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Effect of high pressure on K-value, microbial and sensory characteristics of yellowfin tuna (*Thunnus albacares*) chunks in EVOH films during chill storage

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ABSTRACT

The effect of different high pressure treatments on K-value, total plate count, *enterobacteriaceae* and organoleptic characteristics of yellowfin tuna chunks packed in ethyl vinyl alcohol (EVOH) films during chill storage (2 ± 1 °C) was studied. 50 g of fresh tuna chunks were placed in EVOH multilayer film pouches and vacuum packed for the trials. Tuna chunks were subjected to 100, 200 and 300 MPa for 5 min at 25 °C. Control was vacuum packed tuna without pressure treatment. K value, microbiological analysis and sensory characteristics were evaluated at periodic intervals. The K-value of the samples was found to decrease with increase in pressure when compared to the control. However, K-value was found to increase in all the samples during storage. Higher pressure treatment showed a decrease in the total plate count in the samples which increased during the storage. The *Enterobacteriaceae* decreased with increasing pressure and during storage. Control samples were sensorally acceptable up to 20 days of storage. During the storage period of 30 days 200 MPa treated tuna chunks was found most acceptable based on above parameters.

Industrial relevance: High pressure processing is a non thermal processing method which has wide applications in food industry. Fish is a highly perishable commodity and it has a limited shelf life in chill conditions. High pressure treatment has been found to effectively extend the shelf life of various products. In this study, HPP has been effectively demonstrated for enhancing the storage period of tuna at low temperature. Such studies can be commercially applied by fish processing industries to preserve the valuable catch and can enhance the marketing potential of tuna.

1. Introduction

High pressure processing (HPP) is one of the recent technologies which represent cold sterilisation for food preservation. The use of HPP in food processing is of great interest because of its ability to inactivate food borne microorganisms and enzymes, at low temperature without the need for chemical preservatives. It is also effective in preserving the organoleptic attributes of many foods (Wimalaratne & Farid, 2008). High pressure processed foods were first commercialised in Japan in 1992 (Murchie et al., 2005). The technology has been applied to an increasing range of food products including smoothies, ham, guacamole, salsa, rice products, fish and shellfish after the successful application of HPP of fruit juice and jams. Studies done on meat and fish have shown that high pressure may be a useful processing tool for muscle products (Ohshima, Ushio, & Koizumi, 1993a, 1993b). HPP generally does not affect the natural appearance, taste or flavour of foods (Hoover, 1997; Hoover, Metrick, Papineau, Farkas, & Knorr, 1989; Van Loey, Smout, & Hendrickx, 2003).

Sea foods are highly perishable and usually spoil faster than other muscle foods. They are more vulnerable to post-mortem texture deterioration than other meats (Simpson, 1998). Usually changes in fish quality are due to bacterial growth, but changes in fish tissues result from autolytic reactions controlled by native enzymes, such as the adenosine triphosphate (ATP) break-down process (Kennish & Kramer, 1986). Saito, Arai, and Matsuyoshi (1959) reported that K-value is one of the most appropriate indicators of freshness. K-value as freshness indicators for several finfish freshwater species was studied by Kiesvaara, Hattula, and Karppinen (1990). Anion-cation exchange columns to separate ATP and related compounds have been described by (Jones, Murray, Livinstong, & Murray, 1964; Kennish & Kramer, 1986; Saito et al., 1959).

Raw fish contains a wide variety of microorganisms. More often spoilage is as a result of the production of off-odours and off-flavours caused by bacterial metabolism (Castell & Anderson, 1948). Studies on the inactivation of microorganisms by HPP processing has been investigated as an attractive nonthermal processing technique for producing minimally processed high quality food (Hoover, 1993; Knorr, 1993). The microbial inactivation kinetics of HPP has also been studied (Buzrul & Alpas, 2004; Buzrul, Alpas, & Bozoglu, 2005; Chen, Hoover, & Kingsley, 2005; Chen, Joerger, Kingsley, & Hoover, 2004;

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Kilimann, Hartmann, Delgado, Vogel, & Ganzle, 2005; Ross, Taub, Doona, Feeherry, & Kustin, 2005; Yamamoto, Matsubara, Kawasaki, Bari, & Kawamoto, 2005). The quality deterioration of fresh fish is characterised sensorally by an initial loss of 'fresh fish flavour'. These will progressively become more pronounced and lead to rejection of the fish (Lone & Huss, 1996).

Tuna is one of the largest commercially important of all fishes. Yellowfin tuna (*Thunnus albacares*) are large pelagic fish that prevail in the tropics and subtropics. Currently, there is a good demand for fresh and frozen tuna in the Japanese market and worldwide. Fresh tuna is consumed in raw condition in the form of sashimi. The present study aims to study the effect of different high pressure treatment on the K-value, sensory characteristics and bacterial counts in vacuum-packed tuna chunks during chill Storage.

2. Material and methods

2.1. Preparation of fish sample

Yellowfin tuna (*T. albacares*) collected from landing centre at Fort Cochin were filleted and brought to the laboratory in iced condition in sterile bags. 50 g of tuna were packed within 10 h of being caught in EVOH multilayer film and vacuum sealed for HPP. The tuna samples were kept in insulated icebox with 1:1 ratio of ice: sample. It was transported to Mysore by overnight journey of 8 h. During this time the temperature of the fish was maintained at 1–2 °C. It was then subjected to three different high pressure levels of 100, 200 and 300 MPa for 5 min at a temperature of 25 °C within a period of 20–24 h after catch. After high pressure processing, the samples were kept in insulated icebox with 1:1 ratio of ice: sample and the ice box was kept in chill room maintained at 2 ± 1 °C. Periodically the melted ice water was replaced with fresh ice.

2.2. High pressure processing equipment

High pressure processing of the samples was carried out at Defence Food Research Laboratory (DFRL), Mysore, India using the equipment Model No FPG9400:922 supplied by M/s Stansted Fluid Power Ltd. United Kingdom. The equipment consists of a pressure chamber of cylindrical design. The pressure vessel was of 2 L capacity (570 mm length, 70 mm diameter) and 30% propylene glycol in water as pressure transmitting liquid. Separate pressure pump generates high pressure for system compression, and also a temperature control device monitors the temperature. After setting manually the required parameters for high pressure processing, the process is automatically controlled. The initial temperature of the pressure transmitting fluid was 15 °C and that of sample was 4 °C. For every 100 MPa increase in pressure, the adiabatic heating of pressure transmitting fluid was 3–4 °C.

2.3. K-value

K value may be defined as the ratio of the sum of inosine and hypoxanthine to total concentration of other nucleotides. Nucleotide degradation was determined using high performance liquid chromatography (HPLC) according to the method described by Ryder (1985). Lichrospher™ C-18 reverse phase stainless steel column (4 mm I.D.× 25 cm) having particle size of 10 µm was used for separation of these nucleotides. Sample preparation was done by extracting 5 g fish muscle in 25 ml of 0.6 M perchloric acid at 0 °C, after centrifugation at 3000 g for 10 min. Supernatant was neutralised with 1 M KOH. 0.45 µm Millipore syringe filter was used for filtering the extracted sample. Nucleotide standards and potassium phosphate were purchased from Sigma chemical company. 0.06 M dipotassium hydrogen phosphate and 0.04 M potassium dihydrogen phosphate were dissolved in HPLC grade water (Millipore) and adjusted pH at 7.2 was used as the mobile phase. It was filtered by using 0.45 µm filter before injection to the column. Flow rate was maintained at 1 ml/min and absorbance of eluent at 254 nm was measured. 20 µl of each reference compound of 0.0276 mM was used to calibrate the system. K value computation was done by the method described by Saito et al. (1959).

2.4. Reagents used

Chemicals used for the experiments were of Sigma brand supplied by Sigma-Aldrich Chemicals Private Limited, Bangalore, India.

2.5. Microbiological analysis

Samples were periodically analysed at 5 day interval for aerobic total plate count in each treatment. Samples were taken aseptically (5 g), stomached for 2 min in 0.85% normal saline. 10-fold serial dilutions of the samples were made using normal saline and 0.1 ml aliquots of the appropriate dilutions was plated on plate count agar. Plates were incubated for 48 h at 37 °C.

Enterobacteriaceae were enumerated by the method described by Koutsoumanis and Nychas (1999). 1 ml serial dilutions of fish homogenate was inoculated into 15 ml of molten violet red bile glucose (VRBG) agar medium cooled to 45 °C, mixed well and allowed to set for 15 min. Plates were incubated at 37 °C for 18–24 h. The colony characteristics were large colonies with purple haloes. Average counts were computed and expressed as colony forming units of *Enterobacteriaceae* per gramme (cfu g^{-1}) of the sample.

2.6. Physical properties of the EVOH film

The total thicknesses of pouches were determined as per ASTM (1964). Tensile strength and elongation at break of the packaging material was done as per IS: 2508-1984. Heat seal strength in both machine and cross direction N/25 mm width% was performed as ASTM, 1973, Water vapour transmission rate as per IS: 1060 Part II-1960. Oxygen permeability of the film was carried out using gas permeability apparatus (Gas and steam permeability, Ats Faar, Societa' Per Azioni, Milano, Italia, ASTM, 1982). Overall migration test was performed by using distilled water as food stimulants (IS: 9845-1998).

2.7. Sensory analysis

Sensory analysis of tuna chunks was done by 12 non-trained panelists using 9-point hedonic scales described by Meilgaard, Civille, and Carr (1999). Tuna chunks were cooked with 2% salt for 10 min and provided in a coded plate. The panelists were asked to score for appearance, colour, odour and overall acceptability of the samples. A score of above 4 was considered as the margin of acceptance.

2.7.1. Statistical analysis

Two factor analysis of variance was carried out to find the direct and interaction effect of pressure and period of storage days on physio-chemical variables viz; K-value, total plate count and total *Enterobacteriaceae*. Once ANOVA was found significant, Tukey's test was also performed to compare the means of different levels of pressure and period of storage days. The sensory analysis of the samples were carried out by using Kruskal Wallis one way ANOVA to find the significant difference among the respondence on the overall acceptability during the period of storage days at each level of pressure. All the statistical analyses were carried out by using SAS. 9.2.

Table 1
Physical properties of EVOH pouches.

Physical properties of EVOH pouches	
Total thickness (μm)	139 ± 0.58
Tensile strength (machine direction) kg/cm^2	178.99 ± 2.02
Tensile strength (cross direction) kg/cm^2	158.69 ± 1.62
Elongation at break (machine direction)%	201.12 ± 1.89
Heat seal strength (machine direction)(kg cm^{-2})	150.64 ± 1.59
Heat seal strength(cross direction)(kg cm^{-2})	113.11 ± 1.15
Water vapour transmission rate (g/m^2/24 h at 90 ± 2% RH and 37 °C)	139.02 ± 6.71
Oxygen transmission rate (cc/m^2/24 h at 1 atm pressure difference at 25 °C)	3.86 ± 0.29
Overall migration residue (mg/l) Water extractives at 49 °C for 24 h	21.5 ± 2.36

3. Result and discussion

Table 1 shows the physio-chemical properties of packaging material. Thickness of the film was of 139 ± 0.58 μm. EVOH film showed good tensile strength and heat seal strength. Very less oxygen transmission rate and water vapour permeability indicated its good barrier property. The high oxygen barrier characteristics of the EVOH material were provided by the hydroxyl group of the structure which confers them with a high cohesive energy, reducing the free volume between the polymeric chains available for gas exchange (Lopez-Rubio et al., 2005). Overall migration of the packaging material showed that it is good for food contact applications.

Fig. 1 shows the K-value of control and pressure treated samples. The initial K-value of the control and the three treated samples were 21.38, 22.45, 21.8 and 20.92 respectively. Multiple comparison tests revealed that there was no significant difference in the mean of K-value for control and treated samples initially. The nucleotides present in the sample do not change with the application of pressure. Ortea, Rodríguez, Tabilo-Munizaga, Pérez-Won, and Aubourg (2010) reported for chilled salmon muscle treated with 135, 170, 200 MPa for 30 min. Statistically there was significant difference on the effect of pressure and period of storage and their interaction of K-value at 1% level of significance. K-value was found to increase in all the samples during storage. It was found that control and 100 MPa showed almost identical values during storage. This is because of weak pressure and holding time (Ortea et al., 2010). 200 and 300 MPa treated samples showed less increase in K-value during storage, compared to control and 100 MPa. Lower K-value was found in high pressure processed tilapia fillets (Ko & Hsu, 2001). The decrease in the K-value was due to protein denaturation and deactivation of enzymes involved in the degradation of ATP and related compounds (namely dephosphorylases) during HPP (Shoji & Saeki, 1989). K-value of 78.99 for 200 MPa and 78.47 for 300 MPa was observed for the samples on 30th day of storage.

Fig. 2 shows the Total Plate Count (TPC) of control and pressure treated samples. Total microbial count is an important criterion for quality evaluation in fresh and frozen seafood products. According to the International Commission of Microbiological Standards for Foods (ICMSF, 1978), the maximum acceptable microbial limit in fresh and frozen fish is 10^7 cfu g^{-1}. Initial load of control and 100 MPa pressure treated samples showed values of 5.65 and 5.24 cfu g^{-1} respectively. A value of 4.62 and 4.11 cfu g^{-1} was observed for 200 and 300 MPa pressure treated sample initially. Statistical analysis revealed that there was significant and constant increase in all the samples during storage. Cruz-Romero, Smiddy, Hill, Kerry, and Kelly (2004) reported similar results in oyster (*Crassostera gigas*) during chill storage. Ramirez-Suarez and Morrissey (2006a) reported low microbial levels of pressure treated samples of albacore tuna minced muscle at low temperature storage. Yagiz, Kristinsson, Balaban, and Marshall (2007) reported that there was no bacterial growth for pressure of 400 and 650 MPa for mahi mahi and rainbow trout during a 6-d storage period after HPP. Other studies have demonstrated that HPP reduces microbial load on various foods such as mango puree (Guerrero-Beltran, Barbosa-Canovas, Moraga-Ballesteros, Moraga-Ballesteros, & Swanson, 2006), green beans (Krebbers, Matser, Koets, & Van den Berg, 2002), pork (Shigehisa, Ohmori, Saito, Taji, & Hayashi, 1991), octopus (Hurtado, Montero, & Borderías, 2001) and albacore tuna (Ramirez-Suarez & Morrissey, 2006b).

Fig. 3 shows the changes in total *Enterobacteriaceae* count after pressure treatment and during storage. Initial load in control, 100 MPa, 200 Mpa and 300 MPa samples were 4.4, 4.6, 2.9, and 2.5 cfu g^{-1} in respectively. Drastic reduction of total *Enterobacteriaceae* in octopus muscle after high pressure treatment was reported by Hurtado, Montero, Borderías, and Solas (2001). During chill storage total *Enterobacteriaceae* count showed a decreasing trend in all the samples. Similar results were reported in meat product pressurised at 300 MPa by Carballo, Fernandez, Carrascosa, Solas, and Jimenez Colmenero (1997). There was no significant reduction in total *Enterobacteriaceae* count between control and 100 MPa, whereas a significant reduction was observed in 200 and 300 MPa treated samples during storage.

Fig. 4 give the sensory scores of the control and treated samples. Control sample was sensorally rejected after 20 days of storage. The 100 MPa treated samples were accepted up to 25 days and the 200 and 300 MPa were acceptable up to 30 days of storage. As the storage period increased, a decreasing trend in sensory score was observed in all samples. Kruskal Wallis one way ANOVA was performed and it was observed that there was significant difference in overall acceptability with changes in pressure and storage period. 200 MPa gave better

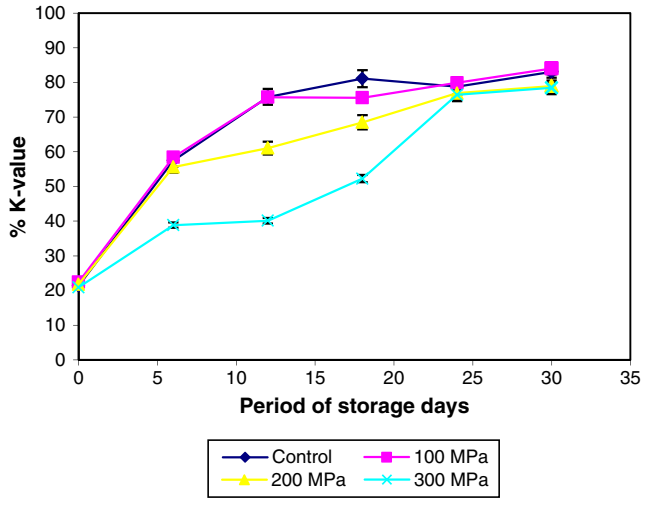

Fig. 1. K-value of tuna both control and treated samples during storage, n = 3.

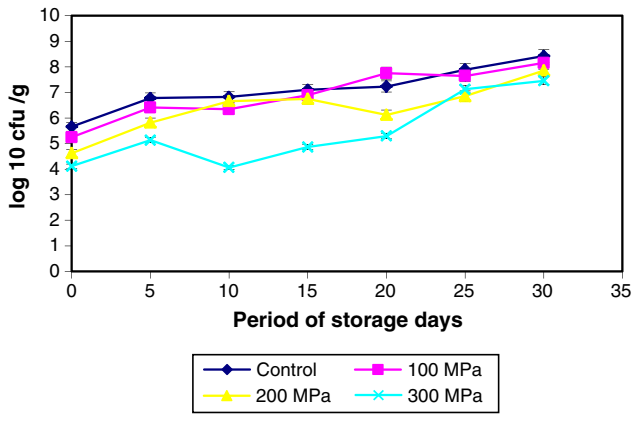

Fig. 2. Changes in total plate count during chill storage, n = 3.

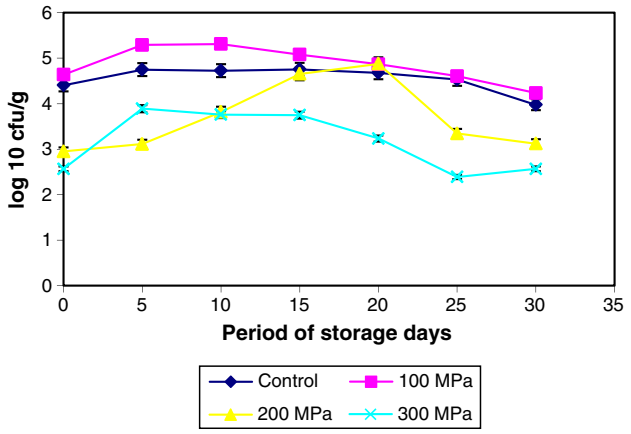

Fig. 3. Changes in total enterobacteriaceae during chill storage, n = 3.

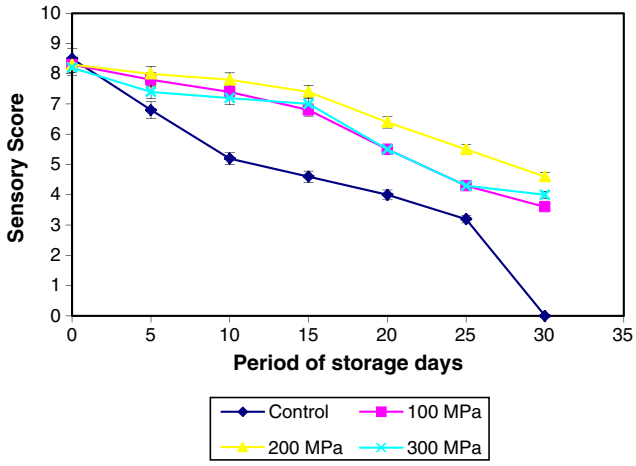

Fig. 4. Changes in overall sensory score of control and treated samples during chill storage, n = 12.

acceptability compared to 100 and 300 MPa treated samples. A sensory score of 4.6 was observed for 200 MPa treated sample and a score of 4.1 on 30th day of storage for 300 MPa. Tuna meat is dark pink/red colour and contains a considerable amount of myoglobin. As the pressure treatment increases the red colour of the tuna meat showed a decrease. High pressure causes changes in the appearance in the fish muscle when pressure is applied (Ohshima et al., 1993a, 1993b). Treatment of pressure above 300 MPa can give sea food opacity similar to that obtained by very light cooking (Hoover et al., 1989). Wada (1992) reported increases in lightness for HPP treated minced sardine and also for HPP thawed carp muscle (Sequeira-Munoz, Chevalier, LeBail, Ramaswamy, & Simpson, 2006; Yoshioka, Yamada, & Maki, 1996).

4. Conclusion

High pressure processing can improve the shelf life of filleted tuna chunks packed in EVOH multilayer films. Increasing pressure treatment had a negative effect on the K-value of the samples during storage. Statistically there was a significant difference on the effect of high pressure on K-value during storage period. Pressure treatment also had the ability to decrease the total bacterial count. However on storage it showed an increase in control and treated samples. *Enterobacteriaceae* count decreased during storage for pressure treated samples. Based on the chill storage studies, control samples had a shelf life of 20 days, 100 MPa had a shelf life of 25 days and after 30 days of storage, 200 MPa treated samples were superior to 300 MPa samples in all aspects studied here. Overall a pressure treatment of 200 MPa was suitable for storing tuna chunks in EVOH material for a period of 30 days. Further studies will have to be conducted to arrive at the optimum pressure levels, holding time and temperature that is required for maximisation of shelf-life of tuna chunks.

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Appendix 1. Sensory score for test panel studies

SENSORY EVALUATION

Assessor: ..... Date: .....

(Please score the sample characteristics by placing the relevant score)
An honest expression of your personal feeling will help us.

Cooked fish:

Attributes	Sample I	Sample II	Sample III	Sample IV
Appearance				
Colour				
Odour				
Flavour				
Taste				
Texture				
i. Firmness				
ii. Succulence				
iii. chewiness				
iv. Toughness				
Overall acceptability				

Please score the sample characteristics according to the following scale

Quality grade description	Score
Like extremely	09
Like very much	08
Like moderately	07
Like slightly	06
Neither likes nor dislikes	05
Dislike slightly	04
Dislike moderately	03
Dislike very much	02
Dislike extremely	01

Comments:

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