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Antioxidant effect of combined fruit peel extracts (CFPE) and BHA on the quality and shelf life of *Sardinella gibbosa* during chilled storage

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Abstract

Effects of combined fruit peel extracts (CFPE) and BHA on the quality and shelf life of sardine (*Sardinella gibbosa*) during chilled storage were investigated. Total phenolic and total flavonoids contents were found to be 137 mg/g GAE and 111.33 mg/g QE respectively. The DPPH radical scavenging activity of CFPE at 6 μ g/mL concentrations was 83.09%, BHA showed at 4 μ g/mL concentration was 91.87% of inhibition. Freshly Sardine was assigned to four treatments: control without treatment (CON); sardine treated with 1% CFPE (TCF1) and sardine treated with 3% CFPE (TCF3) and sardine treated with 2 ppm BHA (TB) to retard lipid oxidation in sardine during storage study 3 days at 4±1 °C. Changes in lipid were assessed by PV, thiobarbituric acid (TBA). Apart from total volatile base nitrogen (TVB-N), total plate count (TPC). CFPE was found to offer protective effects to certain degree against lipid oxidation.

Keywords: Fruit peel extracts, lipid oxidation, sardine fish, chilled storage, shelf life

Introduction

The fish and seafood contain high contents of polyunsaturated fatty acids mainly omega-3 fatty acids because of which, the fatty fish and fishery products are highly prone to lipid oxidation that causes deterioration and reduction of shelf life ^[19]. Lipid oxidation can be minimized or inhibited by the use of antioxidants in the fish and fishery products and therefore the product quality and storage stability can be improved. Synthetic antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Tert- Butylhydroquinone (TBHQ), and Propyl gallate (PG) have been widely used in food processing industry ^[4, 18]. The above have advantages among the food producers mainly because of their lower production cost and higher antioxidant capabilities when compared to natural antioxidants ^[20]. In recent years several studies have reported that the high intake of synthetic antioxidants can be hazardous for human health ^[40].

The natural antioxidants from plants and plant materials in the form of extracts investigated to decrease the lipid oxidation ^[9, 13]. Pomegranate (Punica granatum) is native from Iran to northern India and cultivated over the whole Mediterranean region. India is one of the leading countries in pomegranate production 2442000 MT in ^[24]. Pomegranate peel contains substantial amount of polyphenols such as ellagic tannins, ellagic acid and gallic acid ^[10]. These polyphenols exhibit various biological activities, such as eliminating free radicals, inhibiting oxidation and microbial growth, and decreasing the risk of cardio and cerebrovascular diseases and some cancers ^[6]. Orange (*Citrus sinensis*) belongs to the family Rutaceae. Citrus fruits peel and juices are very important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human health ^[23]. Flavonoids demonstrate their antioxidant activities in three ways: by neutralizing free radicals, by reducing the concentration of local free radicals and by chelating metals ^[5]. Pineapple (Ananas comosus), family Bromeliaceae is one of the most consumed tropical fruits and its consumption has been related to several beneficial properties such as antioxidants ^[12]. The study has shown that pineapples contain phenolic compounds, namely the quercetin, flavones-3-ol, flavones ^[22], p-coumaric acid and ferulic acid ^[14], and vitamin C^[21], anti-inflammatory^[11] and anti-diabetic activities^[41].

The present investigation is to employ natural antioxidants extracted from the peels of fruits

such as pomegranate peel (PP), orange peel (OP) and pineapple peel (PAP). The main purpose of this study was to determine the Effect of combined peel extracts on the storage stability of Sardine fish under chilled conditions.

Methods and Materials

Preparation of fruit peel extracts

Fresh PP, OP and PAP were procured from juice vendors in the local market Tuticorin in ripe condition and peeled. Fresh peel sample was washed under running tap water, dried in dryer at 50 °C until constant weight and ground to powder. 100 gm PP, OP and PAP powder were separately dissolved in 600 ml methanol and then extracted in a water bath at 40 °C for 4 hours and after that, it was extracted in rotary shaker for 12 hours at the speed of 180 rpm. The PP and OP extracts were filtered in whatman paper no. 1 and PAP extracts were filtered in whatman paper no. 42 and these extracts are separately concentrated in a rotary evaporator at the temperature of 50 °C to get crude extracts ^[19; 42].

Combined fruit peel extracts were prepared the extraction of all three fruit peel extracts (pomegranate, orange and pineapple) were mixed in equal ratio or amount (10gm each fruit peel extract). All pomegranates, orange and pineapple peels extract and combined fruit peel extracts were packed in dark amber glass bottle and stored in refrigeration under $3\pm$ °C until required for use.

Calculation of extract yield

Extracts yield (%) =
$$\frac{\text{Weight of residue}}{\text{Weight of powder}} \times 100$$

Analysis of antioxidant activity of different fruit peel and their combined peel extracts

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical-scavenging activity of fruit peel extracts, were determined followed by the method of Zhang ^[43]. The DPPH radical-scavenging activity was calculated using the following formula:

Scavenging activity (%) =
$$\frac{Abs \text{ control} - Abs \text{ sample}}{Abs \text{ control}} X 100$$

Total phenolic content and total flavonoid content of different fruit peel and their combined peel extracts

Total phenolics of fruits peel extracts were determined using the method of Singleton and Rossi and the results were expressed as gallic acid equivalents ^[33]. The total flavonoid content of the extracts were determined according to the colorimetric assay ^[34].

Sample preparation

Fresh sardine (*Sardinella gibbossa*) was procured from the Tuticorin fishing harbour landing centre in fresh condition. Fish was washing, dressing, beheading and again washed thoroughly with chilled water to free from blood and mucus. After dressing the solution of combined peel extracts was made in two concentrations, such as 1% and 3% and BHA such as standard dose 200 ppm using distilled water. Treatments of sardine fish were given by dipping them in

combined peel extracts and BHA solution for 5 minute. The ratio of fish to solution was 1:1. For each experiment, total fish was divided into four batches. One batch was soaked in 1% concentration of fruit peel extract and their combined peel extracts solution, second batch in 3% concentration and other BHA 200 ppm concentration. In each experiment, one batch was kept as control (CON) without any treatment. The control and fruits peel extract treated fish were packed in LDPE pouches and heat sealed. In each experiment, combined fruit peel extracts treated batches including control were stored in a refrigerator at $4\pm1^{\circ}$ C for 72 hrs. In chilled storage condition. A sampling of fish (both treatment and control) was done every day. During sampling four packets of fish from each batch were taken randomly for microbiological and biochemical analysis.

Shelf life of treated Sardine fish under chilled storage condition

The study was conducted as a completely randomized design, with four treatments, chilled types of storage condition and three days sampling intervals at 4 ± 1 °C. The treatments were (i) *Sardinella gibbosa* without any dip treatment (CON), (ii) Treated with combined fruit peel extracts 1 % concentration for five minutes (TCF1), (iii) Treated with combined fruit peel extracts 3 % concentration for five minutes (TCF3), (iv) Treated with BHA for five min (TB). The experiments were done in triplicate.

Analytical techniques

The total volatile basic nitrogen (TVB-N) content of sardine was determined according to the method of Conway ^[7]. The value of TBA was determined according to Tarladgis ^[36]. Peroxide value (PV) expressed in milliequivalents of peroxide oxygen per kg of fat was determined according to ^[16].

Microbiological analysis

Total plate count (TPC) was done following spread plate technique on plate count agar following the standard method given in APHA ^[3].

Statistical analysis

Experiments and analyses were conducted in triplicate. Data obtained were appraised using Statistical Package for Social Sciences (SPSS, version 25). Analysis of variance (one way - ANOVA) was performed to determine the differences between the treatments. The tests for differences were done by using Duncan's Multiple Comparison Test. Significance of differences was defined at P<0.05.

Results and Discussion

Extraction yield

In the present study, the extraction was done using methanol. PPE (pomegranate peel extract) showed the highest extraction yield of 23.74 %, OPE (Orange peel extract) 20.06 % and PAPE (pineapple peel extract) of 17.11% respectively (Table 1). The extraction yield of pomegranate was coherent and the difference occurred could be due to the solvent used for extraction of natural compounds reported by Singh and Immanual (2014) ^[32]. The extraction yield of OPE was higher which can be attributed to the species of orange used for the experiment or the environmental condition, Similar result extraction yield of PAPE was reported by Alejandra *et al.* (2011) ^[1].

 Table 1: Total extraction yield from pomegranate, orange and pineapple peels

Extracts	Yield (%)
PPE	23.74
OPE	20.06
PAPE	17.11

Total phenolic content and total flavonoid content

Total phenolic content of the combined fruit peel extracts (CFPE) were estimated by the folin-ciocalteu methods The Total phenolic content values of the CFPE was 152 mg/g GAE. The total flavonoid content values of the CFPE were 111.33 mg/g QE (Table 2). According to Shinde *et al.*, (2015) the phenolic content in pomegranate peel extract (PPE) was observed to be 212 ± 20.55 mg TAE/g ^[31]. Uchoi *et al.*, (2017) reported that the pineapple have total phenolic and total flavonoids content of 131 mg GAE 100g-1 and 211.2 mg QE 100g-1 respectively ^[38]. It has been shown that orange and pomegranate peel is a good raw material for producing natural antioxidants because of its high content of antioxidants reported by Anagnostopoulou *et al.* 2006; Qu *et al.* 2010^[2; 28].

Table 2: Content of total phenols and flavanoids in CFPE

Antioxidants	Value
TPC mg/g GAE	226
TFC mg/g QE	177.33

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities

Stable DPPH radical has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity ^[17]. DPPH results shown in the (Figure 1), CFPE extracts showed higher radical scavenging activity at the concentration of 6 µg/mL with the inhibitory activity of 83.09% and for BHT 91.87% at a concentration of 4 µg/mL. The PPE extract has the radical scavenging activity of 92.38% at the concentration of 35µg/mL but at the same concentration, BHA showed inhibition of 93.59 % which is almost equivalent to PPE. Pal et al., (2017) reported that the OPE showed the radical scavenging activity 85.68 % at a higher concentration of 700 µg/mL^[27]. Uchio., (2017) reported that the pineapple peels (PAPE) to exhibit maximum Diphenyl-1extract picrylhydrazyl (DPPH) radical scavenging activity of 81.75% at 2 mg-1ml^[39]. Triphathi (2016) reported that the radical scavenging ability of PPE to be 12.5±1.5% at 50 mg/L, 22.7±0.9% for 100 mg/L, 63.7±1.2% for 250 mg/L, 73.6±1.9% for 500 mg/L and 74.5±0.S% for 1000 mg/L concentration [37].



Fig 1: DPPH scavenging activity (%) of combined fruit peel extracts and BHA

Biochemical analyses

Changes in PV

Changes in the peroxide value of sardine treated with

different concentrations of PPE and BHA (Figure 2). The PV found in initial day for TCF1 was (1.6), TCF3 (1.33), TB (1.2) meq/kg. PV of all the samples was significantly (P<0.05) increasing with storage time till 3rd day. The sample taken as control which was not treated with any antioxidant had increased its PV at the maximum up to 3.56 at the 3rd day and it was the highest value among all the samples. A significant difference observed between control and all the treatment samples (P<0.05). The PV recorded in the sample treated at the concentration of TB showed significantly (P<0.05) lower value than other samples TCF1 and TCF3 throughout the storage period. According to Tarkhasi (2016) such changes in peroxide value of edible Coating pomegranate peel extract on silver carp (Hypophthalmichthys molitrix) fillet during refrigerated storage, the initial PV (meq peroxide/kg fish sample) in the control sample was 1 and increased to 15.33 at the end of storage ^[35]. No significantly difference (P>0.05) was observed throughout first days of the storage among the different sample. PV content significantly increased (P<0.05) in all treatments throughout the refrigerator storage, indicating that lipid deterioration continued under the storage temperature conditions. At the end of the storage time significant differences (P>0.05) were observed in the PV between the control (15.33) and each of T1 (14.23) and T2 (12.60) samples. PV of T1 sample was also significantly higher than of T2 samples. Storage time has a significant effect on the PV for each of control and treated samples (P<0.05). The higher antioxidant capacity of pomegranate peel is related to the presence of phenolic compounds, especially ellagic acid and punicalagin that can act as free radical scavengers during the oxidation in the food system^[30].



Fig 2: Changes in peroxide value of sardine treated with combined peel extracts and BHA during chilled storage condition at 4 ± 1 °C

Changes in TBA

Changes in the TBA value of sardine treated with the combined fruit peel extracts and BHA. In case TCF1, TCF3 and TB, the initial TBA value was 0.70, 0.68 and 0.58 mg MA/kg of fat respectively; it increased throughout the storage period for 3 days. The control sample without any antioxidant showed significant (P<0.05) increase in TBA value than the samples treated with the combined fruit peel extracts and BHA. The highest TBA value recorded was 1.61 mg MA/kg for control sample at the day 3. The sample TCF1, TCF3 and TB had the TBARS value 0.79, 0.74 and 0.71 mg MDA/kg at the day 3 and this result showed that there is significant (P<0.05) difference between all the treated samples. Özen and Sover (2018) also reported such changes in TBARS values of mackerel mince during frozen storage. Secondary oxidation products presented low values at the beginning of the storage and gradually increased for all groups throughout storage. The level of TBARS in control samples increased speedily from 2.80 to 8.25 mg MDA/ kg during preliminary 3 month of storage, then increased up to 14.25 mg MDA/kg at the end of storage ^[25]. Whereas pre-treated samples showed a slight increase in TBARS values from 2.34 to 7.57 mg MDA/kg throughout storage study. Ozgul *et al.*, (2010) was reported by stating the treatments with rosemary extract to the sardine fillets significantly reduced the formation of TBARS value ^[26].



Fig 3: Changes in thiobarbituric acid of sardine treated with combined peel extracts and BHA during chilled storage condition at 4 ± 1 °C

Changes in TVB-N

TVB-N was 13.06 mg % at the beginning of the storage in TCF1 and TCF3 (Figure 4). The TVB - N value in TB was 12.13 mg %. The TVB-N content was observed to increase progressively in all treated samples. However, the increment of TVB-N content was rapid in control 14.93 to 16.8 at the end of 3 days storage. The lower TVB-N values were recorded in TB samples during storage ^[39]. The TVB-N values for these treatments significantly increased (P < 0.05) during the chilled storage and are below limit level at the end of chilled storage. The results of TVB-N of all treated sample were significantly increased with increase in storage period and highest an increasing trend was recorded in case of control sample. The increasing trend of TVB-N value fish ham stored at the ambient storage was also reported by Rajalaxmi (2014)^[29].



Fig 4: Changes in total volatile base nitrogen of sardine treated with combined peel extracts and BHA during chilled storage condition at 4 ± 1 °C

Microbiological analyses

Total plate count of TCF1, TCF3 and TB samples stored in chilled storage were comparatively lower than that of control samples (Figure 5). In TCF1, TCF3 and TB, the initial TPC value was 3.5, 3.36 and 3.1 cfu/g respectively. TPC of all the samples significantly (P<0.05) increased with storage time till 3rd day. In the control the TPC reached 3.93 on the 3rd day and it was the highest value among all the samples. The TB Showed lower value compare to other samples. These findings are in agreement with Ibrahium; (2010) ^[15]. The phenolic compounds such as carvacrol, eugenol, thymol, green tea extract, rosemary extract, grapefruit seed extract and lemon extract control the microbiological spoilage in packed fresh cod hamburgers and were studied by Corbo *et al.* (2009) ^[8].



Fig 5: Changes in total plate count (TPC) of sardine treated with combined peel extracts and BHA during chilled storage condition at 4 ± 1 °C.

Conclusion

The present investigation can be concluded that the combined fruit peel extracts were effective in delaying lipid oxidation in muscles of sardine fish thereby enhancing the shelf life during chilled storage condition. The combined fruit peel extract could be a potential source of natural antioxidant which can be effectively incorporated in fish and fishery products. The significant improvement in shelf life was achieved due to the antioxidant and antimicrobial properties of fruit peel extracts. From the above result, it may be concluded that giving dip treatment in crude aqueous fruit peel extracts is an efficient and effective way to maintain good quality and giving longer shelf life to the product.

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