

## Original Research

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# Effect of pomegranate and orange peel extracts on the quality of fish ham under frozen storage

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## Abstract

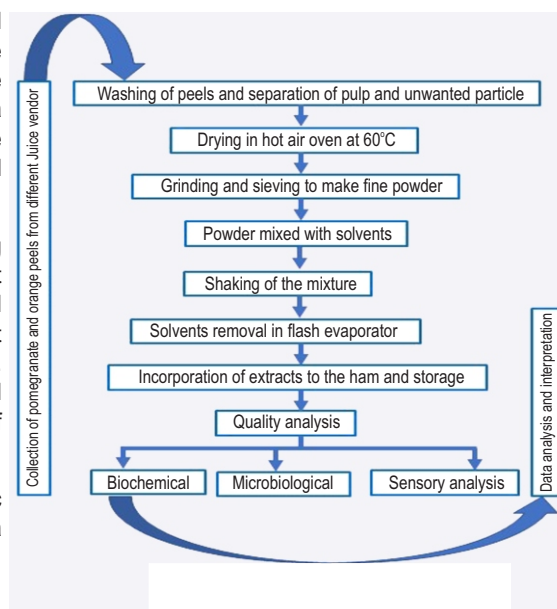
**Aim:** The objective of the present study was to evaluate the effect of pomegranate and orange peel crude extract on fish ham quality in terms of biochemical, microbiological, and sensory parameters under cold storage ( $-18 \pm 2^\circ\text{C}$ ).

**Methodology:** The crude extracts were extracted from dry powder of orange and pomegranate peel using absolute ethanol as solvents. The extracts were standardized in the mackerel cooked meat model system to determine the concentration for the final products. Fish ham was prepared using red meat of tuna (*Thunnus albacares*) and white meat of bull's eye fish (*Priacanthus hamrur*). The prepared fish ham was treated with  $2000 \text{ mg l}^{-1}$  of pomegranate and orange peel extracts and stored under frozen conditions.

**Results:** The results obtained were compared with a control sample containing synthetic antioxidant Butylated hydroxyanisole. The results showed that pomegranate and orange peel extracts significantly ( $p < 0.05$ ) reduced the lipid oxidation and microbial activity. The results of biochemical studies revealed that there was a considerable increase in the quality parameter in all treated samples. The fish ham treated with pomegranate peel extract, orange peel extracts showed formation slower primary, secondary oxidation products and breakdown of nitrogenous compounds.

**Interpretation:** Pomegranate and orange peel can be a substitute for synthetic antioxidants. These findings suggest that the pomegranate and orange peel serve as a choice to use as a food antioxidant for extending the shelf life of fish fishery products.

**Key words:** Antioxidants, Fish ham, Orange peel, Pomegranate peel, Shelf life



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## Introduction

Fish and fishery products are the most important among the internationally traded food item but are also the most perishable food commodity due to high contents of polyunsaturated fatty acids. To prevent the quality deterioration of food items due to lipid oxidation and bacterial growth, synthetic food preservatives Butylated hydroxyanisole, i.e., Butylated hydroxyl toluene, and Tert-Butylhydro quinine are used (Frankel, 1993). The use of synthetic antioxidants can be health hazardous as they can cause urinary bladder and stomach cancer (Ito *et al.*, 1986). So, there is a need to identify natural antioxidants, especially of plant origin to enhance the storage life of fish and fishery products. In general, fruit peels constitute about 50 to 65% of the whole mass of fruits and they are the main byproduct of fruit industry. If they are not processed further, they become waste and produce bad odor, and harbor insects leading to serious environmental pollution (Mandalari *et al.*, 2006). Pomegranate (*Punica granatum*) is native to Asia, mainly to Iran, Afghanistan, and in India it is mainly cultivated in the northern part of India. India is one of the major leading countries in the production of pomegranates.

It is a drought-tolerant crop and can be grown in an area with low irrigation potential, low maintenance cost, high yield, and their fruit have a relatively well storage life (Kanatt, 2008). Citrus fruits are the world's largest fruit sector with annual production of >100 million tons (Shehata *et al.*, 2021). Oranges (*Citrus sinensis*) is one of the citrus fruit it belongs to the family "Rutaceae". The industrial use of oranges generate a large number of fruit wastes like peel, seed, and pulp which has become an environmental hazard if not utilized and disposed of properly. The peels of these fruits are not edible, they possess substantial bioactivity mainly antimicrobial, antioxidant, and anti-cancer activities (Kumar *et al.*, 2020). Recently, there is an increase in the extraction of natural beneficial compounds from horticulture waste and their use in food preservation (Shahidi *et al.*, 2019). Bitalebi *et al.* (2019), reported that the apple peel extract inhibits lipid oxidation and protein oxidation in mince trout during refrigerated storage.

The clove, sage, and kiwifruit peels extract help to extend the shelf life of fish fingers during the storage period up to 30 days under cold storage conditions (Abdel-Wahab *et al.*, 2020). According to Lopes *et al.* (2021) the potato peel extracts are appropriate for emerging starch-based films with enhanced shelf-life of smoked fish fillets and their packaging stability. Similarly, Maqbool *et al.* (2021) reported that beetroot peel treatment help in retarding the spoilage, thus increasing the shelf-life of mahseer steaks under frozen storage. In this regard, researchers across the world are looking for promising natural-based preservatives with excellent antioxidant and antimicrobial activity for enhancing the shelf life of fish and fishery products (Olatunde and Benjakul, 2018). The by-products obtained from the fruit processing industry can offer a well-enriched economic source of natural antioxidants that are capable of replacing synthetic

antioxidants. Because of the above, the present investigation was undertaken to study the antioxidant potential of pomegranate and orange peel extracts and their effect on the storage stability of fish ham under frozen conditions.

## Materials and Methods

**Preparation of extracts:** Pomegranate and orange peels were collected from different juice vendors in Mangalore city. The peels were washed to remove dirt and unwanted materials thereafter peel and pulp were manually removed and the cleaned peels were cut into small particles and oven-dried at 60°C for 12 hrs. Dried peels were ground and sieved using a 1 mm mesh size flour sieve. The extract was prepared by using the method suggested by Iqbal *et al.*, (2008) with slight modification. Twenty-five grams of peel powder was mixed with 150 ml of ethanol. The mixture of peel powder and ethanol were centrifuged at ambient temperature for 12 hr at 190 rpm. The mixture was filtered and the residue was re-extracted with the same solvent. The filtrates of the mixture were placed under a hood in the rotavapor to remove the residual ethanol under vacuum at 40°C. The extracts obtained were stored at -20°C in a sample container for further study.

**Preparation of fish ham:** Fish ham samples were prepared following the method of Siddppaji *et al.* (2002) by mixing the red meat of tuna and white meat bull's eye fish in 3:2 ratio. The prepared fish ham was mixed with 2000 ppm of pomegranate and orange peel extracts and the samples were stored at -18±2°C. Sampling was done at an interval of 30 days and subjected to biochemical, microbiological, and sensory analyses. The results of treated fish ham were compared with the results of control and synthetic antioxidants.

**Biochemical analyses:** Peroxide value was determined by the method of Jacob (1958). Free Fatty Acid was determined by the improved titrimetric method as described by (AOAC, 2016). Thiobarbituric acid reactive substance was determined spectrophotometrically as per Raghavan and Hultin (2003). Total volatile basic nitrogen was determined by the method of Antonopoulos and Vyncke (1989). Trimethylamine was estimated by micro-diffusion method (Chouhan *et al.*, 2015).

**Microbiological analysis:** Total plate counts and total spore former counts were estimated by the standard methods of APHA, (2015).

**Sensory analyses:** Sensory analyses was conducted by the method of Freitas *et al.* (2019). A six-member trained panel group evaluated the treated and untreated fish ham sample at regular sampling periods during refrigerated storage. The sample was warmed at 90°C for 5 min and placed in a coded covered plate. The order of presentation was made using random permutation. The panelists were instructed to evaluate all the samples using a 9-point hedonic scale.

**Statistical analyses:** Statistical analysis was done by using the Statistical Package for the Social Sciences (SPSS) package program version 21.00 (IBM SPSS Statistics, IBM Corp, Somers, NY, USA). Data were analyzed by One-way ANOVA, followed by a Post-hoc deacon and multiple comparison test.

## Results and Discussion

Lipid oxidation is a major cause of quality degradation in fish and fishery products, mainly products that comprise high lipid content. This is the primary deteriorative reaction in several types of food, mainly in fish because of high content of polyunsaturated fatty acids found in the fish (Ackman, 1980). The primary product of lipid oxidation is hydroperoxide, which is measured in terms of peroxide value. The peroxides present in fish lipids are the main indicators of primary oxidation level and its tendency to form rancidity (Gokoglu *et al.*, 2012). The rise in peroxide values at the initial stage of lipid oxidation can lead to degrading the food items (Shim and Lee, 2011). In this study, at the beginning of storage, peroxide value was recorded as 3.63, 3.52, 2.98, and 5.06 Mill eq.  $O_2 \text{ kg}^{-1}$  of fat for the pomegranate peel extract, orange peel extracts, Butylated hydroxyanisole, and control samples, respectively (Fig. 1 a). These values significantly increased with increase in storage time and the values attained at the end of 180 days of frozen storage were 14.39, 18.26, 12.12 and 10.59 in the pomegranate peel extract, orange peel extract, Butylated hydroxyanisole, and control samples, respectively.

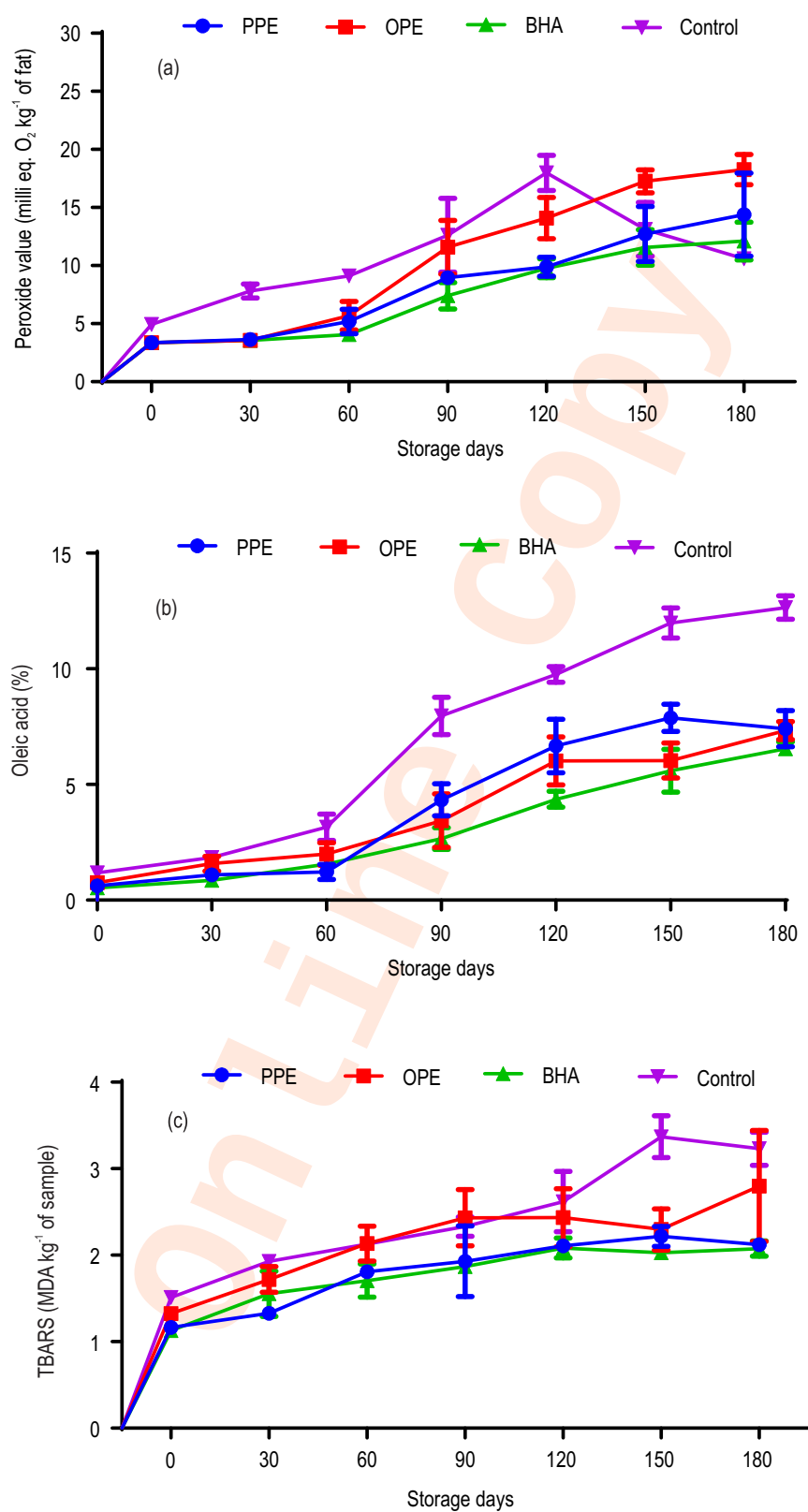
The increase in peroxide value may reflect either increased formation of hydroperoxides or reduced decomposition, (Gordon, 2004). The samples stored without treatments showed a decrease in peroxide value after 150 days of storage and this decrease may be due to decomposition of hydroperoxide (Esfarjani *et al.*, 2019). The reduced peroxide value in fish meat under frozen storage has been reported by Vanitha (2015). The lowest increase of peroxide value was recorded in the case of a sample treated with pomegranate peel extract. This might be due to the effect of phenolic compounds that disturbs the initiation steps of lipid oxidation reactions by scavenging lipid radicals (Lugasi *et al.*, 2005). Our study was also in agreement with the results of Ozogul *et al.* (2016), who reported that trout fillets treated with rosemary and thyme inhibit the highest hydroperoxide content than the control sample. According to Khojah (2020), the coating of pomegranate peel extracts in tilapia fillets delayed peroxide value as edible coatings reduced peroxide value progress and hindered the lipid oxidation in fish fillets. The maximum permissible limit of PV is 10-15 meq/kg fat as per the Connell, (1995) however the PV value of the all the treated samples were not increased beyond the acceptable limit.

The present findings specify that the pomegranate peel extract was most effective in hindering the production of peroxide value of fish ham stored at  $-20^\circ\text{C}$ . The free fatty acid is the hydrolysis product of fat oxidation, it is formed in oil during long-term storage or processing at high temperatures while heating or

frying. The initial free fatty acid value was as 0.62, 0.75, 0.53, and 1.18 % of oleic acid in the pomegranate peel extract, orange peel extract, Butylated hydroxyanisole, and control samples, respectively (Fig. 1b.). Thereafter, these values significantly increased with an increase in sampling periods. The FFA value in control and OPE samples were slightly higher than the pomegranate peel extract and BHA sample. Ozogul *et al.* (2016) also reported significant variations in the levels of FFA that occurred between the control and treated sample with different plant extracts in trout fillets. Clove oil and grape seed extract in silver carp inhibit the formation of FFA in the treated sample as compared to the control sample (Shi *et al.*, 2014). At 180 days of storage period, the free fatty acid and BHA in samples treated with pomegranate peel, orange peel extracts did not show any significant values but the values of the control sample were significant with the other group of samples. This might be due to low FFA in the treated sample is related to phenolic content in pomegranate and orange peel, leading to inhibition of lipid oxidation through modification of several enzymic activities (Raikos *et al.*, 2010). This observation corroborates with the findings of Tavakoli *et al.* (2018) who reported lower FFA value in rainbow trout fillets stored in ice supplemented with Reshgak extract. Similar results were also reported by Erkan and Bilen (2010) who reported that the treatment of chub mackerel fillets with essential oils of lemon reduced the formation of FFA.

The TBARS test is widely used to assess the secondary lipid oxidation produced as malondialdehyde during secondary lipid oxidation of PUFA. This aldehyde holds importance in seafood as produces rancid adour in low amount (Jones, 2017). It is considered one of the major parameters of lipid oxidation (Pereira, 2018). The TBARS test in a sample generates an adduct of red color, which can be distinguished spectrophotometrically. At the advent of storage, the TBARS value of fish ham were almost similar in all the samples which gradually increased with increase in storage time (Fig. 1c). After 30 days of storage period, the TBARS value of the treated sample did not show any significant difference ( $p>0.05$ ) whereas the control sample showed a significant difference, this could be due to pomegranate juice and peel are good sources of polyphenols, such as gallotannins, ellagitannins, ellagic acids, gallic acids, catechins, anthocyanins, ferulic acids and quercetins (Calis and Bayazit, 2012). These polyphenols show several bioactivities, such as inhibiting oxidation, eliminating free radicals, and microbial growth in the food.

The TBARS content increased by the 2.12, 2.80, 2.08, and 3.23 at the end of 180 days of storage in the sample treated with pomegranate peel extract, Orange peel extracts, BHA, and control sample respectively. The results of this study, are in line with the findings of Jouki *et al.* (2014) who reported a lower TBARS value rainbow trout fillets treated with quince seed mucilage film during refrigerated storage. The coherent observation was also recorded by Alfaro *et al.* (2013) who they reported that the incorporation of white grape dietary fiber significantly delayed lipid oxidation in minced fish products under frozen conditions. The results of the present showed that the



**Fig. 1:** Changes in peroxide value (a); free fatty acid (b) and TBARS (c) values of fish ham treated with pomegranate peel extract (PPE) and orange peel extract OPE during frozen storage temperature ( $-18 \pm 2^\circ\text{C}$ ).

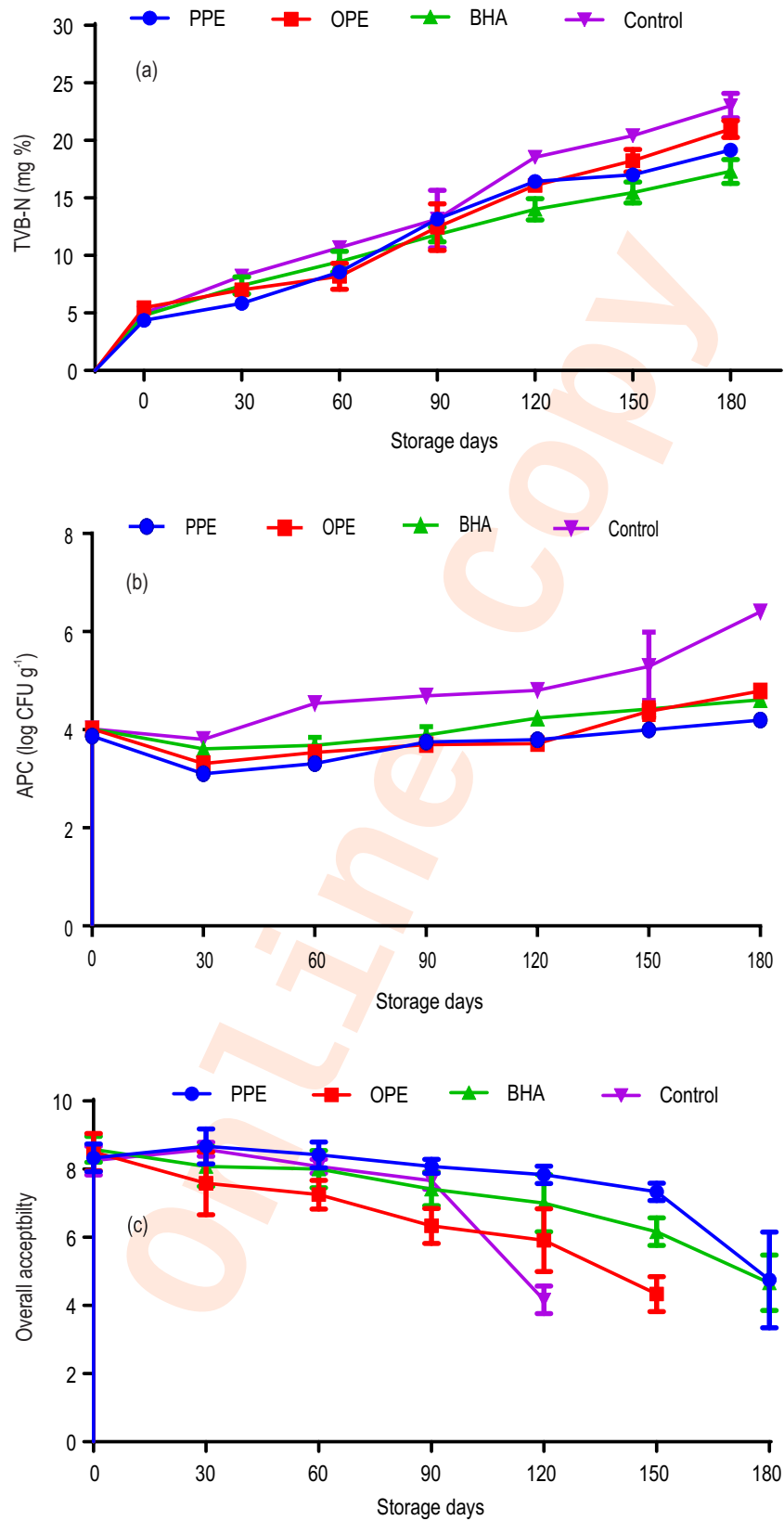


Fig. 2: Changes in TVB-N (a); APC counts (b) and sensory score overall acceptability (c) temperature ( $-18 \pm 2^\circ\text{C}$ ).



secondary oxidative rancidity of the fish ham treated with pomegranate peel extract remained quite low throughout the entire storage period in comparison to control and other treated samples. The TVB-N is the oldest chemical process used to assess fish spoilage as it mainly contains, ammonia, trimethylamine, and dimethylamine. At the beginning of storage, the TVB-N value of fish ham was 7.47, 9.33, 7.48, and 10.36 mg 100<sup>-1</sup> in pomegranate peel extract, OPE, Butylated hydroxyanisole, and control sample respectively (Fig. 2a). These values gradually increased with the increase in storage time with the highest increase recorded in the case of control sample and the lowest sample treated with pomegranate peel extract and Butylated hydroxyanisole.

The increase in level usually 30-35 mg 100<sup>-1</sup> is associated with the action of spoiling bacteria and intrinsic enzymes (Ozogul *et al.*, 2009). The lower value of TVB-N in pomegranate peel extract is possibly due to higher antioxidant and antimicrobial powers (Saloko *et al.*, 2014). At the end of 180 days of storage, the TBV-N value of fish ham reached 19.15, 20.98, 17.30 and 23.03 mg/100g in the pomegranate peel, Orange, Butylated hydroxyanisole, and control sample, respectively. The maximum permissible limit of TVB-N 35 mg/100 g, above this value, fishery products are not fit for consumption (Ludoff and Meyer, 1973), above which, fishery products are not fit for consumption (Ludoff and Meyer, 1973). However, none of the treatments has exceeded the upper limit of TVB-N. The results of APC at the beginning of storage was 3.87, 4.02, 3.91, and 4.11 log CFU g<sup>-1</sup> in pomegranate peel extract, OPE, Butylated hydroxyanisole and control sample respectively (Fig. 2b). These values progressively increased up to 180 days of frozen storage. Maqbool *et al.* (2020) observed inhibition of microbial growth in the Deccan mahseer after treating with the whole reddish extract.

The minimum Aerobic Plate Count value of fish ham was recorded in control sample followed by orange peel extracts and BHA sample. These results confirm with the observations of Firdous *et al.* (2021) who reported that the Amla extracts inhibits the bacterial counts of Indian white prawn fish reached 4.20, 4.79, 4.61, and 6.40 log CFU g<sup>-1</sup> for the pomegranate peel extract, orange peel extracts, Butylated hydroxyanisole, and control sample respectively. The lower Aerobic plate count value in fish ham treated with pomegranate peel extract compared orange peel extracts might be due to the rich source of tannin ellagitannins and phenolic acids as reported by Prashanth *et al.* (2001) and Supayang *et al.* (2005) in the pomegranate peel extracts. Phenolic compounds like eugenol, thymol, carvacrol, in green tea, rosemary extract, grapefruit seed extract, and lemon extract control the microbiological quality is packed fresh cod hamburgers (Corbo *et al.*, 2009). Similarly, Wahab *et al.* (2020) observed that the fish finger treated with clove, sage, and kiwifruit peels extracts controlled the microbial growth under cold storage condition.

The above results of APC prove that the fish ham treated with pomegranate peel extract was virtuous in inhibiting bacterial

counts as compared to the untreated samples and other treated samples. The score of sensory attributes overall acceptability significantly decreased with increase in storage time (Fig. 2c) due to changes in the biochemical and microbial parameters during storage period. The control sample was rejected by the sensory panelist at 120 days of frozen storage but meanwhile, some biochemical parameters were within the acceptable limit. Whereas samples treated with orange peel extract were acceptable up to 150 days of storage. The positive sensory score for the treated sample as compared to control confirms no negative effect on the sensory attributes. Fish ham treated with pomegranate peel extract and orange peel extracts was acceptable up to 180 days of frozen condition. The panelists' scores for evaluation of fish ham sensory attribute's overall acceptability were interrelated with the changes in biochemical and microbiological parameters. Alsagoff *et al.* (2019) strongly confirmed the microbial obligation for the occurrence of muscle discoloration, off odor, slimy texture, and lipid putrefaction throughout storage. Lamyael (2012) reported that the addition of ginger extract was effective in maintaining the sensory properties of sausages even after 90 days during frozen storage.

Application of pomegranate and orange peel extracts showed considerable effects on biochemical quality and sensory attributes of fish ham. They are good sources of phenolic compounds that contain potent antioxidant activity. The results of this study, revealed that pomegranate and orange peel extracts can reduce the primary and secondary oxidation products under frozen storage and also inhibit the microbial growth of microorganisms. The findings of our work conclude that pomegranate peel extract can maintain the post-harvest quality of fish and fishery products. Therefore, the extract can replace the synthetic antioxidants in the food processing industry.

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### Add-on Information

**Authors' contribution:** J Pal: Conducted the study, planning, and manuscript writing; C.V. Raju: Monitoring and guided the research; G. Pandey: Assisted in research work; H.H. Tripathi: Analysis of data; L. Prasad: Manuscript editing.

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**Ethical approval:** Not applicable.

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