency of 28 kHz and 40 kHz respectively for treatment time of 40 minutes. The highest difference (0.56 mg N/100 g) and lowest difference (0.10 mg N/100 g) of TVB-N content between both the frequencies was observed at treatment time of 20 and 30 min respectively.

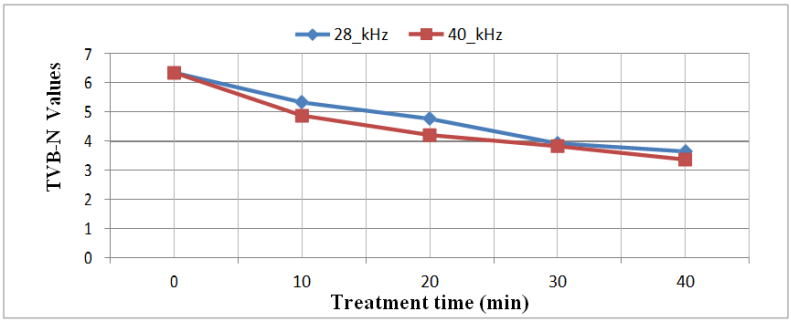

Fig. 5. Variation in TVB-N content (mg N/100 g) with different treatment time at different frequency.

The variations in TVB-N values for treated and raw pomfret were studied under refrigeration storage shown below (Fig. 6). Control sample was stored at two different temperatures (4°C, 27°C) whereas the entire treated sample was stored at 4°C. The control sample which was stored at 27°C crosses the acceptable level 30 mg N/100 g of flesh suggested for fish products (Li *et al.*, 2013; Aubourg *et al.*, 2013) within five days of storage (33 mg N/100 g) while the control sample which was stored at 4°C was remain safe up to 10 days of storages and crosses the acceptable limit after 15 days (40.60 mg N/100 g) of storages. TVB-N values increases slowly till 10 days reaching the values of 17.52 mg N/100 g (classified as good) and then increases rapidly due to autolytic deamination caused by proteolysis (Bugueno *et al.*, 2003; Ando, 1999). TVB-N values increased significantly in all treated samples but did not reach the levels as those of the control sample. The ultrasound treatment of 40 kHz was found to be more effective in reducing the production of TVB-N. Samples treated at 28 kHz remained in good category till 20 days of storages with TVB-N values of 27.92 mg N/100g. However samples treated at 40 kHz remained in good category throughout the storage duration till done (25 days) much lower values TVB-N values of 27.16 mg N/100g. The effect of ultrasound treatment on TVB-N has been given in Fig. 6 below. For both the cases, ultrasound was found to be most influential factor. Ultrasound treatments were found promising to obtain minimum TVB-N values. The 28 kHz frequency was also effective in minimizing TVB-N content of the pomfret fillets whereas it was more prominent in 40 kHz treated frequency for 40 min. This reduced TVB-N values might be due to reduction in microflora and their putrefying activity caused by cavitation phenomenon. The apparent increase in TVB-N during storage is more possibly initiated by microbial associations, autolytic activities, and complete microbial reductions of trimethylamino oxide to tetramethylammonium. The spoilage pattern of fresh seafood generally shows an increase in TVB-N concentration, which closely parallels the bacterial population (Siripatrawan *et al.*, 2009). Sala *et al.* (1995b) reported increased TVB-N in high intensity ultrasound treated salmon, mackerel, cod and hake fillets. They also added such difference in above two parameters was due to microbial activity. None of the treated samples reached the upper limit of acceptability of TVB-N (30–35 mg N/ 100g) (Connell, 1995) for fish.

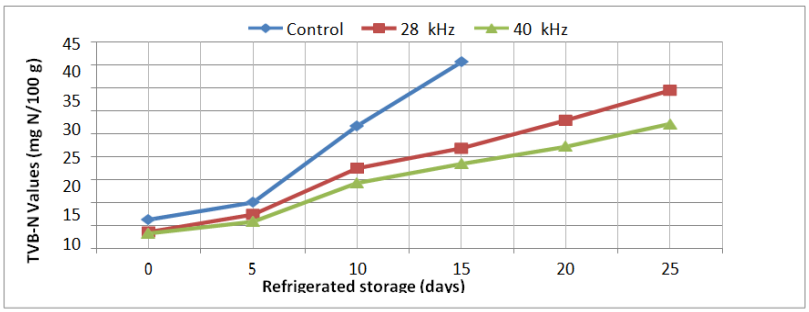

Fig. 6. Variation in TVB-N content (mg N/100 g) with different treatment time at different frequency.

Colour analysis. Raw pomfret fillets had lowest L* values which increased significantly ($p < 0.05$) after the ultrasound treatment. The increased was found to be highest (9.65%) in high frequency that is 40kHz treated sample for 40 min it was found only 4.07% in case of 28 kHz treated sample for same time. The value of b* also increases significantly after ultrasound treatment the initial value of b* was 1.40 which increases by 68.57 % to 2.36 in case of 40 kHz ultrasound treatment for 40 min however this change was less in case of 28 kHz ultrasound treatment, a change of 33.57 % was observed in this case. The increase in brightness by ultrasound treatment was due to both loss of active pigment and protein coagulation changed the sample surface properties, increasing light reflection and creating white appearance (Kruk *et al.*, 2011).

Pomfret fillets showed the decreased a* values in treated samples indicating loss of redness. The a* values decreases to 50.61 % and 51.23 % in 40 kHz and 28 kHz treated sample respectively for treatment time of 40 min. A reduction of a* values observed in salmon after ultrasound treatment due to reduction of total lipid content suggest decrease in oxidation values (Pedros-Garrido *et al.*, 2017). Angsupanich, and Ledward (1998)

suggested that these changes in colour parameters probably due to denaturation of the myofibrillar and sarcoplasmic proteins. However changes in color by thermal treatment were correlated to globin denaturation, precipitation of sarcoplasmic proteins, protein coagulation and/or haem displacement (Eni *et al.*, 2010). Lipid oxidation is another possible reason suggested for the colour due to degradation of highlysaturated carotenoids (Cruz-Romero *et al.*, 2007). In the present study also, the colour changes observed with in ultrasound processed pomfret fillets correlates well with the recorded results.

Further statistical analysis of total colour difference (ΔE) values showed that there was significant ($P < 0.05$) increase in these parameters with ultrasound treatment. The smaller values of ΔE were lesser in the deviation in colour with respect to the reference. Silva and Silva (1999) classified the values ΔE as imperceptible, a very small, a small, distinct, very distinct, great, and very great colour difference as shown in Table 1 below.

A great (7.01) colourdifference was observed in sample which was treated at 40 kHz for 40 min and very small (0.55) colour difference was found in sample treated at 28 kHz for 10 min. In storage conditions great colour difference was observed in sample treated at both the frequency. In a sample which was treated at 28 kHz frequency a colour difference of 10.01 was observed and in 40 kHz treated sample a colour difference of 11.55 was observed on 25 day of storage as compared to 0 day of storage. Pedros-Garrido *et al.* (2017) reported similar results in high intensity ultrasound treated salmon, mackerel, cod and hake fillets.

Table 1: ΔE values and their corresponding color difference (Silva and Silva 1999).

ΔE Values	Color difference
0-0.2	imperceptible
0.2–0.5	very small
0.5–1.5	small difference
1.5–3.0	distinct
3.0–6.0	very distinct
6.0–12.0	great
>12	very great

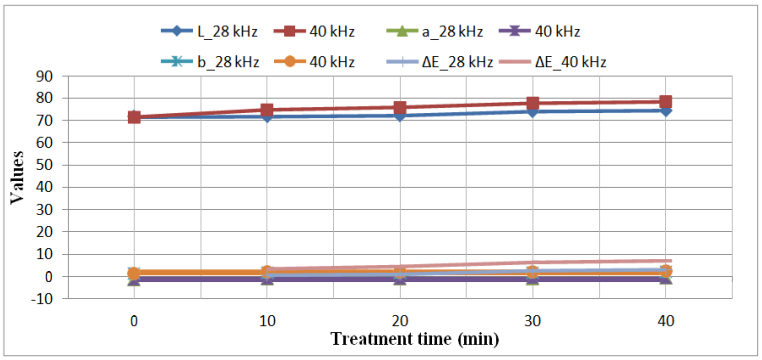

Fig. 7. Showing variation in different colour parameters with treatment time.

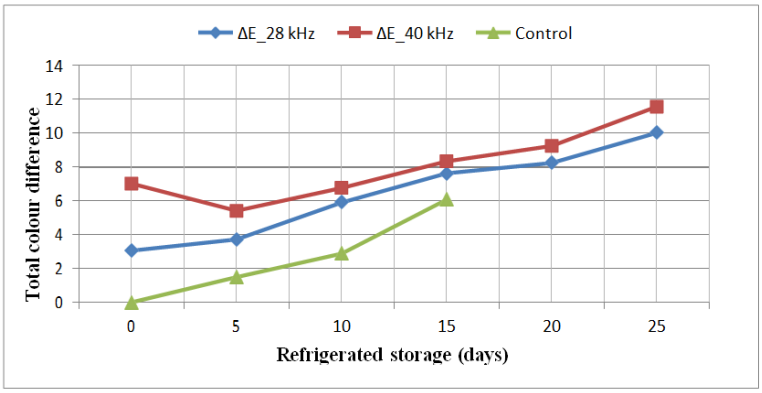

Fig. 8. Showing variations in total colour difference in storage.

During storage, increased L* and b* values and reduced a* values were obtained for all the samples. Raw samples showed slight change in L* values and significant ($P < 0.05$) changes in a* and b* values and developed a darker appearance till 25 days. A statistically significant ($P < 0.05$) increase of 5.08% and 2.83% for L* and 51.82% and 43.30% for b* and decrease of 69.14% and 65.88% for a* was obtained on 25 day for pomfret fillets processed at 28 kHz, 40 kHz, treatment respectively compared to day 0 samples for 40 min treatment time at the end of storage. Pedros-Garrido *et al.* (2017) reported similar results in high intensity ultrasound treated salmon, mackerel, cod and hake fillets. These changes in L*, a*, and b* parameters by high frequency might be due to "reversible" denaturation process of myoglobin. The a* values is also related with the astaxanthin amount in fresh atlantic salmon (Xiang *et al.*, 2012). Protein solubility, metmyoglobin (MetMb) and oxymyoglobin (OMb) were largely associated with changes in L* and b* by thermal treatment; more precipitation of sarcoplasmic proteins, increased MetMb, and a decrease in OMb resulted in increased L* and b* (Dai *et al.*, 2013). Compared to ultrasound treated samples higher L*

values in thermally treated samples might be due to the quick denaturation of heme proteins (hemoglobin and myoglobin) and oxidation of carotenoids. Astaxanthin is an important carotenoid which contributes to the redness index in salmon muscle (Yagiz *et al.*, 2010) and is also a lipophilic compound (Page & Davies 2006). Anincreased L* and b* value and decreased a* value with increased frequency has been reported for different seafood species such as bluefish and salmon.

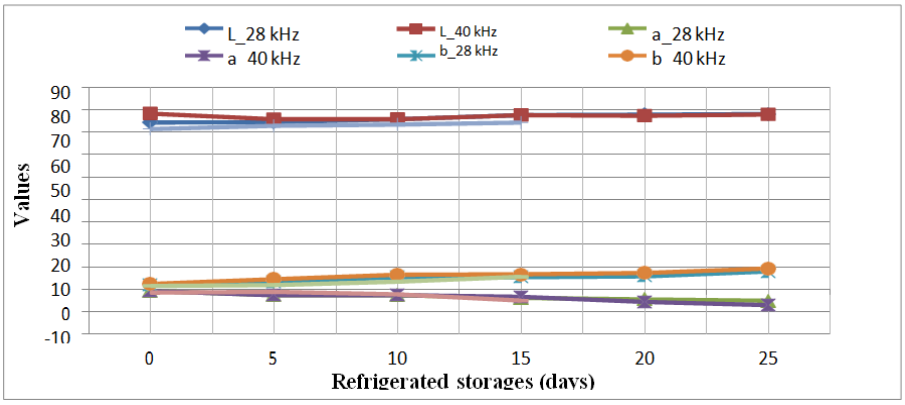

Fig. 9. Changes in colour parameters with storage time.

Textural profile analysis. Hardness is most critical textural attribute in fish or seafood and can be defined as a property which depends on the connective tissues consisting of mainly collagen and the myofibrils composed of myosin and actin ultrasound processing significantly ($p < 0.05$) changes the different textural parameters. The highest hardness 33.35 N an increase of 22.11% compared to control sample was observed after treatment of 40 minutes however in case of 28 kHz treated sample an increase of 20.50% (32.91 N) was observed and the least hardeness of 28.72 N which was 6.26 % more than the control sample (27.31 N) was observed at treatment of 28 kHz for 10 min. was observed in control sample as compared to treated sample. The increase in the value of hardness as compared to control treatment was due to increase in temperature during the ultrasound treatment as well as due to connective tissues as explained above. Similar trend is found in other textural parameters such as cohesivness, springiness, gumminess and chewiness as shown in the Table 2 below. Cohesivness shows an increase of 8% and 15.27% at 28 kHz and 40 kHz frequency respectively however in case of Springiness an increase of 59.90% and 26.30% was observed at 28 kHz and 40 kHz respectively as compared to control sample for treatment time of 40 min. In case of Gumminess an increase of 30.56% and 41.03% was observed at 28 kHz and 40 kHz respectively however highest increase was found in case of chewness which increases 108.81% and 78.16% at 28 kHz and 40 kHz respectively for 40 min treated sample (Table 2). Springiness and chewness are tested to determine the gel textural characteristics of muscle protein increased in springiness values in high frequency treated pomfret fillets led to increases in gumminess and hardness which might be due to unfolding action of actin and sacroplasmic protein formation of the hydrogen bonded networks. Ozuna *et al*. (2013) studied the influence of high intensity ultrasound application on textural properties of pork meat brined at different NaCl concentration and found similar results. Protein networks formed by ultrasound treated are different from protein network formed with heat treatment. The difference in gel structure resulted in higher value of textural parameters for ultrasound treated sample as compared to thermally treated pomfrets fillets. In pork, ultra sonication process treatment resulted in decreased activity of certain proteolytic enzymes which led to firmer texture (Zhang *et al.*, 2014). This increase in hardness in treated samples compared to control could be due to myofibrillar protein denaturation and aggregation**.**

Tables 2: Changes in textural parameters of ultrasound treated pomfret fillets.

Properties	Frequency	Treatment time				
	(kHz)	0 min	10 min	20 min	30 min	40 min
Hardness (N)	28	27.31	28.72	29.21	31.33	32.91
	40	27.31	29.02	29.61	31.10	33.35
Cohesiveness	28	0.491	0.501	0.516	0.522	0.531
	40	0.491	0.552	0.554	0.561	0.566
Springiness	28	0.612	0.747	0.795	0.824	0.979
	40	0.612	0.751	0.752	0.768	0.773
Gumminess(N)	28	13.38	14.38	15.07	16.35	17.47
	40	13.38	16.01	16.40	17.44	18.87
Chewiness(N)	28	8.189	10.74	11.98	13.47	17.10
	40	8.189	12.03	12.31	13.39	14.59

During storage reduction was observed in all the textural parameters and this decrease was significantly ($P < 0.05$) higher in control samples followed by high frequency treated samples. Control samples showed reduced hardness by 46.64% on day 15 of storage and, reduction in hardness by 26.37% and 24.671% was observed in samples processed at 28 kHz, 40 kHz, and respectively at the end of storage compared to day 0 samples as shown in Fig. 10 below. These changes in texture during storage might be due to enzymatic degradation which induces many physical mechanisms, among them the gaping, which contribute to muscle tenderization (Ozuna *et al.*, 2013). The variation of textural properties of fish muscle derived from intrinsic differences mainly depends upon the structure of fish muscle tissue, which is attributed to the internal factors related to the structures of contractile protein, the framework of connective tissue, lipid oxidation, and some external factors, such as methods of sample handling and conditions of cold storage (Aussanasuwannakul *et al.*, 2012). Among texture attributes, firmness also termed as hardness, an essential evaluating parameter of fish freshness is closely associated with the human visible acceptability of fish products. This vital index depends largely on the structure of connective tissue (Casas *et al.*, 2006). Some studies (Johnston *et al.*, 2000) have indicated that textural attributes pose significant positive correlations with the density of muscle fiber.

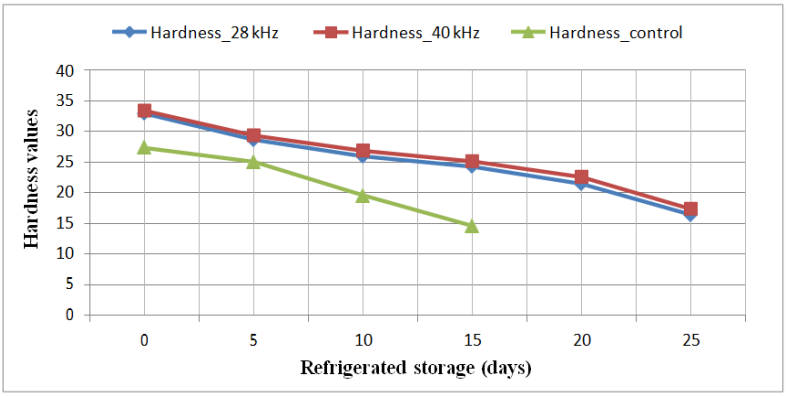

Fig. 10. Variation in hardness with storage time.

CONCLUSIONS

Pomfret *(P. argenteus)* is highly perishable and spoilage occurs during transportation, and retail display due to enzymatic and microbial activity. Ultrasound processing has a wide variety of applications in the processing and evaluation of products. The present study show that US is very effective in reducing microbial loads in pomfret fillets with maximum reduction at 40 kHz treated for 40 min. The colour parameters L* (lightness) and b* (yellowness) increases but a* (redness) decreases for both the frequency treated samples with increase in treatment time. All the textural parameters (hardness, cohesiveness, springiness, gumminess and chewiness) increase in both frequency treated sample. TVB-N content of both frequency treated samples shows a decreasing trend with increasing treatment time. From this results it was concluded that texture and colour the two major quality parameters of fish significantly affected by ultrasound processing and there significant reduction in TVB-N content and microbes at both the frequency. In case of storage stability and self-life evaluation the changes in different quality attributes of LDPE packed pomfret fillets was assessed at refrigerated storage (4 °C) interval of 5 days for 25 days. In case of storage stability and self-life evaluation the changes in different quality attributes of LDPE packed pomfret fillets was assessed at refrigerated storage (4 °C) interval of 5 days for 25 days.

FUTURE SCOPE

Pomfret (*P. argenteus*) is prone to rapid spoilage from enzymatic and microbial activity during transportation and retail display. Ultrasound processing, with its diverse applications, plays a crucial role in both the processing and assessment of such perishable products.

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Conflict of Interest. None.

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