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# Antioxidative Effect of Pineapple Peel Extracts in Refrigerated Storage of Indian Mackerel

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## Abstract

Natural antioxidants were extracted from pineapple peel (*Ananas comosus* L, var. queen) waste by cold extraction method [referred as pineapple peel extracts (PPE)] and applied as a preservative against lipid oxidation in Indian mackerel steaks stored at refrigerated condition for 15 days. The antioxidant substances such as total phenols and total flavonoids were determined. The antioxidant potential of PPE was assessed using *in vitro* assays including, ferric reducing power and DPPH radical scavenging activity. Total phenolic and total flavonoids content were found to be 131 mg GAE $100g^{-1}$ and 211.2 mg QE $100g^{-1}$ respectively. The $IC_{50}$ of PPE for DPPH radical scavenging was 0.55 $mgml^{-1}$. Reducing power ($OD=A_{700nm}$) of PPE was found to be 0.272 at the concentration of 500$\mu gml^{-1}$. Lipid changes during refrigerated storage after treating with PPE were monitored by measuring peroxide value (PV), free fatty acids (FFA) and thiobarbituric acid reactive substance (TBARS). These parameters revealed that the BHA which was used as a standard for comparison was more effective than the PPE. However, when the PPE treated samples were compared with the changes in control samples, PPE was found to offer protective effect to certain degree against lipid oxidation. Thus, it can be concluded that the antioxidant compounds present in the pineapple peel can be extracted and used as natural antioxidants.

**Keywords:** Pineapple peel extracts, natural antioxidant, lipid oxidation, Indian mackerel, refrigerated storage

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## Introduction

In recent years, antioxidative molecules from natural resources have gained much attention due to their health beneficial properties. However, identification of cheap resources, its abundance and activity potential are essential criteria for selection of any natural antioxidant. Pineapple is an important fruit in the world (Cabrera et al., 2000) as well as in India. According to Indian Horticulture Database (2014), the total fruits production in India was 88977 (In 000′MT) out of which 1736 (000′ MT) was contributed by pineapple. Reports have shown that 40-80% of pineapple fruit is inedible and discarded as waste (Bankoffi & Han, 1990). Pineapples produce 9.12% of core, 13.48% of peels, 14.49% of pulp, 14.87% of crown, and 48.04% of finished products (Ayala-Zavala et al., 2010). Chandapillai & Selvarajah (1978) reported that in pineapple canneries, approximately 75% of the fruit, in the form of core, crown end, peeled skin, etc., is discarded as waste, causing problems of disposal and pollution.

Fatty fish species possess high nutritional importance due to their high content of polyunsaturated fatty acids (PUFAs) which includes eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The beneficial functions of EPA and DHA to human health are well established. Nevertheless, PUFA are more susceptible to oxidation and associated with the development of rancidity and loss of nutritional value (Frankel, 1998a; Hsieh & Kinsella, 1989). The high levels of moisture, free amino acids, polyunsaturated fatty acids, presence of autolytic enzymes and near neutral postmortem pH, render fish as an easily perishable commodity (Cakliet al., 2007). Apart from good amounts of PUFAs, the heme pigments and trace amounts of metallic ions present in the dark flesh of fatty fish makes the lipid more prone to oxidation (Hsieh & Kinsella, 1989).

Lipid peroxidation in fatty foods not only brings about chemical spoilage but also produces free radicals such as peroxyl and hydroxyl radicals, which are toxic in nature and associated with carcinogenesis, mutagenesis, and aging diseases (Yagi, 1987; Nasr et al., 1996). Many degenerative human diseases including cancer, cardio- and cerebro-vascular diseases have been identified as a possible consequence of free radical damage to major bio-molecules such as lipids, proteins and nucleic acids (Choi & Lee, 2009). To retard such a quality loss in food industry and to protect the consumer, synthetic antioxidants have been used to decrease lipid oxidation during the processing and storage of food and food products (Boydet al., 1993). However, the use of synthetic antioxidants has raised questions regarding food safety and toxicity upon long term consumption (Chang et al., 1977). Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA) have been used but restricted recently, due to their possible carcinogenicity (Mahdavi & Salunkhe, 1995), liver swelling and changing the liver enzyme activities (Martin et al., 1968).

Plant and fruit extracts have a great potential as alternatives to chemical preservatives and can be referred to as bio-preservatives or green additives. Pineapple and its waste products (peel, core and top) contain good quantity of phenolic compounds, flavonoids and ascorbic acids which are important natural antioxidants. Flavonoids are mainly present as colouring pigments in plants and also act as antioxidants at various stages of processing and preservation (Terao et al., 1994). Pineapples have been studied for their nutrients including fibre, protein, carotenoids, mineral composition, total polyphenols content, etc. (Gorinstein et al., 1999; Alejandra et al., 2011). Limited research has been carried out on the antioxidant capacity of pineapples and its peel. de Oliveira et al. (2009) has studied the total phenolic content and antioxidant activities of methanolic extracts of pineapple (*Ananas comosus*) residues (including pulp, seeds and peels from a local juice factory) using DPPH radical and super-oxide anion scavenging activity. The study on total polyphenols of pineapple (Calendar, India) extracted with different solvents was reported by Hossain et al. (2011) and the results showed that the polyphenolic contents of the extracts were found to be highest in methanol and exhibited the highest antioxidant properties. Moreover, there are limited reports on application of pineapple peel extracts as fish preservative. Therefore, the present work was aimed at assessing antioxidant activity of pineapple peel extracts and determining its efficiency on Indian mackerel steaks under refrigerated storage for 15 days.

## Materials and Methods

Pineapple peel (*Ananas comosus* L, var. queen), mix of fully and half ripened,were collected from local market, Mangalore, Karnataka. The peel was washed, cut into small pieces, dried in the hot air oven at 60°C for 72 h and ground into fine powder using grinder mixer. Extraction was (50 g each in two 500 ml conical flasks) carried out with absolute ethanol at a ratio of 1:5 in an orbitary shaker (Orbitek LJ Scigenics Biotech, Chennai, India) at 180 rpm for 10 h. Extracts were filtered with Whatman No. 42 and the filtrate was concentrated by vacuum rotary evaporator (Superfit Rotavap PBU-6D, Mumbai, India) at 50°C. The yellow color concentrate obtained (referred as PPE) was transferred to amber glass container and stored at 3±1°C in a refrigerator until used.

The yield of dried powder was calculated as the percentage ratio of dried powder obtained to the weight of fresh peel. Whereas, the residue after vacuum evaporation was weighted to obtain the extracts yield.

% Extracts yield (concentrated) = weight of residue/ weight of powder x 100

Total Phenolic Content (TPC) of PPE was determined according to the method of Singh et al. (2001) with slight modifications. About 50 mg of extract was dissolved in 50 ml of 10% ethanol. Aliquots of 300 µl sample/ deionized water (for control) were mixed with 1.5 ml freshly diluted Folin-Ciocalteau (FC) reagent. After 3 min, 1.2 ml of 7.5% sodium carbonate solution was added and mixed. The reaction mixture was kept in dark for 30 min at room temperature and its absorbance was measured at 765 nm against deionized water in a UV-Vis spectrophotometer (Systronic VIS Double Beam Spectro 1203, Ahmedabad, India). The concentration of the total polyphenols was determined using gallic acid standard curve and expressed as mg of gallic acid equivalents (GAE) 100 $g^{-1}$ PPE.

The total flavonoid content (TFC) in extracts was estimated as described by Christ & Muller (1960)

with slight modification. Samples (1 mg) were dissolved in 1 ml methanol/water mix (50:50 v/v). An aliquot of 0.5 ml sample solution was mixed with 1.5 ml of methanol and 0.1 ml $CH_3COONa$ (1 M) was added. After 6 min of incubation at room temperature, 0.1 ml $AlCl_3$ (10%, w/v) was added and the total volume was made up to 5 ml. The solution was mixed in a vortex and incubated for 30 min at room temperature. The colour development was measured at 430 nm against the blank. Quantification was expressed by comparing the absorbance of quercetin calibration curve as the standard for flavonoids (0 to100 $\mu gml^{-1}$). Results were expressed as mg quercetin equivalents (GE) 100 $g^{-1}$ dry weight.

The DPPH free radical scavenging capacities of PPE and BHA were measured according to the method of Shimada et al. (1992) with slight modifications. Stock solution of PPE and BHA was made at concentration of 2 and 0.02 $mgml^{-1}$, respectively. An aliquot of different concentrations was mixed with 3.9 ml of 100 μM DPPH solution by incubation at room temperature in darkness for 30 min. The absorbance after incubation was measured at 517 nm. Appropriate control was maintained using ethanol. The DPPH radical scavenging activity was computed using the equation:

DPPH free radical scavenging activity (%) = [(Absorbance of control — Absorbance of sample)/ (Absorbance of control)] × 100

The results were expressed as $EC_{50}$, (the effective concentration of antioxidant that decrease the initial DPPH radical concentration by 50%). The lower EC50 value indicates higher antioxidant activity.

FRAP assay was determined as described by Oyaizu (1986).

Indian mackerel (*Rastrelliger kanagurta*) weighing 200-210g in the length range of 22-23 cm (n=10) were purchased from local landing centre, Mangalore, Karnataka. The fish were dressed and thoroughly cleaned using potable water. The following process scheme was used for the treatment of samples (Fig. 1).

The proximate components (moisture, crude protein, total lipids and ash) were analyzed as described in AOAC (1997). The analysis was carried out in triplicates for all the parameters.

Fish were washed, gutted and beheaded

↓

Cut into steaks

↓

Steaks were submerged into respective BHA, PPE and Control (DW) solution (10±2°C) at a ratio of 1:1 separately for 40 min

↓

Solutions were drained and steaks were packed in polyethylene bags

↓

Stored under refrigerated condition (3-4°C)

Fig. 1. Flow Chart for preparation of fish steaks sample

pH was determined using EUTECH instruments pH Tutor. Ratio of 1:10 (w: v) sample to distilled water was taken for preparing homogeneous mixture.

TBARS values of samples were determined as per Raghavan (2005).

Free fatty acids content was determined as per AOAC (1990).

FFA (% oleic acid) = Titre value of alkali x Normality of alkali/ Weight of fat x 28.2

Peroxide values of the samples were determined as per AOAC (1990).

PV = Titre value X N of $Na_2S_2O_3$/ Weight of fat X 1000

Statistical analyses of data were carried out using statistical package for social science software (IBM SPSS Statistics 20.0, Chicago IL. USA). Analysis of variance (ANOVA) and the significant difference among the treatments was carried out by Duncan's Multiple Range Test (DMRT). The level of significance was 5%.

## Results and Discussion

Yield, TPC and TFC of PPE is presented in Table 1. The total polyphenolic content of PPE was found to be 131 mg GAE $100g^{-1}$ of PPE. One group of active molecules of great interest to the food industry is the phenols extracted from fruits, vegetables, and agro-processing by-products (Corbo et al., 2008). Pineapple, grape, pomegranate, berries etc. are well

established to be the good source of polyphenols. Shen (2013) stated that gallic acid (31.76 mg $100g^{-1}$), catechin (58.51 mg $100g^{-1}$), epicatechin (50.00 mg $100g^{-1}$) and ferulic acid (19.50 mg $100g^{-1}$) were found to be the main polyphenolics in pineapple peels.The solvent used for extraction influence the yield and nature of polyphenols in the extract (Hossain et al., 2011; de Oliveira et al., 2009). Hossain et al. (2011) had studied the effect of solvents on the polyphenols extraction from pineapple (var Calendar, India) and found that the methanol was the suitable solvent to get more yield (21.50%) followed by ethyl acetate (4.90%) and water extract (4.30%). But, methanol was not used for extraction in the present work since methanol should not be used in food application. The yield of ethanolic pineapple peels extracts in the present study was 14.54%. Recent studies showed that phenolic compounds from fruit extract were effective in delaying lipid oxidation in frozen horse mackerel (*Trauchurus trauchurus*) fillets and alsothe fruit polyphenols inhibit the depletion of endogenous α-tocopherol, ubiquin one-10 and total glutathione in minced mackerel (*Scombers combrus*) muscle and horse mackerel fillets (Pazoas et al., 2005; Pazos et al., 2006).

The total flavonoid content in PPE was found to be 211.2 mg QE 100 $g^{-1}$ (Table 1). As a general categorization proposed by Shi et al. (2003), phenolic compounds in grape and pineapple can be divided into two groups: phenolic acids (precursors of flavonoids) and flavonoids. The four most flavonoids present in the fruits and plants extracts are Catechin, Epicatechin, Myricetin, and Quercetin.

Table 1. Yield of PPE and content of total phenols and flavanoids in PPE

| Extract / Antioxidants | Value |
|---|---|
| PPE (%) yield | 14.54 |
| TPC (mg GAE $100g^{-1}$) | 131.00 |
| TFC (mg QE $100g^{-1}$) | 211.20 |

DPPH radical scavenging activities of PPE and BHA at different concentrations are shown in Fig. 2. BHA showed high DPPH radical scavenging activity even at very low concentration. But PPE showed moderate free radical scavenging activity. PPE was found to exhibits maximum DPPH radical scavenging activity of 81.75% at 2 $mg^{-1}$ml. The $IC_{50}$ was found to be 0.55 $mgml^{-1}$. The $IC_{50}$ of the extracts defines the amount of the extracts needed to scavenge 50% of the DPPH radical. The effect of antioxidants on DPPH radical scavenging is generally due to their hydrogen donating ability (Siddhuraju & Becker, 2007). The presence of a second hydroxyl group in the ortho or para position of phenolic derivative is known to increase antioxidative activity due to the additional resonance stabilisation and o-quinone or p-quinone formation (Graf, 1992). Thus, the presence of higher numbers of hydroxyl groups in catechin and tannic acid of any fruit extract will most likely to increase the DPPH radical scavenging activity. On the other hand, the ferulic acid is reported to have negative effect on antioxidant activity (ThiagoInacio et al., 2008).

BHA= Butylated hydroxyanisole, PPE= Pineapple peel extracts

Antioxidant potential of phenolic compounds was estimated for their ability to reduce Fe(III) to Fe(II) and the results are presented in Fig.3. As per the assay, more the absorbance (OD) at 700 nm, higher the reducing power of the antioxidant. The reducing power of PPE was lower when compared to ascorbic acid. At 500 $\mu gml^{-1}$ concentration the absorbance was found to be 0.272. The reducing capacity measures the ease of the compounds in donating electrons (Medina et al., 2007). Results indicate that PPE contained substances which could reduce the ferric ions to ferrous form.

The proximate compositions of the samples are shown in Table 2. Initially, there was a slight decrease in the moisture content of all the samples and the value was almost constant upto 9 days of

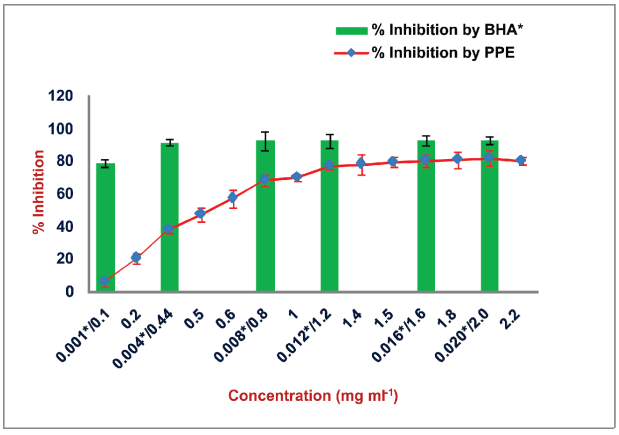


Fig. 2. DPPH radical scavanging capacity of BHA and PPE

Table 2. Proximate composition of control, BHA treated and PPE treated Indian mackerel steaks in refrigerated storage

| Parameters | Samples | 0th day | 3rd day | 6th day | 9th day | 12th day | 15th day |
|---|---|---|---|---|---|---|---|
| Moisture (%) | CTRL | 75.22±0.33$^{a}$ | 72.34±0.25$^{ab}$ | 72.85±0.40$^{b}$ | 72.22±0.51$^{ab}$ | 73.58±0.27$^{c}$ | 73.34±1.33$^{ab}$ |
| | BHA | 75.08±0.82$^{a}$ | 72.55±0.13$^{b}$ | 73.00±0.15$^{b}$ | 72.79±0.51$^{b}$ | 72.40±0.39$^{b}$ | 73.90±0.64$^{b}$ |
| | PPE | 75.23±0.49$^{a}$ | 72.18±1.72$^{a}$ | 71.72±0.25$^{a}$ | 71.66±0.24$^{a}$ | 71.35±0.30$^{a}$ | 72.14±0.66$^{a}$ |
| Ash (% DMB) | CTRL | 4.72±0.03$^{a}$ | 4.01±0.18$^{a}$ | 4.38±0.01$^{c}$ | 4.31±0.04$^{c}$ | 3.89±0.05$^{b}$ | 4.01±0.03$^{b}$ |
| | BHA | 4.41±0.06$^{a}$ | 4.00±0.29$^{a}$ | 3.77±0.03$^{b}$ | 3.71±0.04$^{a}$ | 3.07±0.02$^{a}$ | 3.71±0.03$^{a}$ |
| | PPE | 4.56±0.02$^{a}$ | 3.66±0.07$^{a}$ | 3.39±0.04$^{a}$ | 3.84±0.02$^{b}$ | 3.14±0.01$^{a}$ | 3.76±0.02$^{b}$ |
| Protein (% DMB) | CTRL | 61.09±0.88$^{a}$ | 68.69±0.00$^{a}$ | 73.03±2.02$^{a}$ | 71.99±1.01$^{a}$ | 77.59±1.75$^{a}$ | 74.86±2.67$^{a}$ |
| | BHA | 61.07±1.33$^{a}$ | 68.37±1.75$^{a}$ | 71.29±0.00$^{a}$ | 71.95±1.01$^{a}$ | 71.84±1.01$^{a}$ | 69.27±1.01$^{a}$ |
| | PPE | 60.51±1.51$^{a}$ | 68.47±1.01$^{a}$ | 69.83±1.01$^{a}$ | 76.74±1.75$^{a}$ | 76.82±1.75$^{a}$ | 75.30±1.75$^{a}$ |
| Lipid (% DMB) | CTRL | 34.18±0.05$^{a}$ | 28.27±0.12$^{b}$ | 21.21±0.20$^{a}$ | 20.37±0.15$^{a}$ | 18.47±0.04$^{a}$ | 18.69±0.15$^{a}$ |
| | BHA | 34.51±0.22$^{a}$ | 27.61±0.12$^{a}$ | 24.81±0.04$^{b}$ | 22.85±0.06$^{b}$ | 24.74±0.06$^{c}$ | 24.59±0.09$^{b}$ |
| | PPE | 34.92±0.02$^{a}$ | 27.85±0.05$^{ab}$ | 26.62±0.10$^{c}$ | 19.26±0.04$^{a}$ | 19.75±0.38$^{b}$ | 18.55±0.04$^{a}$ |

CTRL= Control, BHA Treated = Butylated hydroxyanisole, PPE treated = Pineapple peel extracts. (n=3, Mean±SD)
DMB= Dry matter basis
Different letters in the same column indicate significant differences ($p<0.05$)

storage. Further, there was a negligible increase in the moisture content up to 15 days of storage. This could be attributed to the changes taking place in the muscle fibre architecture. During storage, due to changes in the moisture level, the protein content also vary as the solid content per unit weight differs with the moisture content. In general, reduction in the total lipid was observed in all the samples and one could expect the degradation of lipids due to oxidation as mackerel is a good source of unsaturated fatty acids which are known for their susceptibility to oxidation process. In the present investigation, the reduction in lipid content was more pronounced in control followed by PPE and BHA treated samples. Significant difference ($p<0.05$) was observed in the lipid quantity amongst the control, BHA and PPE treated samples during the storage period. However, there was no remarkable change in the ash content throughout the storage period.

The changes in PV of the samples during refrigerated storage are given in Fig. 4. The measure of peroxide value indicates the formation of primary lipid oxidation products which include fatty acid

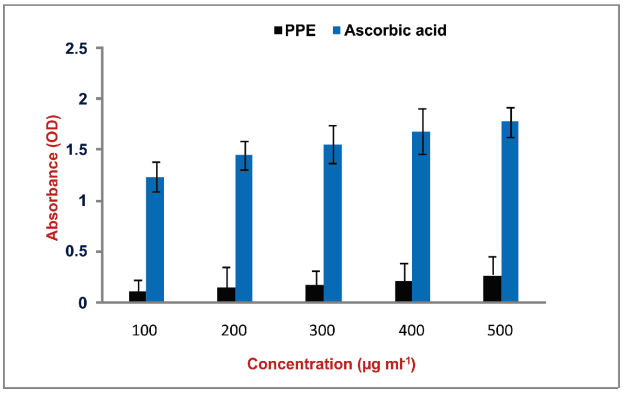


Fig. 3. FRAP assay of Ascorbic acid standard and PPE

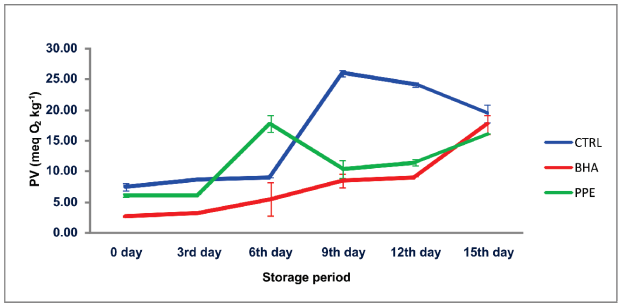


Fig. 4. PV content of control, BHA treated and PPE treated Indian mackerel steaks in refrigerated storage
CTRL= Control, BHA treated = Butylated hydroxyanisole, PPE treated = Pineapple peel extracts

peroxy radicals. The peroxy radicals are highly reactive and further abstracts the hydrogen from another hydrocarbon chain. This reaction yields hydroperoxide and form a new free radical. Mackerel contains high amount of PUFA due to which its shelf life is limited. In this study, the initial PV of control, PPE and BHA treated samples were 7.47, 6.09 and 2.76 meq $O_2$ $kg^{-1}$ fat, respectively. It has to be mentioned that the dip treatment was given for 40 min at below room temperature. In control, the treatment was with distilled water. From the results, it could be made out that the oxidation takes place during the treatment itself which could be effectively controlled by BHA followed by PPE.

The slightly higher initial PV (0 day) in control sample could be due to exposure of steaks surface in distilled water leading to easy hydration. Hydration of molecule increases molecular mobility and reactivity. Water accumulating around molecular surfaces dissolves catalyst and increases their diffusion and interaction with lipid; and mobilizes active metals present in the muscle (Schaich et al., 2013). During the storage period, the PV increased abruptly in control samples during 6 to 9$^{th}$ day (9 to 26.01 meq $O_2$ $kg^{-1}$ fat). Though there was an increase in PV of PPE treated sample, overall PV was lower compared to control till 15$^{th}$ day. The better retardation of lipid oxidation process was found in BHA treated samples compared to PPE. The slight decrease in PV of control sample after 9 days of storage could be due to decomposition of hydroperoxide formed (Boselliet al., 2005; Al-Bulushiet al., 2005).

Changes in Thiobarbituric acid reactive substances (TBARS) values of the samples are shown in Fig.5. TBARS value is an index of lipid oxidation and is well correlated with the oxidized flavour of animal foods. Lipid hydroperoxides formed during the primary phase of oxidation are very unstable. They undergo cleavage and yield free alkoxy radicals, which are further broken down to aldehydes, ketones and hydrocarbons. It is well known that unsaturated lipids produce malonaldehyde in the terminal step of the autoxidation process. The samples treated with PPE were relatively stable to oxidation than the control sample as revealed by the changes in TBARS value. The initial TBARS value (0 day) was observed to be considerably higher which could be attributed to enhanced mobility of radicals due to hydration of the molecules during dip treatment (Schaich et al., 2013). TBARS values are expected to increase with storage time but interestingly it was observed that the value decreased on 9$^{th}$ day of storage. This decrease could be attributed to their reaction with free amino acids, proteins and peptides of fish muscle to form Schiff's base (Dillard & Tappel, 1973).

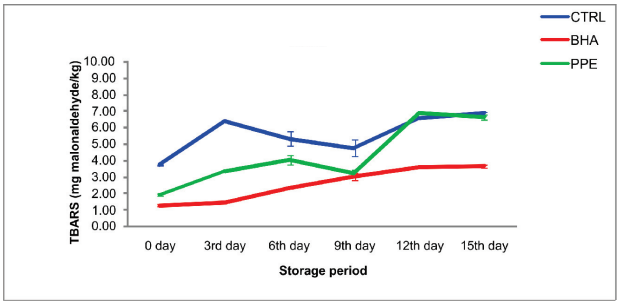


Fig. 5. TBARS content of control, BHA treated and PPE treated Indian mackerel steaks in refrigerated storage
CTRL= Control, BHA treated = Butylated hydroxyanisole, PPE treated = Pineapple peel extracts

Among all the samples, BHA treated samples showed better lipid stability against oxidative deterioration. Numerous studies carried out in marine fatty fish like mackerel, have reported inhibition of lipid oxidation by the use of grape based natural antioxidants (Pazoset al., 2005a; 2010; Sánchez-Alonso et al., 2007; Sánchez-Alonso & Borderías, 2008). Yerlikaya & Gokoglu (2012), have reported that the grape seed extracts (GSE) were effective in retarding the increase of TBA levels in bonito (*Sarda sarda)* fillets during frozen storage and Özen et al. (2011) reported the formation of lipid hydroperoxides and TBARS was significantly inhibited by the addition of GSE in chub mackerel (*Scomber japonicus*) minced muscle during frozen storage.

Results of FFA are presented in Fig.6. FFA is formed as a result of enzymatic decomposition of lipid in fish (Hardy, 1980). The initial level of FFA was 1.65%, 1.08% and 0.97 for the control, BHA and PPE treated sample, respectively. BHA in control sample reached maximum level of 6.48% on the 12$^{th}$ day of the storage. There were significant differences ($p<0.05$) in FFA between the control and PPE treated groups. The lowest FFA was observed for BHA while the highest FFA was found for the control. This indicated that PPE can be effectively employed for lowering the lipolytic activity in fish muscle.

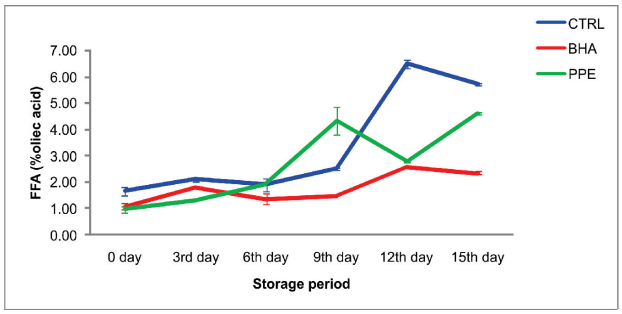


Fig. 6. FFA of control, BHA treated and PPE treated Indian mackerel steaks in refrigerated storage CTRL= Control, BHA treated = Butylated hydroxyanisole, PPE treated = Pineapple peel extracts

Lipid hydrolysis developed at a slower rate in the samples treated with PPE during the initial stage of storage. Previous workers have also reported similar results (Kenar et al., 2010; Ozogul et al., 2010). An increase in FFA (lipolysis) results from the enzymatic hydrolysis of lipids (Hwang & Regenstein, 1993). The connection between lipolysis and lipid oxidation is that the free polyunsaturated fatty acids oxidise more readily than intactlipid (Ashton, 2002).

In light of the experimental results, it can be concluded that the pineapple peel extracts were effective in delaying lipid oxidation in muscles of Indian mackerel thereby enhancing the shelflife during refrigerated storage. Pineapple peel extracts could be a potential source of natural antioxidant which can be effectively incorporated in fish and fishery products.

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## References

Al-Bulushi, I.M., Kasapis, S., Al-Oufi, H. and Al-Mamari, S. (2005) Evaluating the quality and storage stability of fish burgers during frozen storage. Fish Sci. 71: 648-654

Alejandra, R. and Emperatriz, P.D.D. (2011) Chemical composition and bioactive compounds in pineapple, guava, and soursop pulp. Interciencia. 36: 71-75

AOAC (1990) Official Methods of Analysis. 15thedn., Association of Official Analytical Chemists, Washington, DC

AOAC (1997) Official Methods of Analysis. 20th edn., Association of Official Analytical Chemists, Washington, DC

Ashton, I.P. (2002) Safety and Quality Issues in Fish Processing (Allan Bremner, H. Ed), 507 p, Washington, DC, CRC Press

Ayala-Zavala, J.F., Rosas-Domínguez, C., Vega-Vega, V. and González-Aguilar, G.A. (2010) Antioxidant enrichment and antimicrobial protection of fresh-cut fruits using their own by products: Looking for integral exploitation. J. Food Sci. 75: 175-181

Boselli, E., Caboni, M. F., Rodriguez-Estrada, M. T., Toschi, T. G., Daniel, M. and Lercker, G. (2005) Photoxidation of cholesterol and lipids of turkey meat during storage under commercial retail conditions. Food Chem. 91: 705-713

Boyd, L.C., Green, D.P.G., Giesbrechi, F.B. and King M.F. (1993) Inhibition of oxidative rancidity in frozen cooked fish flakes by tert-butylhydroquinone and rosemary extract. J. Sci. Food Agri. 61: 87-93

Cabrera H.A.P., Menezes H.C., Oliveira, J.V. and Batista, R.F.S. (2000) Evaluation of residual levels of benomyl, methyl parathion, diuron, and vamidothion in pineapple pulp and bagasse (Smooth cayenne).J. Agric. Food Chem. 48: 5750-5753

Cakli S, Kilinc B, Cadun A, Dincer T and Tolasa S. (2007) Quality differences of whole ungutted sea bream (*S. aurata*) and sea bass (*D. labrax*) while stored in ice. Food Chem. 18: 391-397

Chandapillai, M.M. and Selvarajah T. (1978) Food and Agriculture Malaysia, 257 – 274 p., 2000Ed. Chin H.F., Faculty of Agriculture UPM, Malaysia

Chang, S. S., Ostric-Matijasevic, B., Hsieh, O. A. L.andHuang, C. (1977) Natural antioxidants from rosemary and sage. J. Food Sci. 42(4): 1102-1106

Choi, Y. and Lee, J. (2009) Antioxidant and antiproliferative properties of a tocotrienol rich fraction from grape seeds. Food Chem. 114: 1386-1390

Christ, B. and Muller. K.H. (1960) Determination of the amount of flavonol derivatives in drugs. Archiv der Pharmazie. 293: 1033-1042

Corbo, M. R., Speranza, B., Filippone, A., Conte, A., Sinigaglia, M. and Del Nobile, M. A. (2008) Study on the synergic effect of natural compounds on the microbial quality decay of packed fish hamburger. Int. J. Food Microbiol. 127: 261-267

de Oliveira, A.C. Valentim, I.B. Silva, C.A. Bechara, E.J.H. Barros, M.P. Mano, C.M. and Goulart, M.O.F. (2009) Total phenolic content and free radical scavenging

activities of methanolic extract powders of tropical fruit peels. Food Chem. 115: 469-475

Dillard, C. J. and Tappel, A. L. (1973) Fluorescent products from reaction of peroxidizing polyunsaturated fatty acids with phosphatidyl ethanolamine and phosphatidylalanine. Nippon Suisan Gakk. 8: 183-189

Frankel E.N. (1998) Free radical oxidation. In: Lipid Oxidation, pp 13-22, (Frankel, E. N. Ed). The Oily Press, Scotland

Gorinstein, H., Zemser, M., Haruenkit, R., Chuthakorn, R., Grauer, F., Martin-Belloso, O. and Trakhtenberg S. (1999) Comparative content of total polyphenols and dietary fiber intropical fruits and persimmon. J. Nutr. Biochem. 10: 367-371

Graf, E. (1992) Antioxidant potential of ferulic acid. Free Radical Bio. Med. 13: 435-448

Hossain, M.A. and Rahma, S.M.M. (2011) Total phenolics, flavonoids, and antioxidant activity of tropical fruit pineapple. Food Res. Int. 44: 672-676

Hsieh, R. and Kinsella, J. (1989) Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. Adv. Food Res. Nutr. 33: 233-341

Indian Horticulture Database (2014) National Horticulture Board, Ministry of Agriculture, Government of India. 289 p

Kenar, M., Ozogul, F. and Kuley, E. (2010) Effects of rosemary and sage tea extracts on the sensory, chemical and microbiological changes of vacuum-packed and refrigerated sardine (*Sardina pilchardus*) fillets. Int. J. Food Sci. Tech. 45: 2366-2372

Mahdavi, D. L. and Salunkhe, D. K. (1995) Toxicological Aspects of Food Antioxidant. In: "Food Antioxidants", (Mahdavi, D. L., Deshpande, S. S. and Salunkhe, D. K. Eds) Marcel Dekker, New York

Martin, A. D. and Gilbert, D. (1968) Enzyme Change Accompanying Liver Enlargement in Rats Treated with 3-tert-butyl-4- hydroxyanisole. J. Biochem. 106: 22-23

Medina, I., Gallardo, J. M., Gonzaalez, M. J., Lois, S. and Hedges, N. (2007) Effect of molecular structure of phenolic families as hydroxycinnamic acids and catechins on their antioxidant effectiveness in minced fish muscle. J. Agri. Food Chem. 55: 3889-3895

Nasr, C. B., Ayed, N. and Metche, M. (1996) Quantitative Determination of the Polyphenolic Content of Pomegranate Peel. Z. Lebensm. Unters. Forsch. 203: 374-378

Oyaizu, M. (1986) Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 44: 307-315

ÖzalpÖzen, B., Eren, M., Pala, A.,Özmen, I. and Soyer, A. (2011) Effect of plant extracts on lipid oxidation during frozen storage of minced fish muscle. Int. J. Food Sci. Tech. 46: 724-731

Ozogul, Y., Ayas, D., Yazgan, H., Ozogul, F., Boga, E.K. and Ozyurt, G. (2010) The capability of rosemary extract in preventing oxidation of fish lipid. Int. J. Food Sci. Technol. 45: 1717-1723

Pazos, M., Alonso, A. and Fernandez-Bolanos, J. (2006) Physicochemical properties of natural phenolics from grapes and olive oil by-products and their antioxidant activity in frozen horse mackerel fillets. J. Agric. Food Chem. 54: 366-373

Pazos, M., González, M.J., Gallardo, J.M., Torres, J.L. and Medina, I. (2005) Preservation of the endogenous antioxidant system of fish muscle by grape polyphenols during frozen storage. Eur. Food Res. Technol. 220: 514-519

Pazos, M., Iglesias, J., Maestre, R. and Medina, I. (2010) Structure-activity relationships of polyphenols to prevent lipid oxidation in pelagic fish muscle. J. Agric. Food Chem. 58: 11067-11074

Raghavan, S. (2005) Distribution and effectiveness of exogenous antioxidants in a mixture of minced cod muscle and canola oil. 111 p. Ph.D. Thesis, Department of Food Science, University of Massachusetts, Amherst

Sánchez-Alonso, I., Jiménez-Escrig, A., Saura-Calixto, F. and Borderías, A.J. (2007) Effect of grape antioxidant dietary fibre on the prevention of lipid oxidation in minced fish: evaluation by different methodologies. Food Chem. 101: 372-378

Sánchez-Alonso, I., Jiménez-Escrig, A., Saura-Calixto, F. and Borderías, A.J. (2008) Antioxidant protection of white grape pomace on restructured fish products during frozen storage. LWT - Food Sci. Tech. 41: 42-50

Schaich, K., Shahidi, F., Zhony, Y. and Eskin, N.A.M. (2013) Lipid oxidation. In: Biochemistry of Foods (Eskin, N.A.M and Shahidi, F., Eds), pp 419-478, Elsevier Inc. (USA)

Shi, J., Yu, J., Pohorly, J. E. and Kakuda, Y. (2003) Polyphenolics in grape seeds biochemistry and functionality. J. Med. Food 6: 291-299

Siddhuraju, P. and Becker, K. (2007) The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) walp.) seed extracts. Food Chem. 101(1): 19

Shimada K., Fujikawa K., Yahara K. and Nakamura T. (1992) Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. J. Agr. Food Chem 40: 945-948

Singh, R. P., Jayaprakasha, G. K. and Sakariah, K. K. (2001) A process for the extraction of antioxidants from pomegranate peels. Submitted for Indian Patent 392/Del

Terao, J., Piskula, M. and Yao, Q. (1994) Protective effect of epicatechin, epicatechingallate, and quercetin on lipid peroxidation in phospholipid bilayers. Arch. Biochem. Biophys. 308: 278-284

Thiago Inacio, B. L., Roberta, G. C., Nidia, C. Y. and Neli, K. H. (2008) Radical scavenging activity of orsellinates. Chem. Pharm. Bull. 56: 1551-1554

Yagi, K. (1987) Lipid Peroxides and Human Disease. Chem. Phys. Lipids, 45: 337-341

Yerlikaya, P. and Gökoðlu, N. (2012) Protective effect of grape seed extract treatment before and after freezing on lipids of bonito. International Conference of Agricultural Engineering CIGR-Ag Eng Valencia, Spain