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Effect of high-pressure processing on the physicochemical stability of yellowfin tuna (*Thunnus albacares*) during chilled storage

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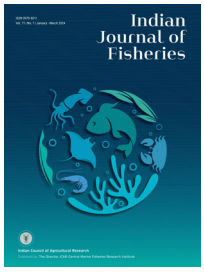

Abstract

The effect of pressurisation on the physicochemical properties of yellowfin tuna (*Thunnus albacares*) during low-temperature storage was studied. Tuna chunks were vacuum sealed in EVOH multilayer pouches and subjected to pressure treatments of 100, 200, and 300 MPa at 25°C for 5 min. Unpressurised samples, which underwent no pressure treatment, were maintained as controls. All samples were stored at 2±1°C throughout the study for subsequent analysis. At regular intervals, all samples were evaluated for biochemical indicators, including pH, trimethylamine (TMA), total volatile base nitrogen (TVB-N), and thiobarbituric acid (TBA), and physical parameters such as hardness and hue. pH in the samples increased slightly on storage. TBA content enhanced proportionally with the level of pressurisation of the samples and exhibited higher content in 300 MPa-treated samples. In contrast, TMA and TVB-N content in the tuna chunks showed an inverse relationship with pressurisation level, and lower levels of volatile base nitrogenous compounds were found in pressurised samples than in unpressurised samples. Application of pressure increased the hardness and lightness (L* values) of the samples. The control samples became unacceptable on the 20th day of storage, whereas all pressurised samples maintained better quality even up to 30 days. Among the samples, those treated with 200 MPa, exhibited the lowest lipid oxidation along with minimal changes in colour and texture.

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Introduction

High-pressure processing (HPP) is a technology with several commercially valuable characteristics, making it a safe and reliable option for consumers. HPP is applied in muscle foods for tenderising, as well as for reducing bacterial and pathogenic risks (Ohshima *et al*., 1993; Xiang *et al*., 2010). Pressurisation can be applied to ensure safety and retain the natural and nutritional attributes of food (Cheftel and Culioli, 1997). Since pressurisation is usually carried out at low or moderate temperatures, the covalent bonds of protein remain intact, while the weaker bonds (e.g., hydrogen bonds) are disrupted. This process alters enzyme properties and minimises the loss of flavour bearing compounds (Torres and Velazquez, 2005). HPP is being used for processing a wide range of food products like meat, fish, dairy and fruits (Hayashi, 1989). Improved shelf life in pressurised minced albacore tuna during frozen and chilled storage was reported by Ramirez-Suarez and Morrisey (2006).

Tuna is the third most commercially important fish species traded in the global market. The most landed species are skipjack tuna (50.7%), followed by yellowfin tuna (31%) and bigeye tuna (10.8%) (Miyake *et al*., 2010). Sashimi-grade tuna, which is consumed as raw meat, fetches a high price in the international markets, especially Japanese markets. Besides, non-sashimi grade tuna is marketed in both chilled and frozen forms, with the fresh chilled form earning the highest unit value (Miyake

et al., 2010). Fish is known for its short shelf life, particularly under tropical climatic conditions where spoilage tends to occur swiftly. The limited shelf life of chilled raw tuna is attributed to quality degradation, which is due to both microbial spoilage and physicochemical changes that occur during handling, storage and distribution. The colour and texture of seafood have a significant role in determining its commercial value. Discolouration on the surface of fish and shellfish can result from the oxidation of pigments, which may occur through both enzymatic and non-enzymatic pathways. Fish with high lipid content are highly susceptible to oxidation, resulting in the formation of oxidative products that adversely impact their natural attributes and quality (Hultin *et al*., 1982). Copper (Cu^{2+}), one of the most vital metal ions, has a significant role in marine lipid oxidation (Khayat and Schwall, 1983).

The trimethylamine produced from trimethylamine oxide during the storage of seafood serves as a key indicator for evaluating its quality. In general, volatile bases in fish muscle are formed through the action of autolytic enzymes, spoilage microorganisms, or chemical processes. The changes in trimethylamine, total volatile base nitrogen and thiobarbituric acid are key quality tests used to assess the quality of seafood during storage (Ozogul, 2000). pH is another quality index that directly influences the sensory attributes of seafood (Flores and Crawford, 1973). Changes in the pH of seafood result from bacterial growth and fish deterioration due to enzyme action, which alters the content of free hydrogen and hydroxyl ions through oxidation-reduction processes (Varlik *et al*., 2000). High-pressure processing reduces the microbial load and enzymatic activity, thereby maintaining the quality of seafood.

High-pressure processing (HPP) is widely recognised for enhancing the microbiological safety and storage stability of chilled fish products by effectively suppressing microbial proliferation. Previous studies have demonstrated that the combined use of HPP and marination can significantly prolong the refrigerated shelf life of marlin meat by delaying the development of spoilage related biochemical changes when compared with untreated samples (Chien *et al.*, 2025). In sardine, pressure treatment has been shown to modify colour, texture, and volatile compound profiles while maintaining overall product acceptability throughout refrigerated storage (Nartea *et al*., 2025). Likewise, the integration of high-pressure processing with brine salting has been reported to improve both microbial quality and physicochemical characteristics of mackerel fillets (Huang *et al*., 2022). Collectively, these findings highlight the capacity of HPP to extend shelf life while preserving essential quality attributes in fish products. Despite these advances, limited information is available on the response of yellowfin tuna to different pressure intensities during chilled storage. Therefore, the present study aimed to evaluate the effects of HPP at 100, 200, and 300 MPa with a 5 min holding period, on the physicochemical quality of yellowfin tuna steaks vacuum packed in EVOH pouches and stored at 2±1°C. Tuna steaks packed in EVOH pouches without pressure treatment were used as the control.

Materials and methods

Sample preparation

Yellowfin tuna (*Thunnus albacares*) with a mean length of 72 cm and an average body weight of 3.3 kg were procured from the Thoppumpady fish market, Kerala, India. The fish were thoroughly washed, dressed, and portioned into uniform chunks weighing approximately 50 g each. The portions were vacuum-sealed in multilayer EVOH pouches, and fourteen pouches were prepared for each treatment group. High-pressure processing was carried out at 100, 200, and 300 MPa for a holding time of 5 min at 25°C using a high pressure processing unit (Model FPG9400:922, Stansted Fluid Power Ltd., UK) equipped with a 2 l capacity cylindrical pressure vessel. The pressure build-up rate was maintained at 600 MPa min^{-1} to limit temperature rise resulting from adiabatic compression. Distilled water containing 30% propylene glycol was used as the pressure transmitting medium. A holding time of 5 min was selected based on its reported effectiveness in reducing spoilage microorganisms and enzyme activity while retaining the quality attributes of fish (de Alba *et al*., 2019). Following treatment, the samples were stored under chilled conditions (2±1°C) and analysed at 5 day intervals throughout the storage period.

Analysis of physicochemical parameters

The pH of each sample was measured following the standard protocol recommended by APHA (1998). TMA and TVB-N levels were determined using the microdiffusion technique described by Conway (1962). Malonaldehyde (MDA) levels were assessed based on TBA reactivity ((Tarladgis *et al*., 1960).

Sample hardness was analytically determined using a Universal Testing Machine (Lloyd instruments LRX plus, UK) according to Bourne's method (1978). Tuna samples of equal size were prepared to ensure consistency across analyses. A 50 mm diameter cylindrical probe was used with a maximum load of 100 N and a crosshead speed of 12 mm min^{-1}. Each sample was compressed to 40% of its initial height, and force-over-time data were recorded. Mean values of hardness-1 were calculated, representing the maximum force (resistance) during the first compression cycle.

A Hunter Lab MiniScan® XP Plus spectrocolourimeter (Model D/8-S, Hunter Associates Laboratory Inc., Reston, VA, USA) was used to assess the colour characteristics of the samples. The instrument employed an 8° diffuse geometry sphere, an 8 mm viewing area, and a D65 standard illuminant. Colour parameters L* (lightness), a* (red-green), and b* (yellow-blue) were recorded.

Statistical analysis

Data analysis was performed with an adequate sample size for each test to ensure statistical validity. Statistical analysis was performed using SAS version 9.2, with significance determined at $p<0.05$ using Analysis of Variance (ANOVA).

Results and discussion

Changes in pH

The initial pH values of unpressurised and pressurised samples (*i.e.*, 100, 200, and 300 MPa) were 5.87, 5.89, 5.90, and 5.92, respectively. A slight increase in pH was observed in the samples with increasing pressurisation. The pH increased with storage time

in both unpressurised and pressurised samples (Fig. 1). Pressurised samples exhibited relatively lower pH values than the unpressurised samples over the course of the storage study. Throughout the storage period, the differences in pH between the treatment groups were statistically significant ($p<0.05$). Nuray and Gonca (2010) reported an increase in pH of pressure treated fresh gilthead sea bream compared to unpressurised samples during refrigerated storage. The higher pH levels in the samples over time may be linked to microbial activity and the generation of basic nitrogenous compounds during spoilage (Cann *et al*., 1983). The unpressurised sample recorded a pH value of 6.16 on the 20th day of storage. In contrast, the samples subjected to pressures of 100, 200, and 300 MPa exhibited pH values of 6.18, 6.14, and 6.11, respectively, after 30 days of preservation.

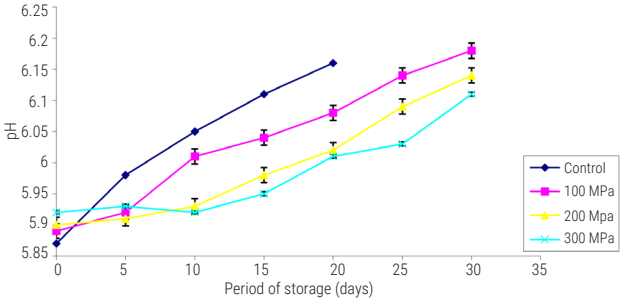

Fig. 1. Changes in pH of unpressurised and pressurised tuna chunks during chilled storage

Changes in TBA

The TBA values for both unpressurised and pressurised samples during the storage period are presented in Fig. 2. Lipid oxidation deteriorates the quality of seafood. TBA is the measure of malonaldehyde product during lipid oxidation. The initial TBA values were 0.608, 0.577, 0.787, and 0.639 mg malonaldehyde kg^{-1} for unpressurised, 100, 200, and 300 MPa treated samples, respectively. Samples treated at 200 MPa showed significantly higher TBA values than the unpressurised and 100 MPa samples ($p<0.05$), while the 300 MPa treatment also differed significantly from the other treatments. Nonetheless, throughout storage, all TBA values remained below the generally accepted threshold of 1–2 mg MDA kg^{-1} in seafood products (Lakshmanan, 2000). Throughout the storage period, all samples had TBA values significantly below this acceptable limit. The elevated

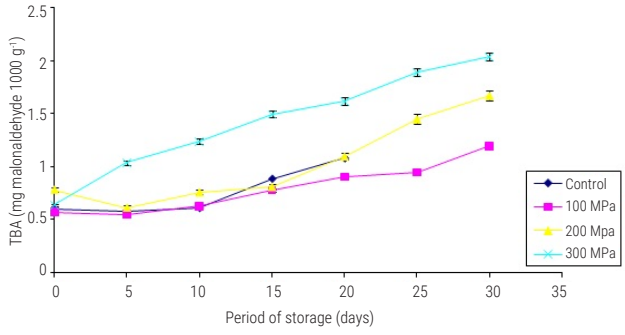

Fig. 2. Changes in TBA of unpressurised and pressurised tuna chunks during chilled storage

TBA values detected in the 300 MPa treated samples during storage indicate enhanced lipid oxidation under high pressure conditions. This phenomenon may be linked to pressure induced oxidative reactions, as described by Ohshima *et al*. (1992) and Tanaka *et al*. (1991), who reported that the denaturation of haemoproteins under pressure can release metal ions, which subsequently catalyse lipid auto-oxidation in fish tissue. Similar trends were observed in cod (*Gadus morhua*), where TBA levels rose in samples treated at 400, 600, and 800 MPa after 7 days of refrigerated storage (Angsupanich and Ledward, 1998). Yagiz *et al*. (2007) observed that lipid oxidation intensified in the dark muscle of rainbow trout when pressure levels exceeded 300 MPa.

Changes in TMA

Fig. 3. presents the changes in TMA values of tuna steaks. During the initial storage period, TMA values for unpressurised as well as 100, 200, and 300 MPa pressurised tuna chunks were 4.3, 4.2, 4.2, and 3.9 mg N_2 100 g^{-1}, respectively. Bacterial degradation and enzymatic activity generate TMA from the reduction of trimethylamine oxide (Oehlenschlager, 2006). An increase in TMA content is characteristic of spoilage; consequently, it has been extensively used as an objective indicator of seafood quality (Chang *et al*., 1976). TMA content in all samples showed an increasing trend with respect to the storage period. The limit of acceptability in TMA level is 10-15 mg N2 100 g^{-1} (Dalgaard *et al*., 1993). The TMA value of the unpressurised sample reached 10 mg N_2 100 g^{-1} at the time of rejection. In contrast, pressurised samples maintained lower TMA content throughout storage. Erkan *et al*. (2011) similarly reported suppressed TMA formation in pressurised horse mackerel stored under refrigeration. The reduction in TMA content in pressurised samples may be attributed to the inhibitory effect of pressure on bacteria and TMAO reductase, which are responsible for TMA formation (Wu and Bechtel, 2008).

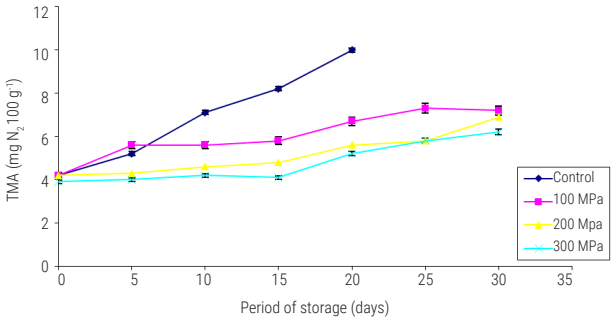

Fig. 3. Changes in TMA of unpressurised and pressurised tuna chunks during chilled storage

Changes in TBA

Changes in TVB-N content of unpressurised and pressurised tuna chunks stored under chilled conditions are presented in Fig. 4. The initial TVB-N content in unpressurised samples (28 mg N_2 100 g^{-1}) was higher than that of pressurised samples, with values of 19.5, 18.3, and 18.2 mg N_2 100 g^{-1} for the 100, 200, and 300 MPa

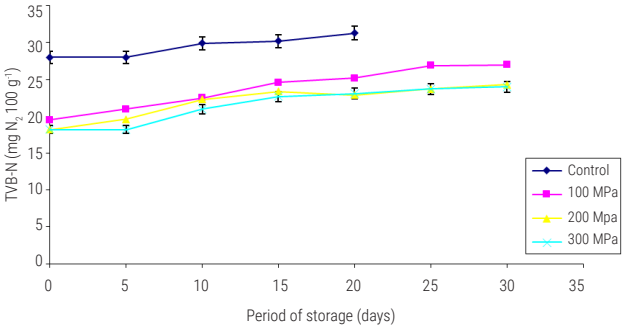

Fig. 4. Changes in TVB-N of unpressurised and pressurised tuna chunks during chilled storage

treatments, respectively. These results are consistent with findings by Erkan and Uretener (2010). Both pressurised and unpressurised samples exhibited an increasing trend in TVB-N content throughout the storage period. However, pressurised samples maintained significantly lower TVB-N levels compared to the unpressurised control. Seafood is considered acceptable for consumption when its total volatile basic nitrogen (TVB-N) content remains below 30–35 mg N_2 100 g^{-1} (Connell, 1995). On the 20th day of storage, the unpressurised sample exhibited a TVB-N content of 31.3 N_2 100 g^{-1}. In contrast, the 100, 200, and 300 MPa treated samples showed TVB-N values of 27.0, 24.3, and 24.0 mg N_2 100 g^{-1}, respectively, on the 30th day of storage. Nuray and Gonca (2010) reported similarly higher TVB-N levels in pressure treated gilthead sea bream during chilled storage. Zare (2004) suggested that the decline in TVB-N levels in pressure treated tuna could be due to the suppressive effect of high pressure on the bacterial activity involved in the breakdown of TMAO.

Changes in L* values

Fig. 5. illustrates the variation in L* values, indicating the lightness of tuna samples subjected to different pressure treatments during storage. Initially, both the control and 100 MPa treated samples had an L* value of 30.50, with no significant difference between them ($p>0.05$). In contrast, samples processed at 200 and 300 MPa exhibited higher initial L* values of 37.61 and 40.20, respectively, demonstrating an increase in whiteness correlated with pressure intensity.

This enhancement in lightness is likely a result of protein denaturation, particularly in the myofibrillar and sarcoplasmic fractions, which alters

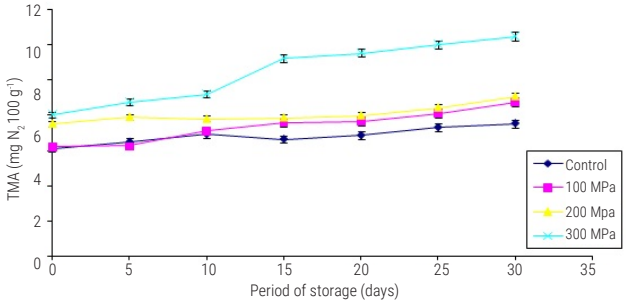

Fig. 5. Changes in L* value of unpressurised and pressurised tuna chunks during chilled storage

the reflective properties of fish muscle (Ledward, 1998; Zare, 2004). Similar effects of pressure on lightness have been reported in various fish species, including bluefish (Oshima *et al*., 1993), sheepshead (Ashie *et al*., 1996), hake (Hurtado *et al*., 2000), and turbot (Amanatidou *et al*., 2000; Chevalier *et al*., 2001), where treatment above 100 MPa increased L* values. In the present study, the 300 MPa treated sample consistently showed the highest L* values during storage. By the 20th day of storage, the L* value in the unpressurised sample increased to 34.25. In comparison, by the 30th day, the L* values of the pressurised samples increased to 43.76, 45.39, and 62.35 for the 100, 200, and 300 MPa treatments, respectively.

Changes in a* values

Fig. 6 illustrates the changes in a* values (red/green component) of both unpressurised and pressurised samples during storage. A significant reduction in redness ($p<0.05$) was observed in the samples treated at 300 MPa. Similar results were reported by Yagiz *et al*. (2007) in pressure-treated rainbow trout and mahi-mahi, where processing at 300 MPa led to significantly lower a* values during storage. The characteristic dark purple-red colour of tuna is primarily attributed to the presence of myoglobin. Upon exposure to oxygen, myoglobin forms oxymyoglobin, which imparts the bright red hue typical of fresh meat. However, during storage, especially under high pressure conditions, myoglobin undergoes oxidation to form metmyoglobin, resulting in a brownish discolouration and reduced redness (Zare, 2004). Throughout the storage period, all pressurised samples exhibited lower a* values compared to unpressurised samples, indicating a loss of redness due to pressure induced oxidative changes.

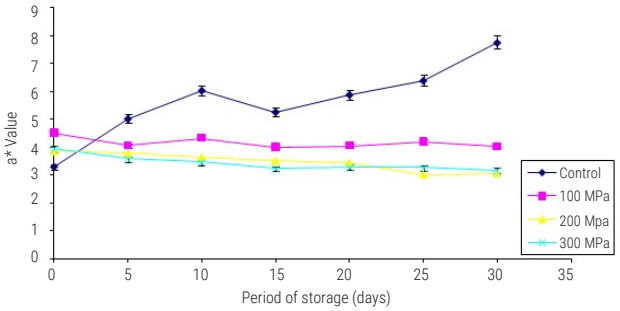

Fig. 6. Changes in the a* value of unpressurised and pressurised tuna chunks during chilled storage

Changes in b* values

Fig. 7. illustrates the variation in b* values, representing yellowness, for both control and pressure treated samples over the storage period. At the beginning of storage, the b* value for the unpressurised samples was 4.20, whereas those subjected to 300 MPa treatment showed a higher initial value of 7.06. An upward trend in b* values was observed across all samples during storage. Notably, the 300 MPa treated samples reached a maximum b* value of 10.6 on the 30th day of storage. The increase in b* values is attributed to the cooked appearance induced by high pressure treatment (Yagiz *et al*., 2007). Chevalier *et al*. (2001) reported a similar increase in b* values in turbot (*Scophthalmus maximus*) muscle when subjected to 100–200 MPa with holding times of 15 and 30 min at 4°C. Likewise, Sequeira-Muñoz *et al*. (2006) observed an increase in b* values in carp fillets treated at pressures exceeding 140 MPa.

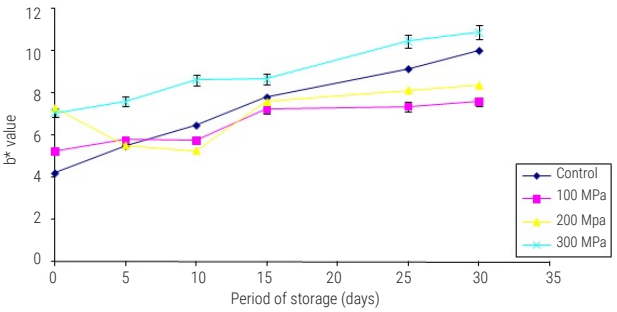

Fig. 7. Changes in b* value of unpressurised and pressurised tuna chunks during chilled storage

Changes in hardness

Fig. 8. shows the hardness values of unpressurised and pressurised samples. The unpressurised sample exhibited a hardness of 1.20 N, while samples treated at 100, 200, and 300 MPa showed hardness values of 1.33 N, 2.35 N, and 2.48 N, respectively. The increase in hardness observed at higher pressure levels is likely due to the unfolding of sarcoplasmic and myofibrillar proteins, which promotes the development of new hydrogen bond interactions (Angsupanich and Ledward, 1998). A comparable rise in the hardness of cod (*Gadus morhua*) at 200 and 400 MPa was also documented by Matser *et al*. (2000).

Hardness revealed a statistically significant difference ($p<0.05$) between the pressurised and non-pressurised samples. Storage further influenced texture, with pressure treated samples displaying a continued increase in hardness over time. Ohshima *et al*. (1993) explained that pressure induced compaction of muscle tissue reduces its volume, thereby strengthening protein–protein interactions and enhancing texture, leading to increased toughness and elasticity. There was no significant change in hardness in the unpressurised and 100 MPa-treated samples during storage. However, in samples treated at 200 and 300 MPa, hardness increased to 3.96 N and 4.99 N, respectively, by the 30th day of storage. Yagiz *et al*. (2009) similarly reported higher hardness values in Atlantic salmon treated at 300 MPa compared to those treated at 150 and 100 MPa.

High pressure processing proved to be an effective non-thermal method for enhancing the storage quality of yellowfin tuna under chilled conditions. Changes in physicochemical parameters indicated that samples treated at 100 MPa exhibited only minor differences compared to the unpressurised samples. However, higher pressures, particularly 300 MPa, significantly altered physicochemical properties, including TBA content, L*, a*, b* values, and hardness. Throughout storage, pH showed a gradual increase across all treatments, with pressurised samples maintaining comparatively lower values. Among all treatments, the highest thiobarbituric acid value was recorded in the 300 MPa treated sample, suggesting increased lipid oxidation at higher pressures. Trimethylamine levels decreased as a result of pressurisation, likely due to the partial inhibition of bacterial activity responsible for the reduction of TMAO. Total volatile base nitrogen content showed a general increase during storage in all samples. In terms of colour, lightness (L*) increased and redness (a*) decreased with rising pressure levels, whereas yellowness (b*) increased over the storage period. Textural analysis revealed that sample hardness was positively influenced by both pressurisation and storage duration, with significantly higher values observed in pressurised samples.

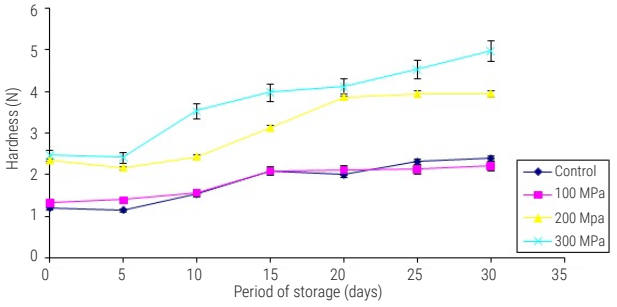

Fig. 8. Changes in hardness of unpressurised and pressurised tuna chunks during chilled storage

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