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Beetroot Peel Extract as a Natural Preservative for Shelf life Extension of Deccan mahseer (*Tor khudree*) Steaks during Chill Storage

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Abstract

The efficacy of solvent free beetroot (*Beta vulgaris*) peel extract in retarding the lipid oxidation of mahseer steaks were evaluated during chill storage. Preliminary analysis of the beetroot peel extract (BPE) for antioxidant activity were evaluated based on the total phenolic content, 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity and 2, 2-azino-bis (3-thylbenzothiazoline-6-sulfonic acid radical scavenging activity. BPE had total phenolic content of 360.47 mg 100 g^{-1}; both DPPH activity (91.62%) and ABTS radical scavenging activity (99.04%) were found to be higher at 20% concentration. Mahseer steaks were given dip treatment in beetroot peel extract (20%, w/v) then packed in LDPE pouches and kept at 0-2°C. The effect of dip treatment on quality changes of steaks were assessed periodically for biochemical (pH, PV, FFA, TBA, TMA, TVB-N), microbiological (total plate count) and sensory characteristics along with control sample. The microbial analysis revealed that the control (CT) sample reached to the limit of acceptability (7.47 log cfu g^{-1}) on 9th day while treated sample remained within the limit up to 15th day of storage. Sensory evaluation also showed the decreasing trend as the storage days increased. The results showed that CT sample exceed the acceptable limits on 9th day of storage, whereas the BPE treated sample was found to be acceptable up to 15th day indicating the positive role of BPE in shelf life extension of masher steaks under chilled storage (0-2°C).

Keywords: Deccan mahseer, beetroot peel extract, biochemical quality, microbial analysis

Introduction

Fish is a highly perishable food commodity and due to its nutritional components such as protein, lipids and minerals, it spoils very rapidly due to enzymatic autolysis, lipid oxidation and microbial deterioration. These process leads to a decrease in shelf life of fish and fishery products (Arashisara et al., 2004; Sathish et al., 2018). In food industries synthetic antioxidants, such as butylated hydroxyanizole (BHA) and butylated hydroxytoluene (BHT) are commonly used as antioxidant agents to preserve the freshness, nutritive value, flavour and colour of the food products (Indrajit, 2013). However, it was revealed that the synthetic antioxidants could be toxic (Schilderman et al., 1995) which could affect human health and with an increasing consumer consciousness, the demand for natural additives is high (Sloan, 2005). These increasing interest demands the food industries to focus on substitution of synthetic antioxidants by natural extracts to preserve the food products.

Selected compounds from vegetable or fruit waste has antioxidant potential and it could be used to prevent the oxidative damage by scavenging oxygen free radicals and also retarding lipid peroxidation (Makris et al., 2007). Antioxidant from vegetable and fruits also help in control of microbial growth (Arnao et al., 2001). Hence industries are giving special attention on extracts from inexpensive or

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residual sources from agricultural based products especially, plant derived constituents which are generally regarded as safe (GRAS) (Smid & Gorris, 1999). The main factor responsible for antioxidant property of plant derived compounds is the presence of bioactive compounds categorised as polyphenols, flavonoids, tannins, alkaloids, terpenoids, isothiocyanates, lectins, polypeptides or their oxygen substituted derivatives (Edeoga et al., 2005).

The increasing interest towards the use of process discards like peels and seeds acquired from fruit and vegetable processing units are due to a rich source of phytochemicals and antioxidants and presence of high amount of phenolic compounds (Goulas et al., 2012) and among these Beetroot (*Beta vulgaris*) ranks the 10 most powerful vegetables with respect to its antioxidant capacity (Kujala et al., 2000). Beetroot from *Chenopodiaceae* family are of different varieties of colour ranging from yellow to red of which, deep red-coloured beetroots are usually consumed either in the raw or in the cooked form (Dias et al., 2009; Zitnanova et al., 2006). Beetroot peel possesses antioxidant activity due to the free radical scavengers and prevent active oxygen-induced and free radical-mediated oxidation of biological molecules (Pedreno & Escribano, 2001). It has shown strong antioxidant activity in comparison to other vegetable peel extracts due to the presence of several active compounds such as carotenoids, glycine betaines, saponins, betacyanins folates, betanins, polyphenols and flavonoids. It is a main source of valuable water-soluble nitrogenous pigments, called betalains, comprising of two main groups, the red betacyanins and the yellow betaxanthins. Beetroot peel is widely used in food and as a colorant due to presence of betalains (John et al., 2017). These peels obtained from various fruits units constitute about 40%-45% of the total weight and if they are not processed in a proper manner (Hegary & Ibrahim, 2012). Thus, reutilization and application of plant by-products like beetroot peel involves the process of converting them into novel preservative sources that are useful to the food industry.

Deccan mahseer (*Tor khudree*), is a fresh water fish in habiting upstream, rivers and lakes and is mostly relished by the tribal people. It is a valuable food fish as well as commercially important game fish. Mahseer fish is highly esteemed owing to their nutrition subsistence and supplemental income to the hilly population. Studies related to the processing of this highly valued fish is limited; and hence, the study was aimed to make use of solvent free beetroot peel extract as a preservative on mahseer steaks to extend the shelf life under chilled condition (0-2°C).

Materials and Methods

Fresh mahseer (*Tor khudree*), having an average weight around of 1.86±0.71 kg and average length of 23.37±2.63 cm were procured from the mountain-streams of Malakkapara forest area in Kerala, India. The fresh fish were immediately iced with the ratio of 1:1 in insulated containers and brought to the laboratory. Fresh beetroot peel was procured from local market. All the chemicals used for the analysis were of reagent grade.

Beetroot peel of about 1 kg was washed thoroughly in water and dried completely in solar drier at 50°C. Extracts preparation was done according to the method described by Mansur & Khalil (2000) with slight modifications. After drying, peels were ground into powder. The dried powder (200 g) was extracted with two-three volumes of water by shaking for 1 hr in rotary shaker (Kemi, KK3.23) and filtered through Whatman No. 1 filter paper. The combined filtrates were concentrated to a volume of 100 mL in a rotary shaker (Buchi, Switerzland). The aqueous extract of beetroot peel (BPE) obtained was centrifuged for 30 min (15000 X g) and the clear supernatant was stored in air-tight container at 4°C until further use.

The total phenolic content (TPC) of beetroot peel extract (BPE) was determined as per the method of Singleton & Rosy (1965). The amount of total phenolics was calculated as catechol equivalents in mg 100 g^{-1}. Antioxidant activity of BPE was measured in terms of hydrogen-donating or radical scavenging ability; using the stable DPPH radical according to procedure of Yen & Wu (1999). The percentage inhibition of the DPPH radical was calculated as per the formula:

$$DPPH\ \% = [(AB - AS)/AB] \times 100$$

Where,

AB = absorbance of control (t = 0 min) and

AS = absorbance of tested sample at the end of the reaction (t = 30 min).

ABTS radical scavenging activity of BPE was determined according to the method of Re et al.

(1999) with slight modification. The activity was expressed as %.

$$ABTS\ \% = [(AB - AS)/AB] \times 100$$

Where,

AB = absorbance of control (t = 0 min) and

AS = absorbance of tested sample at the end of the reaction (t = 2 h).

For storage study, mahseer fishes were beheaded, degutted and finally, steaks of about 2-3 cm were cut and divided into two lots. Lot-I as control (CT) with no treatment and Lot-II was (treated steaks) dipped in 20% BPE. Concentration of the extract was selected based on the antioxidant capacity, as a minimum optimum concentration while the duration of dip treatment of 5 min. was selected based on the previous standardization at various time intervals. After dip treatment the excess liquid was drained off and the steaks were packed in pouches (size: 20 × 15 cm) made of 90 μ-polyester laminated low density polyethylene. The samples were then placed in a polypropylene box with ice and the box was further kept in chiller at 0-2°C. The samples were drawn every 3 days interval for the analysis of biochemical, microbiological and sensory quality parameters. All the analyses were done in triplicate and the mean values were taken.

Proximate composition of the mahseer steak was determined by AOAC (2000) method. The fish muscle was homogenised in distilled water (1:5 w/v) and pH was determined according to APHA (1998) using digital pH meter (Cyberscan 510, Singapore). Peroxide value (PV) and thiobarbituric acid reactive substance (TBARS) was estimated as per the methods of Yildiz et al. (2003) and Tarladgis et al. (1960) respectively. Total volatile base nitrogen (TVB-N) and trimethylamine (TMA) were estimated by the micro diffusion method (Conway, 1950). The Free Fatty Acid (FFA) content in the lipid extract was determined with improved titrimetric method (AOAC, 2000).

The average count of individual bacterial colonies in the triplicates was taken and the count was calculated as cfu g^{-1} of the sample.

TPC (cfu g^{-1}) = (Average count x Dilution factor x 10)/weight of the sample

Sensory analysis of samples was conducted by a panel of experts. Uniform pieces of fish steaks after boiling in 1.5% sodium chloride solution for 10 min. were assessed by the panellists. Scoring was based on a nine-point hedonic scale as described by (Meilgaard et al., 1999). Characteristics like colour and appearance, texture, odour and flavour were evaluated. An overall acceptance score was calculated as an average of all scores. A sensory score of 6 was taken as the borderline for acceptability. All results were expressed as mean ± standard deviation (n=3) for data analysis.

Results and Discussion

The total phenolic content of BPE is depicted in the Fig. 1 and expressed as catechol equivalents in mg 100 g^{-1}. Phenolic content increased with respect to increasing concentration. BPE showed high phenolic content (360.47±0.91 mg 100 g^{-1}) at 100%. Many studies report a correlation between antioxidant activity and phenolic content (Nuutila et al., 2003; Sathish et al., 2014). High phenolic content in beetroot peel can be attributed to the presence of considerable amount of phenolic acids such as ferulic, protocatechuic, vanillic, *p*-coumaric, *p*-hydroxybenxoic and syringic acids (Maraie et al., 2014).

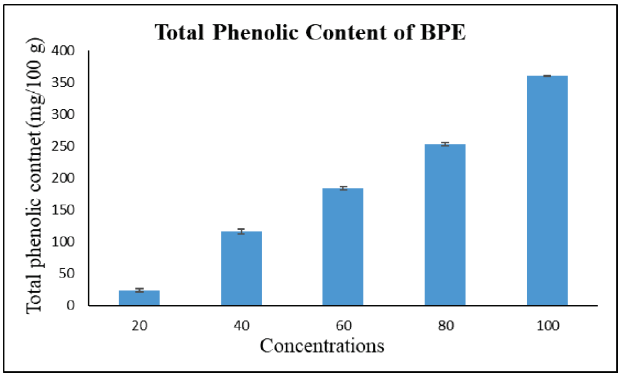

Fig. 1. Total phenolic content of beetroot peel extract (BPE)

DPPH$^{\bullet}$ radical activity is a widely used method to evaluate antioxidant activities and results were shown in the Fig. 2. The highest radical scavenging activity of BPE was 91.17±0.90% at 20%. The scavenging activity increased with increase in concentration of the extract, which is similar to the findings of Nisa et al. (2015). Hydrogen donating ability of beetroot extract was thought to be responsible for the effect on DPPH radical scavenging ability. It may be also related to the presence of imino and hydroxyl groups as well as phenolics substance which are the major contributor for the antioxidant activity of beetroot (Wu et al., 2006).

The $ABTS^{\bullet+}$ radical scavenging activity of the extract was expressed in percentage (%) (Fig. 2). The maximum radical scavenging activity of BPE was observed to be highest at 20% with the respective value of 99.04±0.56. The radical scavenging activity of the extract increased with increasing concentration from 4 to 20%. High ABTS value can be attributed to the abundance of water-soluble betalains in red beetroot extract that had a strong scavenging activity towards $O2^{"}$, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals. Clifford et al. (2015) has found that the beetroot juices inhibited *in vitro* radical formation in the (3-ethylbenzothiazoline-6-sulfonicacid) ABTS• assays by 92% which are nearly similar to our findings.

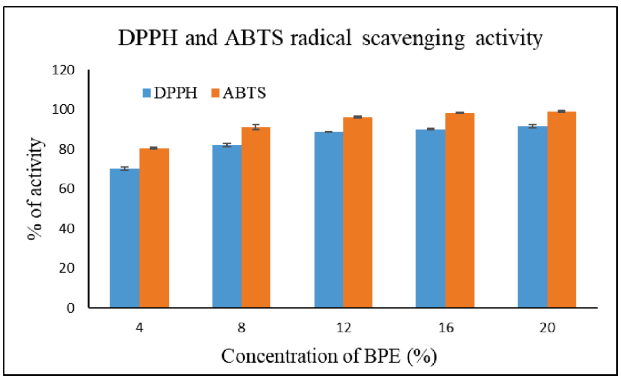

Fig. 2. Antioxidant activities of beetroot peel extract (BPE)

The fresh mahseer steaks used for this study had moisture (75.15±0.52%), protein (21.75±1.23%), fat (4.1±0.35%) and ash content (1.9±0.07%). The high protein content indicates the comparable nutritive value of Deccan mahseer with other fresh water fish such as rohu, mrigal and calbasu (Mukundan et al., 1986). The fat content indicated a semi fatty nature of fish meat which is susceptible to oxidation during storage. Higher moisture content also contributes to faster spoilage due to microbial growth.

Initial pH of fresh Deccan mahseer steaks was 6.2±0.02 and the changes in pH are depicted in Fig. 3A. There was marginal increase in pH in all the samples with respect to the storage period. In CT, the pH values increased gradually up to 6.85±0.02, whereas BPE treated samples showed the pH value of 6.42±0.13 at the end of storage period. Variations in the pH values can be attributed to the production of volatile amines (ammonia) and other compounds due to the microbial action (Kyrana et al., 1997). The initial decrease of pH maybe attributed to the production of lactic acid by anaerobic glycolysis and while the increase of pH could be due to an increase in volatile bases products, e.g. ammonia and trimethyl amine, either by endogenous or microbial enzymes (Delbarre et al., 2006). Similarly, several authors have reported an increase in pH with increase in the storage time (Ozogul et al., 2016, Sofi et al., 2016).

PV measures the amount of hydroperoxides formed i.e., first stage of oxidative rancidity in fish muscle and the changes in PV of mahseer steaks treated with BPE during storage is depicted in Fig. 3B. The peroxide value of fresh fish was found to be 1.03±0.01 mg hydroperoxide kg^{-1} sample. During storage, PV values constantly increased in the CT sample and reached to a maximum value on 9^{th} day (9.46±0.42 mg hydroperoxide kg^{-1}), while BPE treated samples had a peroxide value of 3.53±0.42 mg hydroperoxide kg^{-1}. On the 15^{th} day of storage, CT samples had crossed the limit with value of 10.95±0.03 mg hydroperoxide kg^{-1} whereas; treated BPE sample had PV of 6.69 mg hydroperoxide kg^{-1} which revealed that, the BPE effectively limited the formation of hydroperoxides throughout the storage period. Ojagh et al. (2010) reported much lower peroxide value than the present result (3.43 mg hydroperoxide/kg sample) for rainbow trout fillets (*Oncorhynchus mykiss*) treated with chitosan coating during the refrigerated storage (4±1°C). Shi et al. (2014) and Jianyun et al. (2014) have reported the similar findings in case of silver carp fillets treated with grape seed and clove bud extracts as natural antioxidants during chilled storage.

Changes in TBARS value during chilled storage of steaks are illustrated in Fig. 3C. Initial TBA value of the samples was found to be 0.34±0.01mg malonaldehyde kg^{-1}. Control sample exceeded the acceptable limit of 2 mg MDA kg^{-1} (Connell, 1990) on 9^{th} day of storage period. During the storage CT sample showed gradual increase in TBA value and touched the maximum value of 2.92±0.02 mg malonaldehyde kg^{-1} at the end of storage. However, BPE treated sample reached the maximum value of 2.0±0.03 mg malonaldehyde kg^{-1} on 15^{th} day of storage. Therefore, based on results it is clearly evident that, sample treated with BPE effectively extended the shelf life of mahseer steaks.

Changes in TVB-N content of BPE treated mahseer steaks during ice storage are shown in Fig. 3D. In CT, TVB-N content gradually increased from the initial value of 10.5±0.28 mg% and on 9^{th} day of storage the TVB-N value of CT crossed the limit of

30.68 mg% reaching the maximum value of 38.47±0.35 mg% on 15th day. Whereas BPE treated sample was below the acceptability limit (29.28±0.23 mg%) even at the end of storage. The level of TVB-N in freshly caught fish is typically between 5 and 30 mg N100 g^{-1}, and is generally regarded as the limit of acceptability for ice-stored cold water fish (Connel, 1980). TVB-N delayed formation in treated sample during the storage can be credited to the antimicrobial property of the BPE (Chanda & Parekh, 2008). The results of present study was consistent with the findings of other authors (Arul kumar et al., 2017; Quitral et al., 2009). Consequently, TVB-N was lowest in BPE steaks as compared to the CT samples indicating the preservative effect of beetroot peel extract in controlling the bacterial growth responsible for volatile bases formation during spoilage.

TMA is most commonly used method of quality assessment of fish muscle (Chang et al. 1976). A gradual increase in TMA-N of ice-stored mahseer steaks treated with BPE and control samples are shown in Fig. 4A. Initial values of TMA content of the samples were 2.10±0.10 mg%. The CT samples reached the limit of 10.25±0.12 mg% on 9th day and maximum of 18.45±0.22 mg%. While BPE treated samples (11.45±0.43 mg%) were within the limit of acceptability at the end of 15th day of storage. In fresh water fish a level beyond 10-12 mg TMA-N 100 g^{-1} of fish is considered as spoiled (Connell, 1980). As in case of fresh water fish TMA content may dependent on several factors like sex, species, season of capture, feeding habit.

Lipid hydrolysis during the storage was estimated by changes in value of free fatty acid (FFA) of mahseer steaks as depicted in Fig. 4B. Changes in free fatty acid content in both the samples increased throughout the storage period. The FFA value of CT steaks increased from 1.14±0% to the maximum

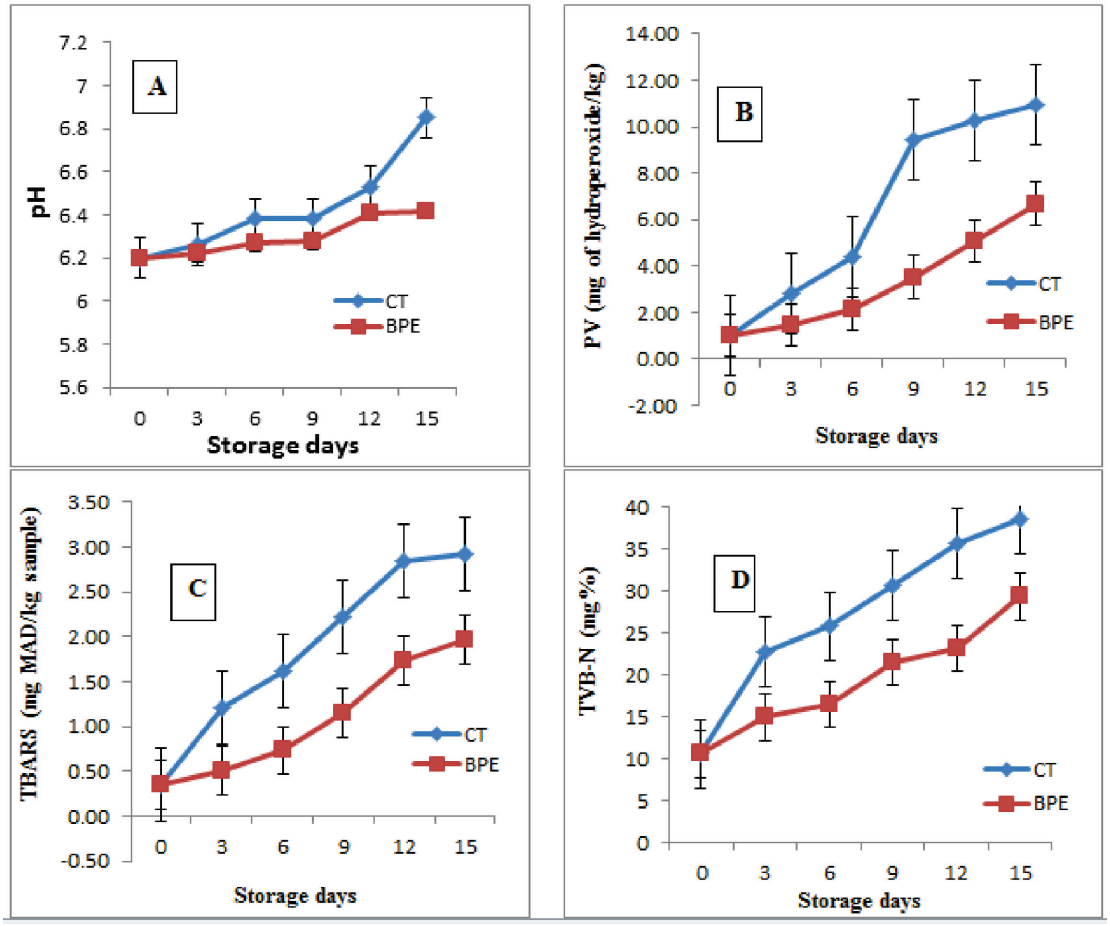

Fig. 3. Changes in biochemical and quality parameters of Mahseer steaks during chilled storage

A: pH values, B: Peroxide values C: TBARS value D: TVB-N values

value of 6.12±0.11% on 9th day, at the end of storage period the value attained 8.82±0.14% of oleic acid, whereas in BPE treated samples the values gradually increased from 1.14±0% to 6.12±0.10% of oleic acid on the 15thday of storage. The results clearly shows effect of BPE is significant to inhibit rate of lipid hydrolysis thus effective in controlling the liberation of free fatty acid and the reason could be the inactivation of lipolytic enzymes and inhibition on the growth of lipolytic bacteria as the beetroot possess the water-soluble fractions of betalains and phenolic substances (Raikos et al., 2016).

The TPC of fresh mahseer was 4.11±0.01 log cfu g^{-1} indicating the fish was of good quality as per the proposed upper limit of fresh fish (5.00 log cfu g^{-1}) by ICMSF (1998) for human consumption. Changes in TPC of BPE treated mahseer steaks throughout storage were shown in Fig. 4C. The TPC values of CT samples gradually increased and exceed the limit of (7.47±0.02 log cfu g^{-1}) on the 9th day of storage. Whereas the samples treated with BPE samples exceed the limits (7.35±0.05 log cfu g^{-1}) on 15th day of storage, which is clearly evident that the BPE effectively extents the shelf life of mahseer steaks by 6 days. Several authors have reported the antimicrobial property of beetroot peel, and activity of bioactive components mostly present towards the outer parts of root and decreasing in order from peel, crown and flesh (Chanda & Parekh, 2008; John et al., 2017).

Changes in overall acceptability score is presented in Fig. 4D. Sensory scores showed a significant decline in both the control (CT) and BPE treated samples with increasing storage period. Sensory deterioration was rapid in control samples, and became unacceptable on day 9. The overall score declined from initial 9.03±0.06 to 3.02±0.01 on 15th day of storage. Whereas the score for sample treated with extract showed decline from initial score of 9.03±0.06 to 6.06±0.03 at the end of storage day. Fish

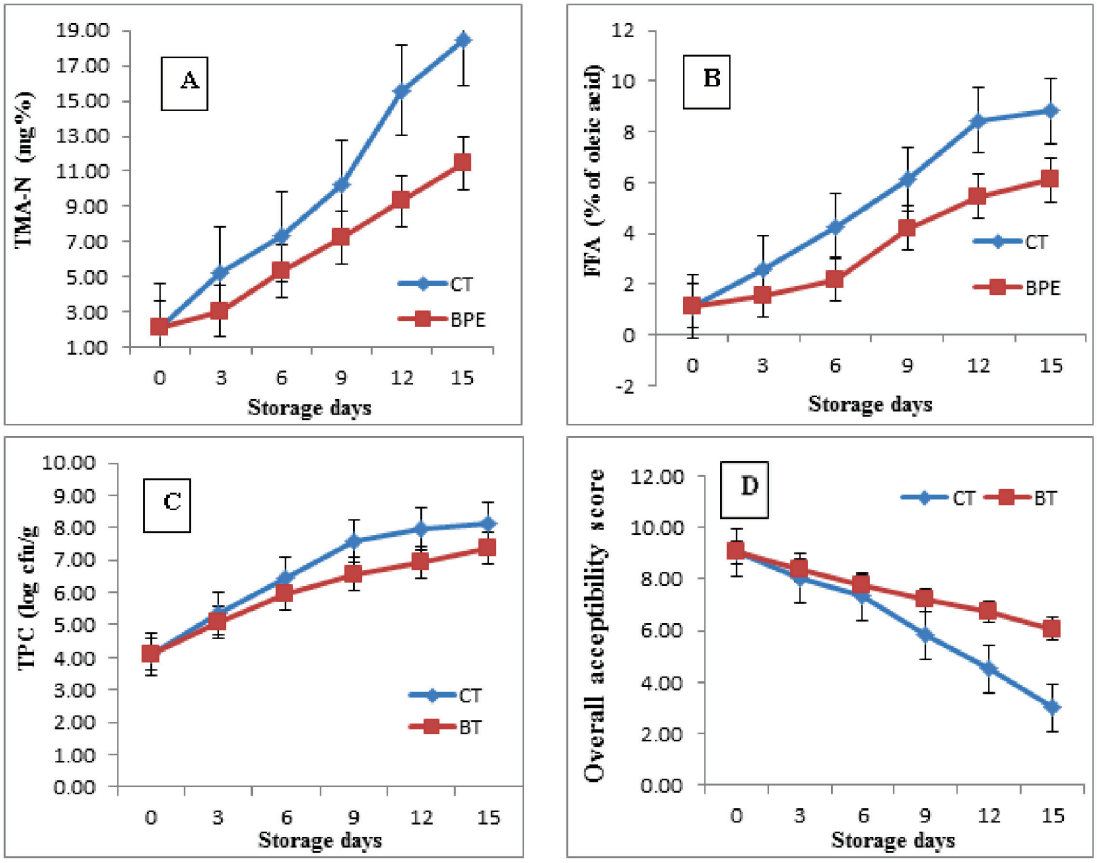

Fig. 4. Changes in quality parameters, microbial analysis and overall acceptability of mahseer steak during chilled storage

A: TMA-N values, B: FFA values C: TPC value D: Overall acceptability score

samples were considered to be acceptable for human consumption until the sensory score reached 6 (Satish et al., 2014). Consequently, control samples were acceptable up to 6th day while the treated sample remained in good condition up to 15th days. Preservative effect of (BPE) could be attributed to the presence of the phenolic contents such as L-tryptophane, *p*-coumaric, protocatechuic, vanillic, and ferulic acids, as well as cyclodopa glucoside derivatives that possess high levels of antimicrobial activity (Baydar et al., 2004; Kujala et al., 2001). Thus, the results of present study agree with the conclusion of the other biochemical and microbiological parameters.

The results of the present study revealed a significant effect of BPE on mahseer steaks for controlling the biochemical indices like TVB-N, TMA-N, PV, FFA and TBARS during storage. The total bacterial count was also suppressed due to the effect of treatment when compared with control sample and although, the overall acceptability scores of the extract treated mahseer steaks was better than control. Therefore, based on the present results the dip treatment in beetroot extract enhanced the storage stability and extended the shelf life of mahseer by six days during the chilled storage. Therefore the present study clearly demonstrated the preservative effect of beetroot peel extract, which could be a potential source of antioxidants, and can be used as a natural preservative for shelf life extension of fish and fishery products.

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