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ORIGINAL ARTICLE

Antioxidant and antimicrobial properties of grape and papaya seed extracts and their application on the preservation of Indian mackerel (*Rastrelliger kanagurta*) during ice storage

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Abstract Antioxidant properties of grape (GSE) and papaya seed extracts (PSE) were tested in vitro at varied concentrations and growth inhibition were seen against gram positive and gram negative bacteria by disc diffusion method. The results revealed that GSE contain four times higher phenolic and six folds higher flavonoid content than PSE. The antioxidant properties of GSE and PSE showed dose dependent activities and were comparatively much higher in GSE. Linoleic acid model of GSE and PSE displayed 67.67 and 46.43 % of inhibition respectively at 500 mg/L. The effect of dip treatment by GSE and PSE at a concentration of 500 and 1000 mg/L respectively on the quality changes of Indian mackerel (*Rastrelliger kanagurta*) in iced condition were assessed using chemical, microbiological and sensory parameters along with chilled whole control (CWC). The inhibition of primary and secondary lipid oxidation products by GSE at 500 mg/L was comparable to BHT at 200 mg/L. GSE exhibited higher antimicrobial activity on gram-positive strains compared to PSE and reduced the formation of volatile bases significantly. On the day of sensory rejection for CWC, the formation of trimethylamine and total volatile base nitrogen were reduced by 32.27 and 31.85 % in GSE samples and 19.01 and 24.70 % in PSE samples respectively. The dip treatment of GSE increased the shelflife of mackerel up to 15 days, PSE by 12 and 9 days for CWC during ice storage. Therefore, it can be concluded that, GSE can be used as a promising natural preservative and a substitute to the synthetic counterparts.

Keywords Grape seed extract · Papaya seed extract · Indian mackerel · Antioxidant properties · Antimicrobial activity · Ice storage

Introduction

Fish quality is very subjective nature and is very complex concept (Bremner 2000) which comprises biochemical, microbiological, nutritional and physiochemical attributes. The freshness of fish degrades after death due to various biochemical reactions such as changes in protein and lipid fractions and microbiological spoilage. This results in the deterioration of sensory quality and nutritional value of fish. Hence preservation assumes greater importance to prevent the loss of this nutritionally rich natural resource.

Indian mackerel (*Rastrelliger kanagurta*) is a pelagic species, which belongs to the family Scombridae. It is quite abundant in the West Coast of India and forms a commercially important fishery due to its high amount of unsaturated fatty acids, taste and relatively lower price. It contributes 7.2 % to the total marine fish landing of India and forms the mainstay pelagic fishery after oil sardine along the West Coast. Normally, consumers prefer freshly landed or iced Indian mackerel over frozen or canned Indian mackerel. However, during glut season, it becomes difficult to preserve the

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freshness of fish due to its susceptibility to spoilage. Therefore its preservation becomes at- most importance. Of the various preservation methods, icing is the most important and ideal medium used for preserving fresh fish (Surti et al. 2001). However, icing may not completely inhibit biochemical reactions and microbial growth that lead to quality deterioration of fish. To retard such a quality loss, synthetic antioxidants and antimicrobial agents have been used to during the processing and storage of fish and fish products (Boyd et al. 1993). Nowadays the use of synthetic antioxidants and antimicrobial compounds has raised questions regarding food safety and toxicity (Chang et al. 1977). On the other hand, the use of natural antioxidants and antimicrobial agents is emerging as an effective method to control autoxidation and microbial spoilage in food.

Grape seed is a by-product of the food industry and forms a cheap source of natural antioxidant due to its phenolic content (Spigno and Defaveri 2007). Seeds constitute a considerable proportion of the grape, ranging from 38 to 52 % on a dry matter basis and constitute 46–69 % polyphenol (Amerine and Joslyn 1967). The antioxidant activity of grapes has been positively associated with their phenolic composition such as anthocyanins, flavonols, flavan-3-ols, procyanidins, and phenolic acids (Bertelli et al. 2004; Fujii et al. 2007; Kedage et al. 2007; Pazos et al. 2006; Shafiee et al. 2003). Additionally, these compounds have shown to reduce hydroperoxide formation and inhibit lipid and protein oxidation (Heinonen et al. 1998). In addition to antioxidant properties, the grape phenolics have also displayed antimicrobial properties (Palma et al. 1999). The antimicrobial property of grape seed extract is due to core structures with 3,4,5- trihydroxyphenyl groups found in epigallocathecin, epigallocathechin-3-ogallate, prodelphinidin and castalagin (Tagurt et al. 2004).

Papaya (*Carica papaya* L.) is native of tropical America but has now spread all over the tropical world. The central cavity contains large quantity of seeds that comprise about 15 % of the wet weight of the fruit. The total global production of papaya averages about 10.0 million metric tons, and India and Brazil are the major producers with annual production of 3.6 and 1.9 million metric tons, respectively. The fruit is rich in phytochemical, especially polyphenols (Sancho et al. 2011). Besides its juicy pulp, the peel and seeds of papaya are valuable too. Although papaya peel and seeds have various uses, the phytochemicals especially phenolic compounds in these parts of papaya have antioxidative and antimicrobial properties.

The overall objective of this study was to identify whether the addition of GSE and PSE can retard both lipid oxidation and microbial spoilage and to extend the shelf life of Indian mackerel during ice storage. In addition, this study provides greater insight into the potential of GSE and PSE as natural and effective sources of antioxidant and antimicrobial compounds for fish processing industry.

Material and methods

Materials

Chemicals

2,2-azino-bis (3-thylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2 carboxylic acid (Trolox), 2,2-diphenyl-1-picryl hydrazyl (DPPH), Linoleic acid, potassium persulphate, Aluminum chloride, catechin, potassium ferric cyanide, Ferric chloride, Ferrozine were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All solvents used were of analytical grade and obtained from E-Merck, Mumbai, India and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Bacterial cultures

Bacterial cultures namely *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2688), *Bacillus subtilis* (NCIM 2063), *Salmonella typhium* (NCIM 2501) *and Pseudomonas fluroscens* (NCIM 2099) were procured from National chemical laboratory, Pune. India. The above cultures were grown in nutrient agar media (Hi Media, Mumbai, India) at 37 °C. Each bacterial strain was transferred from slants stored at 4–5 °C to 10 ml nutrient broth and cultivated at 37 °C for 24 h. Pre-culture was prepared by transferring 1 ml of this culture to 9 ml nutrient broth and cultivated for 48 h.

Preparation of grape and papaya seed extracts

Grapes seeds were collected from local juice centres while papaya seeds were purchased from local juice processing center in Mangalore, Karnataka, India. The samples were washed and cleaned with water and dried in hot air oven at 50 °C for 72 h. Dried grape and papaya seeds were ground to fine powder and stored in separate screw cap bottles before extraction.

Dried powder of grape and papaya seed was used for extraction of bioactive compounds. One hundred grams of each ground material were defatted by shaking three times with four volumes of petroleum ether in a rotary shaker (Orbitek Scigenics, India) for 1 h. The residues obtained after filtration was dried overnight under a hood until all traces of petroleum ether were removed. The dried residues from each material were extracted three times with four volumes of 90 % ethanol by shaking for 1 h and filtered by using filter paper. The combined filtrates from each material were concentrated in a rotary evaporator (Rotavap PBU-6D, India) and placed under a hood to remove the residual ethanol. The obtained aqueous extracts were frozen

overnight and freeze-dried at −60 °C (Modulyod Freeze dryer 230, USA). The freeze-dried extracts were stored in air-tight containers at 5 °C until used for the determination of antioxidant and antimicrobial activity.

Methods

Estimation of total phenolics

The total phenolic content of the GSE and PSE was determined by the Folin-Ciocalteu method and expressed as gallic acid equivalents (mg GAE/g) (Kahkonen et al. 1999). The sample of each extract solution (200 μL) was transferred to a test tube and then mixed thoroughly with 1 mL of Folin-Ciocalteu reagent. After mixing for 3 mins, 0.8 ml of 7.5 % (*w*/*v*) sodium carbonate was added. The mixtures were agitated with a vortex mixer and then allowed to stand in the dark for a further 30 min, after which they were centrifuged at 3300×*g* for 5 mins. The absorbance of extracts and a prepared blank were measured at 765 nm.

Estimation of total flavonoid content

Total flavonoid content of the sample extracts were measured with an Aluminum chloride colorimetric assay and expressed as milligram of (+) catechin equivalent per gram dry mass (mg CE/g dw) (Marinova et al. 2005). An aliquot (1 ml) of extract was added to a 10 ml volumetric flask, containing 4 ml of distilled and deionized water and added 0.3 ml 5 % $NaNO_2$. After 5 min, 0.3 ml 10 % $AlCl_3$ was added and at the sixth min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with double distilled water. The solution was mixed well and the absorbance was measured against a reagent blank at 510 nm with UV-VIS spectrophotometer.

Determination of DPPH radical scavenging activity

DPPH assay of GSE and PSE at different concentrations was performed according to the method of (Yen and Wu 1999). A known volume of 1.5 ml of extract was added to 1.5 ml of 0.1 mM DPPH solution prepared in 99.5 % ethanol and thoroughly mixed by using cyclomixer at high speed and a control was used with same concentration of DPPH and made up to 3 ml using distilled water. The mixture was stored in dark at room temperature for 30 min. After the incubation, the absorbance was measured at 517 nm using UV–VIS double beam spectrophotometer (UV-VIS Spectrophotometer, LaboMed, Inc., UK.). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. DPPH radical scavenging activity was calculated and expressed in terms of percentage of DPPH free radicals scavenged using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = 1 - \frac{\text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

Determination of ABTS radical scavenging activity

ABTS radical scavenging activity was evaluated as per the method of (Arnao et al. 2001).The stock solution included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 50 ml methanol in order to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using a spectrometer (UV-1601 spectrophotometer, Shimadzu, Kyoto, Japan). A fresh ABTS solution was prepared for each assay. Samples (150 μl) with a concentration range of 100–600 mg/L were mixed with 2850 μl of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm. A sample blank at each concentration was prepared using methanol instead of ABTS solution. A standard curve of Trolox ranging from 50 to 600 μM was prepared. The activity was expressed as μmol Trolox equivalents (TE)/ml of phenolic compound.

Determination of ferric reducing antioxidant power

The ability of the extracts to reduce ferric ions to ferrous ions was determined by the method as described by Oyaizu (1986). An aliquot of 1 ml was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1 % (*w*/*v*) potassium ferricyanide. The mixture was incubated at 50 °C for 30 min. After incubation, 2.5 ml of 10 % (*w*/*v*) trichloroacetic acid was added. Finally, 2.5 ml of the solution from the mixture was drawn and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % (*w*/*v*) ferric chloride solution. After 10 min of reaction time, the absorbance of the resultant solutions was measured at 700 nm using UV-VIS Spectrophotometer. Higher absorbance of the reaction mixture indicates higher reducing power.

Determination of metal chelating activity

The chelating activity towards Fe^{2+} was measured by the method of Boyer and Mccleary (1987). Samples (4.7 ml) with a concentration ranging from 0.5–3 mg/l were mixed with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine. The reaction mixture was allowed to

stand for 20 min at room temperature. The absorbance was then read at 562 nm. The blank was prepared in the same manner using distilled water instead of the sample. The chelating activity after the sample blank substraction was calculated as follows:

$$\text{Metal chelating activity } (\%) = \left(A_{blank} - A_{sample}\right)/A_{blank} \times 100$$

Determination of antioxidant activity using linoleic acid model

Linoleic acid auto-oxidation inhibition activity of extracts was measured according to the method as described by Osawa and Namiki (1985). One ml of extract, dissolved in 10 ml of 50 mM phosphate buffer (pH 7.0) was added to a solution of 0.13 ml of linoleic acid and 10 ml of 99.5 % ethanol. The total volume was then adjusted to 25 ml with distilled water. The mixture was incubated in a 30 ml assay tube with a screw cap at 40 ± 1 °C for 5 days in an incubator. The dark room condition was maintained by wrapping with aluminum foil and thicker paper. The degree of oxidation of linoleic acid was measured using the ferric thiocyanate method (Mituda et al. 1966). To 0.1 ml of the reaction mixture, 4.7 ml of 75 % ethanol, 0.1 ml of 30 % ammonium thiocyanate, and 0.1 ml of 20 mM ferrous chloride solution prepared in 3.5 % HCl were added. After 3 min of incubation, the color was measured at 500 nm. Appropriate controls were maintained using ethanol. The antioxidative capacity to inhibit the peroxide formation in linoleic acid was expressed as follows:

$$\text{Lipid peroxidation inhibition } (\%) = 1 - \frac{Abs_{Sample}}{Abs_{Control}} \times 100$$

Method of preparation and dip treatment

A total of 96 Indian mackerel (Rastrelliger kanagurta) weighing 74.20 ± 12.22 g each were purchased from a fish landing centre in Mangalore, India, and were iced immediately in the ratio of 1:1 (fish: ice) at the landing centre and transported to laboratory in chilled condition. The fresh Indian mackerel were washed with potable water and divided into four groups. Chilled whole control (CWC) with no treatment; the second group was given dip treated with BHT at 200 mg/L; the third group was dip treated with GSE at 500 mg/L and the fourth group was dip treated with PSE at 1000 m/L. The time duration of dip treatment was 30 min for the entire groups after standardization at various time intervals. The whole mackerel were packed and sealed in polythene bags and stacked in ice for further analyses. The samples were drawn for quality evaluation periodically every 3 days once from 0 to15 d.

Determination of antimicrobial activity by disc diffusion method

The antibacterial activity of the GSE and PSE was performed by following agar disc diffusion method described by Bauer et al. (1996). Bacterial strains were first grown on Muller Hinton medium for 18 to 24 h at 37 °C. A sterile 10 mm-diameter filter disc dipped with each of extract and was placed on the infusion agar seeded with bacteria. Then, Petri dishes were kept at 4 °C for 1 h and subsequently incubated at 37 °C for 24 h. Ampicillin (10 μg) (Hi Media India) standard discs were used as positive antibiotic controls. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs.

Chemical analysis

Peroxide value (PV) and thiobarbituric acid reactive substance (TBARS) was estimated spectrophotometrically by Richards and Hultin (2002) and Buege and Aust (1978) respectively. Total volatile base nitrogen (TVB-N) and trimethylamine (TMA) were estimated by the microdiffusion method (Conway 1962). pH was determined according to APHA (1998) using a digital pH meter (Polytron system PT 2100, Kinematica, AG) after homogenizing 10 g of the fish sample with the same amount of distilled water. The Free Fatty Acid (FFA) content in the lipid extract was determined with improved titremetric method as described by (AOAC 2000).

Microbiological analysis

Microbiological analysis of mackerel samples was done by taking 25 g of fish muscle aseptically and homogenized with 225 ml of 0.85 % normal, saline for one min. Then the homogenized sample was serially diluted using sterile 9 ml diluents and plated in specific media. Total plate count, counts of *Staphylococcus aureus*, and *E. coli* count were enumerated as per the methods described by Surendran et al. (1985).

Sensory evaluation

Sensory characteristics were evaluated for raw fish and treated whole mackerel by minimum of 12 trained panelists, using a nine point hedonic scale (1-dislike extremely to 9-like extremely) for product acceptability as prescribed by (Meilgaard et al. 1999). Panelists scored for appearance, colour, taste, texture, odour and overall acceptability. During the evaluation sessions, the samples were coded by different letters and presented in random order.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means were determined by Duncan's multiple range test ($p < 0.05$) using statistical software (Statsoft Inc., Tulsa, USA). Values expressed are means of three determinations ± standard deviation.

Results and discussion

Percentage yield, total phenolics and total flavonoids

The percentage yield of extracts, total phenolic and total flavonoid contents of GSE and PSE are presented in Table 1. It was observed that, the maximum yield was obtained for the GSE. The percentage yield of both GSE and PSE were 6 and 2.75 %, respectively.

Total Phenolic content of GSE and PSE were expressed as Gallic acid equivalent per gram of sample. The grapes seeds were observed to possess higher TPC i.e. 1.23 mg GAE/g grape seed when compared to papaya seed it was 0.32 mg GAE/g papaya seed. It was observed that, GSE contains four times higher phenolic content than PSE. Hogan et al. (2009) have reported that, the TPC of Norton grape was 1.8 mg GAE/g for fresh grape. The percentage of phenolic content of Bangalore blue grapes in acetone: water: acetic acid and methanol: water: acetic acid was found to be 46 and 38 % respectively, indicating that, the increase in the phenolic content depends upon the solvent used for extraction (Jayaprakasha et al. 2003). Other researchers have documented a slightly higher phenolic content in red wine ranging from 1.4 to 3.1 mg GAE/g for fresh grape (Yi et al. 1997). Gibis and Weiss (2012) have reported that, the quantification of TPC showed an approximately 26 times higher content in grapes than in rosemary extract. The higher content of phenolics in grapes indicates a better radical scavenging ability, which can prevent lipid oxidation in food products. Maisarah et al. (2013) reported that, the total phenolic content in papaya seed was 3.32 mg GAE/g, which corroborates the results of papaya in the present study. Norshazila et al. (2010) have reported that, the total phenolic content of papaya seeds was 8 mg GAE/100 g for fresh weight, which was comparatively much lower than the results of the present study. Kwang et al. (2012) have reported that, the papaya seeds contain TPC of 6.75 μg TE/ml.

Table 1 Percentage yield of extracts, total phenolic and total flavonoid contents of Grape and papaya seed extracts ($n = 3$)

Seed extracts	Yield (%)	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg CE/g DW)
GSE	6.00	1.23 ± 0.80	1.66 ± 0.30
PSE	2.75	0.31 ± 0.50	0.28 ± 0.30

Bar indicates Mean ± SD ($n = 3$)

Grape and papaya seed extracts significantly varied in total flavonoid contents i.e. 1.662 ± 0.3 and 0.282 ± 0.3, respectively. It was observed that, the GSE had six times higher total flavonoid content when compared to PSE. The results of the present investigation was observed to be similar to the findings of three grape extracts, which varied significantly in total flavonoid content ranging from 0.48 mg CE/g for the cabernet Franc clone 313 to 1.19 mg CE/g for Norton (Hogan et al. 2009). The flavonoid content of the GSE used in present study was slightly higher than that of seven table grape varieties i.e. 0.13 to 0.31 mg CE/g, as reported by Cantos et al. (2002). The flavonoid content of PSE was comparatively lower to that of GSE, which is in accordance with the previous finding of Irondi et al. (2013).

DPPH$^{\bullet}$ radical scavenging activity

Antioxidant effectiveness is measured by monitoring the inhibition of oxidation of suitable substrate. In biological systems antioxidant effectiveness is classified into 2 groups viz evaluation of lipid peroxidation and measurement of free radical scavenging ability. This assay is based on the measurement of reducing ability of antioxidant towards DPPH radical. The ability can be evaluated by electron spin resonance (EPR) or by measuring the decrease of its absorbance. It is used to evaluate antioxidant activity of natural sources and degree of its discoloration is attributed to its hydrogen donating ability of the test compounds, which is indicative of their scavenging potential (Shimada et al. 1992). In the present investigation the DPPH scavenging activity of grape and papaya seed extracts at different concentration is shown in Table 2. The activity of both the extracts increased with increase in concentration ($p < 0.05$). At the same concentration used, the descending order of DPPH radical scavenging activity of the seed extract tested was as follows: GSE > PSE. The DPPH radical activity of GSE increased up to 300 mg/L and thereafter the activity did not show any further increase ($p > 0.05$). The Result suggests that, the GSE showed highest radical scavenging activity of 87.02 % when compared to 61.43 % for PSE at 500 ppm respectively. The high radical scavenging of GSE could be due to flavonoids that can perform scavenging action on free radicals (superoxide, hydroxyl and 1,1-diphenyl-2-picrylhydrazyl (DPPH)), metal chelating properties, reduction of hydroperoxide formation and their effects (Jacob et al. 2008). The presence of the functional group "-OH" in the structure and its position of the flavonoid molecule determine the antioxidant capacity (Arora et al. 1998). Addition of -OH group to the flavonoid nucleus will enhance the antioxidant activity, while substitution by -OCH_3 groups diminishes the antioxidant activity. The other reason for higher scavenging activity of GSE could be

Table 2 Assays on antioxidant activity of GSE and PSE

	GSE	PSE
Sample concentration (mg/L)	DPPH radical scavenging activity	
100	70.29 ± 0.59 [a]	31.90 ± 0.36 [a]
200	77.23 ± 0.84 [b]	38.79 ± 0.59 [b]
300	85.01 ± 0.86 [c]	51.46 ± 0.96 [c]
400	86.05 ± 0.30 [cd]	55.65 ± 0.49 [d]
500	87.02 ± 0.44 [d]	61.43 ± 0.59 [e]
Sample concentration (mg/L)	$ABTS^{\bullet+}$ radical scavenging	
50	26.99 ± 12.58 [a]	4.77 ± 2.92 [a]
100	308.66 ± 11.81 [b]	21.16 ± 4.40 [b]
200	600.05 ± 4.59 [bc]	44.49 ± 3.81 [c]
300	607.55 ± 6.03 [cd]	61.72 ± 2.54 [d]
400	617.27 ± 6.31 [cd]	84.22 ± 8.00 [e]
500	618.94 ± 1.27 [d]	138.38 ± 2.92 [f]
600	622.99 ± 2.40 [d]	149.22 ± 2.09 [g]
Sample concentration (μg/mL)	Ferric reducing antioxidant power	
100	0.24 ± 0.005 [a]	0.07 ± 0.003 [a]
200	0.37 ± 0.040 [b]	0.08 ± 0.001 [a]
300	0.55 ± 0.007 [c]	0.10 ± 0.004 [b]
400	0.72 ± 0.006 [d]	0.12 ± 0.001 [c]
500	0.84 ± 0.022 [e]	0.13 ± 0.005 [d]
Sample concentration (mg/mL)	Metal chelating activity	
0.5	15.45 ± 0.49 [a]	6.28 ± 0.44 [a]
1.0	28.30 ± 0.70 [b]	14.24 ± 0.83 [b]
1.5	45.24 ± 0.57 [c]	29.27 ± 1.02 [c]
2.0	55.30 ± 0.08 [d]	40.22 ± 0.92 [d]
2.5	63.11 ± 0.31 [e]	51.91 ± 1.47 [e]
3.0	76.92 ± 1.56 [f]	62.31 ± 0.30 [f]
Sample concentration (mg/L)	Lipid peroxidation inhibition (%)	
500	67.67 ± 0.56 [a]	46.43 ± 0.84 [a]
750	73.22 ± 0.15 [b]	53.13 ± 0.45 [b]
1000	81.20 ± 0.22 [c]	65.04 ± 0.40 [c]

Mean ± sd, $n = 3$

[a-f] Values in the same rows for each concentrations are followed by different letters are significantly different ($p < 0.05$)

due to high amount of total phenolic content and the presence of monomeric polyphenolic compounds such as (+)- catechin, (−) – epicatechin and epicatechin-3-o- gallate and dimeric and tetrameric procyanidins (Saito et al. 1998).

The lower scavenging activity of papaya seed extract could be due to the presence of P-hydrobenzoic acid and vanillic acid which are simple phenolic compounds as reported by Zhou et al. (2011). The carboxyl group of hydrobenzoic acid and methoxy group of Vanillic acid might explain negative effect on its antioxidative activity. The carboxyl group is the electron withdrawing group which does not benefit the radical scavenging activity of the compound (Thiago et al. 2008). The result indicated that, grape seed were the potential free radical scavengers, which reacted with radicals by donating their hydrogen and acts as primary antioxidants which can be used as a rich source of functional and antiradical compound to prevent lipid oxidation.

$ABTS^{\bullet+}$ cation radical scavenging activity

The assay is based on scavenging ability of antioxidant against long shelf life radical anion ABTS. In this assay ABTS is oxidized by peroxyl radical or other oxidant to its radical cation, $ABTS^{\bullet+}$, which is intensely colored and antioxidant capacity is measured by the ability of the compound to decrease color reaction directly with ABTS radical. ABTS assay measures both the hydrophilic and lipophilic antioxidants since the reagents dissolve well in both aqueous hydrophilic and organic solvent hydrophobic groups (Kwang et al. 2012). ABTS scavenging activity of GSE and PSE at different concentration is shown in Table 2. $ABTS^{\bullet+}$ radical scavenging activity of both extracts increased as the concentration increased ($p < 0.05$). However, the activity varied with the type of extract tested. The dose-dependent scavenging activity was shown in PSE while in case of GSE, there was no significant difference at 200–600 mg/L ($p > 0.05$) and showed a similar trend with the results of the DPPH• assay. The present work is in agreement with the work of Prasad et al. (2010) and Kwang et al. (2012), which exhibited low ABTS•+ scavenging activity of PSE. The extraction of grape seed with different solvents showed varied ABTS•+ radical scavenging activity and it depends upon polarity of the solvent, the isolation procedure and the purity of the active compounds as well as test system used to evaluate the activity (Meyer et al. 1998). The effectiveness to quench the ABTS radical depends upon the number of aromatic rings in the antioxidant, nature of hydroxyl groups and molecular weight (Hangerman et al. 1998). Hence different assays should be conducted to verify the antioxidant activity of various compounds, in which mode of action could be different. The results strongly suggests that GSE is a strong $ABTS^{\bullet+}$ radical cation inhibitor as compared to PSE.

Ferric reducing antioxidant power

Reduction capability of seed extracts can serve as a significant indicator of their potential antioxidant activity. This assay is based on the reaction which measures reduction of ferric (Fe^{3+}) 2,4,6 tripyridyl-5-triazine (TPTZ) to a colored ferrous (Fe^{2+}) (TPTZ) product. The Fe^{2+} formed from the reduction process was then monitored by measuring the formation of perl's Prussian blue. Increasing absorbance of the reaction mixture at 700 nm indicated an increase in the reducing power; however, it is limited to hydrophilic antioxidants. This kind of reduction process is based on the tendency of an antioxidant to donate electron (Medina et al. 2007). The reducing ability of the GSE and PSE were evaluated at different

concentrations (100, 200, 300, 400, and 500 μg/ml) as shown in Table 2 and were compared with a reference, Butylated hydroxyl toluene (BHT) at 200 ppm. All the extracts were capable of reducing Fe^{3+} and did so in a linear dose dependent manner. Among the two extract tested, GSE showed highest ferric reducing activity at all concentrations ($p < 0.05$) and showed highest reducing power at 500 μg/ml i.e., 0.848 ± 0.022 which is almost equivalent to BHT at 200 mg/L i.e., 1.019 ± 0.006, thus indicating GSE could easily donate electron to Fe^{3+}, and reducing it further to Fe^{2+}. The ability of grape seed to show good reducing power could be attributed to catechin and epicatechin possessing the higher number of higher hydroxyl groups. The results of high reducing power of GSE were in agreement with highest DPPH and ABTS radical scavenging activity of the present work. Zhang et al. (2011) also reported that, GSE was able to reduce Fe^{3+} to Fe^{2+} and with increase in concentration ferric reducing power increases. In Contrast, papaya seed extract showed lower reducing power and the results were in agreement with lower phenolic content, DPPH and ABTS radical scavenging activity of the present study. Zhou et al. (2011) have reported that, ethyl acetate extract of PSE possessed higher reducing power than ethanol, *n*-butanol and water fractions, which is comparable to the results of the present study. The results suggested that, a reducing power of the compound appears to be related to degree of hydroxylation and extent of conjugation in polyphenols which was seen in GSE when compared to PSE.

Metal chelating activity

An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which catalyze hydroperoxide decomposition and Fenton-type of reaction in which ferrous ions catalyze the conversion of hydrogen peroxide to hydroxyl radical with the production of ferric ion. Excessive accumulation of metal ions lead to oxidative stress due to increased formation of reactive oxygen species (ROS), which are responsible for DNA damage, lipid peroxidation, protein modification and other effects (Jomova and Valko 2011). Therefore, it was considered as important to screen Fe^{2+} chelating ability of extracts. The assay used to determine the chelating activity of Fe^{2+} was based on the chelation of this metal ion with Ferrozine to yield red colored complex. In the presence of chelating agents, the complex formation is disrupted and red color of the complex decreases. Measurement of the rate of color reduction therefore allows estimation of the chelating activity. GSE and PSE extracts were assayed for their metal chelating activity at different concentrations as depicted in Table 2, and this activity was compared with the chelating activity of synthetic metal chelator EDTA at 0.05 Mm. GSE chelated more iron than PSE ($p < 0.05$), although both extracts were less efficient than commercial chelator EDTA. The maximum metal chelating activity of GSE and PSE were seen at 3 mg/mL which was 76.92 and 62.13 % respectively and was comparatively lower than synthetic metal chelators EDTA. Chelating activity of EDTA at 0.05 and 0.10 Mm showed 84.65 % ± 0.31 and 96.42 % ± 0.58 respectively. Metal chelating activity of the compound depends upon number of hydroxyl groups in ortho position. The low chelating activity of PSE was possibly due to presence of methoxy group. Zhou et al. (2011) have reported that, the chemical structures of papaya seed extract were identified as p-benzoic acid and vanillic acid. The methoxy group of vanillic could interfere in metal chelating activity of PSE and that may be the reason for low metal chelating activity than GSE. Methoxy group could stabilize phenol radicals owing to its electron donating abilities but did not contribute in chelating activities (Danilewicz 2003).

Antioxidant activity by linoleic acid emulsion system

Lipids rich in unsaturated fatty acids are highly susceptible to oxidative processes. Specially, Linoleic acid and arachidonic acid are targets of lipid peroxidation. The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical (Gao et al. 2000). Hydroxyl radicals eliminate hydrogen atom from the membrane lipids, which results in lipid peroxidation.

Lipid oxidation inhibitory activity has been measured by ferric thiocyanate method (FTC). It measures amount of peroxide produced during initial stage of lipid oxidation. The FTC assay consists of ammonium thiocynate and Fe^{2+} in acidic solution. H_2O_2 induced by lipid oxidation oxidizes Fe^{2+} to Fe^{3+} resulting in the formation of a red colored Fe^{3+}-thiocyanate complex. The levels of oxidation are determined by measuring the absorbance at 500 nm (Kosem et al. 2007; Yu et al. 2007). On the other hand, TBA measures the amount of malondialdehyde (MDA) produced during the second stage of the lipid oxidation. This method is based on the MDA reaction with thiobarbituric acid to obtain a red pigment, resulting from the condensation of two molecules of TBA with one molecule of MDA. The levels of oxidation are determined by measuring the absorbance at 535 nm (Fernandez et al. 1997; Zarena and Sankar 2009).

The inhibitory capacity of GSE and PSE against the oxidation of Linoleic acid model system was tested. As shown in Table 2, the percentage of inhibition of lipid oxidation by grape and papaya seed was measured at three different concentrations. At a same concentration, grape seed was observed to have higher inhibitory activity ($p < 0.5$) than papaya seed extracts and with the increase in concentration inhibitory activity of both the extracts increased with a maximum inhibition of 81.02 and 65 % respectively for GSE and PSE. Ramchandani et al. (2010)

also reported that, GSE could inhibit lipid peroxidation in the mouse liver microsome model system by 70–80 %. Moreover, Jayaprakasha et al. (2001) have reported that, ethanolic and water extracts of grape seed showed 80 % inhibition of the Linoleic acid peroxidation after 100 h. The mechanism involved in the interference of GSE inhibition of lipid oxidation is either by iron chelating activity or by scavenging of superoxide radicals, which are responsible for reduction of ferric to ferrous, catalyzed by Fenton reaction and the iron chelating activity (Ramchandani et al. 2010).

Antibacterial activity

The antibacterial activity of grape and papaya seed extracts is shown in Table 3. Among two extracts tested against gram positive and gram negative bacteria, GSE showed higher antimicrobial activity compared with PSE. GSE was more effective against *Staphylococcus aureus* and *Bacillus subtilis* compared to gram negative bacteria like E. coli. These results are in agreement with reports given by Jayaprakasha et al. (2003) and Serra et al. (2008). The higher activity against the gram positive strains may be due to the fact that, gram positive bacteria has less stable cell wall, which make it permeable to some antimicrobial agents. The antimicrobial properties of grape seed could be due the presence of core compounds with 3,4,5-trihydroxyphenyl found in epigallocatechin, epigallocatechin-3-ogallate and prodelphinidin, which might play an important role in their antibacterial activity (Tagurt et al. 2004). The galloyl groups present in the structure of compounds present in GSE could exhibit antimicrobial activity. The mechanism of antimicrobial activity is due to outer cell membrane or cytoplasmic membrane of the bacterium is essentially composed of phospholipid bilayer and proteins and is the major site of interaction with antimicrobial compounds.

Chemical analyses

Changes in peroxide value (PV)

The peroxide value (PV) is a measure of the primary degree of oxidation of seafood during the storage period. In the present study, the impact of GSE and PSE on lipid oxidation of mackerel during ice storage over 15 days is as shown in Table 4. In this study, PV in all treatments increased gradually during the storage. Initially the PV of all samples was observed to increase slowly from 1.60–1.65 mg hydroperoxide/kg sample. On 6th day, the PV of CWC was significantly ($p < 0.05$) higher than that of other treatment groups and there was no significant difference in PV of GSE and BHT treated mackerel. The PV of CWC reached the limit of acceptability on 9th day of ice storage whereas, GSE and PSE treated mackerel were in acceptable limit up to 15 and 12 days respectively. The increase in PV of CWC and PSE indicated that, the samples were in propagation stage of lipid oxidation with a lower rate of decomposition of hydroperoxides. GSE exhibited excellent antioxidant properties to prevent lipid oxidation by scavenging the free radical chains compared to PSE. The inhibition mechanism of hydroperoxide formation in fish muscle with influence of phenolic compounds is to interfere with the initiation or propagation steps of lipid oxidation reactions by scavenging lipid radicals and forming low-energy antioxidant radicals that do not readily promote oxidation of unsaturated fatty acids (Frankel 1998a). The other mechanism for inhibition of lipid oxidation in fish muscle is reducing capacity of phenolic antioxidants (Medina et al. 2007).

During 15 days of storage, higher PV was observed in CWC than in GSE and PSE treated mackerel. The results indicated that, GSE and PSE were effective in retarding the formation of hydroperoxide. Other authors have reported similarly that phenolic compounds of GSE demonstrated strong antioxidant properties in limiting peroxide formation in fish muscle (Shi et al. 2014; Jianyun et al. 2014).

Changes in thiobarbutirc acid reactive substances (TBARS)

The TBARS value is an index of secondary lipid oxidation measuring malonaldehyde content. The TBARS value of all the treatment groups showed an increasing trend in malonaldehyde content with storage period as shown in Table 4. The changes in TBARS content of all treated groups showed significant difference with CWC ($p < 0.05$) but there was no significant difference ($p > 0.05$) between GSE and BHT treated mackerel throughout the storage period. The initial TBARS value of CWC, BHT, GSE and PSE samples were 0.32 mg

Table 3 Effect of grape seed extract (GSE) and papaya seed extract (PSE) on the growth of pathogenic microorganisms ($n = 3$)

	Zone of inhibition in mm				
Microorganisms	*S. aureus*	*B. subtilis*	*P. fluroscens*	*S. typhium*	*E.coli*
Grape seed extract(GSE)	27.04 ± 0.86	30.03 ± 0.83	18.66 ± 0.25	13.01 ± 0.32	15.13 ± 0.32
Papaya seed extract (PSE)	25.42 ± 1.04	25.00 ± 0.76	–	15.15 ± 0.25	17.20 ± 0.25
Ampicillin (positive antibiotic control)	55.02 ± 1.32	51.50 ± 0.40	26.02 ± 0.45	21.33 ± 0.76	25.16 ± 1.60

Bar indicates Mean ± SD ($n = 3$)

Table 4 Changes in peroxide value (PV), thiobarbituric acid reactive substances (TBARS), free fatty acid (FFA), trimethylamine nitrogen (TMA-N), total volatile base nitrogen

Parameters	Storage days						
	Treatments	0	3	6	9	12	15
PV(mg hydroperoxide kg^{-1})	CWC	1.62 ± 0.07^{a}	3.48 ± 0.07^{b}	6.03 ± 0.34^{c}	10.25 ± 0.14^{d}	14.30 ± 0.07^{d}	16.94 ± 0.69^{d}
	BHT	1.65 ± 0.12^{a}	2.54 ± 0.08^{a}	3.54 ± 0.18^{a}	4.22 ± 0.12^{a}	6.71 ± 0.15^{a}	8.11 ± 0.27^{a}
	GSE	1.65 ± 0.12^{a}	2.65 ± 0.13^{a}	3.71 ± 0.16^{a}	4.50 ± 0.16^{b}	7.20 ± 0.07^{b}	9.05 ± 0.28^{b}
	PSE	1.60 ± 0.07^{a}	3.15 ± 0.14^{b}	5.33 ± 0.09^{b}	8.61 ± 0.19^{c}	10.07 ± 0.16^{c}	12.05 ± 0.54^{c}
TBARS(mg malonaldehydekg^{1})	CWC	0.32 ± 0.12^{a}	0.93 ± 0.07^{b}	1.41 ± 0.16^{c}	1.70 ± 0.25^{c}	1.93 ± 0.08^{c}	2.25 ± 0.18^{c}
	BHT	0.32 ± 0.12^{a}	0.38 ± 0.09^{a}	0.48 ± 0.14^{a}	0.74 ± 0.15^{a}	1.14 ± 0.11^{a}	1.27 ± 0.12^{a}
	GSE	0.32 ± 0.12^{a}	0.50 ± 0.14^{a}	0.61 ± 0.13^{a}	0.74 ± 0.13^{a}	1.27 ± 0.12^{a}	1.36 ± 0.13^{a}
	PSE	0.32 ± 0.12^{a}	0.76 ± 0.05^{b}	1.10 ± 0.12^{b}	1.32 ± 0.14^{b}	1.68 ± 0.24^{b}	1.99 ± 0.30^{b}
FFA(% oleic acid)	CWC	1.45 ± 0.14^{a}	3.00 ± 0.14^{c}	5.11 ± 0.19^{c}	6.41 ± 0.19^{c}	7.84 ± 0.08^{c}	9.02 ± 0.31^{d}
	BHT	1.45 ± 0.14^{a}	2.41 ± 0.16^{a}	4.26 ± 0.14^{a}	5.81 ± 0.17^{a}	6.75 ± 0.22^{a}	7.30 ± 0.24^{a}
	GSE	1.45 ± 0.14^{a}	2.61 ± 0.18^{ab}	4.60 ± 0.17^{ab}	5.58 ± 0.07^{ab}	6.91 ± 0.30^{a}	7.60 ± 0.26^{a}
	PSE	1.45 ± 0.14^{a}	2.92 ± 0.22^{c}	4.91 ± 0.22^{bc}	6.02 ± 0.22^{b}	7.23 ± 0.16^{b}	8.72 ± 0.28^{c}
TMA-N (mg N_2 100 g^{-1})	CWC	3.22 ± 0.08^{a}	6.40 ± 0.10^{c}	10.12 ± 0.15^{d}	15.09 ± 0.76^{c}	19.22 ± 0.02^{d}	23.18 ± 0.11^{d}
	BHT	3.22 ± 0.08^{a}	4.10 ± 0.10^{a}	7.01 ± 0.15^{a}	9.52 ± 0.18^{a}	11.88 ± 0.10^{a}	15.22 ± 0.08^{a}
	GSE	3.22 ± 0.08^{a}	4.35 ± 0.22^{a}	7.41 ± 0.14^{b}	10.22 ± 0.11^{a}	14.5 ± 0.23^{b}	16.5 ± 0.23^{b}
	PSE	3.22 ± 0.08^{a}	5.45 ± 0.12^{b}	8.98 ± 0.10^{c}	12.22 ± 0.06^{b}	15.01 ± 0.15^{c}	18.26 ± 0.13^{c}
TVB-N(mg N_2 100 g^{-1})	CWC	8.45 ± 0.23^{a}	18.14 ± 0.46^{d}	26.71 ± 0.26^{d}	35.25 ± 0.51^{d}	39.86 ± 0.34^{d}	49.91 ± 0.25^{d}
	BHT	8.45 ± 0.23^{a}	9.28 ± 0.15^{a}	14.32 ± 0.30^{a}	21.05 ± 0.74^{a}	28.14 ± 0.91^{a}	34.01 ± 0.57^{a}
	GSE	8.45 ± 0.23^{a}	11.06 ± 0.20^{b}	17.67 ± 0.62^{b}	24.02 ± 0.31^{b}	30.10 ± 0.63^{b}	36.07 ± 0.54^{b}
	PSE	8.45 ± 0.23^{a}	13.30 ± 0.16^{c}	19.61 ± 0.56^{c}	26.54 ± 0.69^{c}	34.92 ± 0.31^{c}	41.00 ± 0.20^{c}
Ph	CWC	6.18 ± 0.02^{b}	6.22 ± 0.01^{b}	6.57 ± 0.03^{c}	6.72 ± 0.01^{d}	6.82 ± 0.02^{d}	6.93 ± 0.01^{c}
	BHT	6.20 ± 0.02^{b}	6.25 ± 0.01^{b}	6.36 ± 0.02^{b}	6.47 ± 0.01^{b}	6.57 ± 0.02^{b}	6.70 ± 0.02^{b}
	GSE	5.92 ± 0.01^{a}	6.10 ± 0.01^{a}	6.22 ± 0.01^{a}	6.31 ± 0.02^{a}	6.38 ± 0.02^{a}	6.47 ± 0.03^{a}
	PSE	6.19 ± 0.02^{b}	6.23 ± 0.02^{b}	6.40 ± 0.02^{b}	6.53 ± 0.04^{c}	6.64 ± 0.01^{c}	6.73 ± 0.02^{b}

(TVB-N) and pH of Indian mackerel treated with grape seed extract (GSE) and papaya seed extract (PSE) during ice storage

Mean ± sd, $n = 3$

[a-d] Values in the same column for each attribute are followed by different letters are significantly different ($p < 0.05$)

MAD/kg sample and reached to a maximum of 2.25, 1.27, 1.36 and 1.99 mg MAD/kg sample respectively after 15 days of ice storage. The increase in TBARS value indicated formation of secondary lipid oxidation products. The present investigation revealed that, GSE and PSE treatment to mackerel helped to inhibit the malonaldehyde formation, GSE was observed to be more effective in comparison to PSE and the results are followed by the similar trend of inhibition of TBARS formation treated with 100 ppm of grape procyanidins and inhibited formation of TBARS in fish muscle by 80 % as reported by Iglesias et al. (2012). The mechanism of the protective effect on lipid oxidation and lowering of TBARS content may be due to the fact that grape seed extract are rich in proanthocyanidins which has multiple mechanism for its antioxidant activity and ability to sequestrate radicals, chelate metals and synergise with other antioxidants (Lu and Foo 1999). The results of the present investigation are in agreement with findings reported by Rababah et al. (2006) and Shirahigue et al. (2010), who observed a reduction in TBARS in chicken meat with grape seed extract during refrigerated storage. Jongberg et al. (2012) have reported that, phenolic compounds from natural sources have effective inhibitory action on lowering the secondary lipid oxidation of chill stored beef patties treated with grape seed extract at 500 ppm.

Changes in free fatty acids (FFA)

The free fatty acid is a suitable means for assessment of fish quality during storage and can be used as a quality index of fish and other food products (Hui and Tung 1997). It is well known that FFA is a result of enzymatic decomposition of lipid in fish and fisheries products (Hardy 1980). In the present study, increase in free fatty acid content was detected from the 0 day, observed to increase throughout storage period significantly ($p < 0.5$). On the day of sensory rejection, the FFA content for CWC, BHT, GSE and PSE treated samples (9, 15,

15, 12th day), were observed to be 6.41, 7.30, 7.60 and 7.23 % as oleic acid respectively. The results indicated that, the treated samples reduced the deterioration of lipid effectively shown in Table 4. These results might be attributed to the inhibition of enzymatic action to liberate free fatty acids by both GSE and PSE, but the influence of inhibition of enzymatic hydrolysis was more pronounced in GSE treated samples. The result of the present study is in agreement with findings of Bensid et al. (2014) who reported that, the effect of polyphenols from thyme, oregano and clove extracts lowered the formation of free fatty acids. The prime factor of hydrolytic rancidity in fish during ice storage takes place as a result of endogenous enzyme viz., lipases and phospholipases activity and later on by microbial activity. The inhibitory effect of plant polyphenols on endogenous enzyme activity in fish based muscle system occurred in first stage (days 2–6) while as antimicrobial effect of polyphenols were observed in the second stage (days 10–23) to a lower FFA formation as reported by Bensid et al. (2014).

Changes in trimethylamine nitrogen (TMA-N)

Trimethylamine (TMA) level in fish is an important factor in the subjective evaluation of fish quality because of its close association with fish spoilage (Chang et al. 1976). A level beyond 10–15 mg TMA-N/100 g of fish are considered as spoiled (Govindan 1985). The initial TMA-N value of mackerel was 3.22/100 g and was equal to all four batches of samples, which steadily increased to 23.18 per 100 g for CWC, 15.22 per 100 g for BHT, 16.50 per 100 g for GSE and 18.26 per 100 g for PSE on the 15th day of ice storage as shown in Table 4. The CWC reached a maximum limit of acceptability on 9th day of ice storage whereas; GSE and PSE treated mackerel were in acceptable limit up to 12th day of ice storage. The effect of treatment on all groups showed a significant difference ($p < 0.05$) with that of CWC during the entire storage period. A reduction of 32.27 and 19.01 % of TMA-N was observed with GSE and PSE treated samples on the corresponding day respectively. The rate of accumulation of TMA-N was 1.33, 0.88 and 1.00 per 100 g per day for CWC, GSE and PSE treated samples respectively. The different rates of accumulation of TMA-N might be due to inhibitory effect of GSE and PSE over the growth of bacteria and enzymatic activity. Gao et al. (2014) who reported that, polyphenolic compounds of rosemary extract along with nisin were effective in controlling enzymatic and microbial spoilage in pompano fillet during chilled storage.

Changes in total volatile base nitrogen (TVB-N)

Total volatile base nitrogen (TVB-N), is mainly composed of ammonia, primary, secondary and tertiary amines (Beatty 1938) as a result of degradation of proteins and non-protein nitrogenous compounds, which is chiefly caused by microbial activity (Ruiz-Capillas and Moral 2001). Changes in the TVB-N content of mackerel over storage time are shown in Table 4. On 9th day, the TVB-N level of CWC (i.e., 35.25 ± 0.51 mg of TVB-N/100 g) exceeded the spoilage limit of 35 mg of TVB-N/100 g of flesh suggested for fish products, while TVB-N level of both GSE and PSE treated samples reached the limit of acceptability after 15 and 12 days respectively. At the end of a 15- days storage period, TVB-N content of fish samples treated with GSE (36.07 ± 0.54 mg of TVB-N/100 g) or PSE (41.00 ± 0.20 mg of TVB-N/100 g) increased by 4–5 times as compared to 6 folds for CWC reflecting reduction of 31.85 and 24.70 % of TVB-N for GSE and PSE treated samples on the corresponding day respectively. The effect of treatment on all the groups showed a significant difference ($p < 0.05$) with that of CWC during the entire storage period. Our result indicated that phenolic compounds from GSE had a good antimicrobial activity and is more effective in inhibiting enzyme and microbial activity compared to PSE. The present results are supported by the findings of Ünalan et al. (2011) stating that, phenolic compounds from pomegranate and rosemary extract helps to lower TVB-N formation in halibut fillets. Similar findings were reported by Quitral et al. (2009) on lowering TVB-N content of Chilean jack mackerel (*Trachurus murphyi*) during ice storage with the effect of rosemary and orgeno polyphenols.

Changes in pH

Changes in pH can be used as a spoilage indicator in fishery products. Indian mackerel used in the present study had an initial muscle pH of 6.18 (Table 4) and limit of acceptability of marine fish is considered to be 6.5–7.5 (Gopakumar 2002). The pH of CWC showed faster increase with the storage period reaching a value 6.93 at the end of storage. The present result of increase in pH of Indian mackerel throughout the ice storage period was in the same line as reported by Shinde et al. (2012). GSE treated samples showed lower pH with the effect of treatment and was comparatively lower than other treatment groups ($p < 0.05$), which could be due to acidic nature of the GSE solution used for treating the samples. A pH value 6.47 and 6.73 were observed at the end of storage period for GSE and PSE treated mackerel. The increase in pH with the storage period could be attributed to the production of volatile base compounds such as ammonia by bacterial activity (Kyrana et al. 1997), which was low in treated samples as it is apparent from the results of total plate count (Table 5).

Changes in drip loss

Drip loss is one of the important physical quality attributes which reflects the decreased weight in the final product as well as loss of some water soluble nutrients from the muscle. The

Table 5 Changes in drip loss, total plate count and sensory attributes of Indian mackerel with the effect of grape seed extract (GSE) and papaya seed extract (PSE) during ice storage

Parameters	Storage days						
	Treatments	0	3	6	9	12	15
Drip loss (%)	CWC	0 ± 00^{a}	2.54 ± 0.08^{d}	4.50 ± 0.1^{c}	8.13 ± 0.10^{d}	12.99 ± 0.04^{d}	18.64 ± 0.04^{d}
	BHT	0 ± 00^{a}	1.24 ± 0.08^{a}	2.80 ± 0.15^{a}	5.89 ± 0.06^{a}	8.14 ± 0.06^{a}	11.62 ± 0.07^{a}
	GSE	0 ± 00^{a}	1.54 ± 0.04^{b}	3.08 ± 0.08^{b}	6.95 ± 0.05^{b}	9.17 ± 0.17^{b}	13.44 ± 0.16^{b}
	PSE	0 ± 00^{a}	1.71 ± 0.08^{c}	3.28 ± 0.12^{b}	7.15 ± 0.15^{c}	10.44 ± 0.04^{c}	15.99 ± 0.09^{c}
Total plate count (log cfu/g)	CWC	4.5 ± 0.06 ^{a}	5.4 ± 0.15^{b}	6.0 ± 0.06 ^{c}	8.0 ± 0.14 ^{c}	8.50 ± 0.14^{c}	9.3 ± 0.13^{c}
	BHT	4.5 ± 0.11 ^{a}	5.0 ± 0.07^{ab}	5.8 ± 0.15 ^{b}	6.8 ± 0.04 ^{b}	7.7 ± 0.07^{b}	8.5 ± 0.08^{b}
	GSE	4.5 ± 0.19 ^{a}	4.7 ± 0.11^{a}	5.3 ± 0.04^{a}	6.5 ± 0.07 ^{a}	7.3 ± 0.06^{a}	7.8 ± 0.08^{a}
	PSE	4.5 ± 0.10 ^{a}	4.9 ± 0.54^{ab}	5.8 ± 0.11^{b}	6.8 ± 0.06 ^{b}	7.8 ± 0.05^{b}	8.3 ± 0.05 ^{b}
Overall acceptability score	CWC	8.97 ± 0.09 ^{a}	7.07 ± 0.10^{a}	6.00 ± 0.11^{a}	5.01 ± 0.05 ^{a}	2.57 ± 0.09^{a}	1.49 ± 0.08^{a}
	BHT	9.00 ± 0.07 ^{b}	8.92 ± 0.07^{c}	8.04 ± 0.13^{c}	7.06 ± 0.06 ^{c}	6.25 ± 0.13^{c}	5.29 ± 0.11^{d}
	GSE	9.00 ± 0.07 ^{b}	8.82 ± 0.06^{c}	7.87 ± 0.06^{c}	7.05 ± 0.09 ^{c}	6.07 ± 0.08^{c}	5.13 ± 0.06^{c}
	PSE	9.00 ± 0.05 ^{b}	7.89 ± 0.02^{b}	6.96 ± 0.06 ^{b}	6.29 ± 0.06 ^{b}	5.18 ± 0.08^{b}	4.17 ± 0.04^{b}

Mean ± sd, $n = 3$

$^{a\text{-}d}$ Values in the same column for each attribute are followed by different letters are significantly different ($p < 0.05$)

drip loss was significantly higher ($P < 0.05$) for CWC samples when compared to treated one as shown in Table 5. A value of 8.13 % was observed for CWC samples on the day of sensory rejection. The parallel values for BHT, GSE and PSE treated samples, drip loss value of 5.89, 6.95 and 7.15 % were observed respectively. Superior drip loss in CWC samples could be due to denaturation of myofibrillar proteins which result in the decrease of water holding capacity. The muscle constitutes myofibrils which constitute a significant fraction of muscle bundle in fish, such a phenomenon highly modifies water distribution inside meat leading to loss of less tightly bound water. The shorter shelf life of untreated Indian mackerel could be due to high drip loss of fish, which enhances hydrolytic and oxidative processes. Increase in drip loss was also reported for mackerel and Japanese sardine with storage period by Hamada-Sato et al. (2002).

Changes in microbiological characteristics

It has been estimated that about one third of the world's food production is lost annually on account of microbial spoilage. Microorganisms associated with aquatic food products usually reflect the microbial population in their environment. In a living fish bacteria are present in the skin and gut, but are prevented to enter the fish muscle. Ones a fish dies, its autolysis begins, then bacteria can enter and decompose the muscle (Aberoumand 2010). Changes in total plate count (TPC) on mackerel throughout the storage are shown in Table 4. The initial TPC was around 4.5 log cfu/g, indicating that mackerel was of good quality. The GSE treated samples showed significantly lower TPC compared to other treatment groups ($p < 0.05$), whereas BHT and PSE treated samples did not show any significant difference ($p > 0.05$) and were significantly lower than CWC. The CWC reached limit of acceptability on 9th day of ice storage whereas, GSE and PSE treated samples reached the limit of acceptability on 15th and 12th day respectively. A difference of around 1.5 log cycles was observed between the untreated and GSE treated samples at the end of 15th day of storage while difference of 1 log cycle was reported between control and PSE treated samples. Similar reduction of 1 and 2 log cycle was reported for crown god treated Indian mackerel (Winarni et al. 2012). The result clearly demonstrated that, the GSE had strong inhibitory effect on microbial growth of stored mackerel and extended the shelf life of Indian mackerel.

Changes in sensory quality

Sensory evaluation was performed according to attributes mentioned in the experimental section, and results are expressed in Table 5. For all types of fish samples, a quality decrease was detected by the panel as a result of increase in the storage days. A prolonged shelf life was obtained for Indian mackerel treated with GSE and PSE stored under ice when compared to CWC. Thus, control was found to be unacceptable at day 9, when compared to treated mackerel. The limiting factor was the flesh odour of raw mackerel; better scores were also obtained in treated mackerel when considering other attributes such as external odour and gills appearance. This enhancement in sensory quality is in agreement with the results previously mentioned for microbiological and chemical quality indices. The sensory scores treated groups declined

gradually for 15 days of ice storage. Initially, the fish was very fresh with characteristics glossy appearance, bright eyes, reddish gills, sea weedy odor and very firm texture. Changes in sensorial properties varied among the treatments, but as the storage time increased, all the sensory attributes decreased. The initial glossiness of the fish showed a decreasing trend with the storage period. Using sensory score of 9 as limit of acceptability, the observed shelf life was 9 days in CWC samples, 12 days for PSE treated samples and 15 days each for BHT and GSE treated samples, whereas a difference of 3 days were seen in PSE treated samples and 6 days in BHT and GSE treated samples. Previous works demonstrated an increased shelf-life and sensory quality enhancement of Indian mackerel by means of treatment with Aloe Vera and God fruit extracts by Winarni et al. (2012). The results of the sensory analysis indicated that GSE treated mackerel was found to be of superior quality compared to other treatment groups and improved sensory characteristics of Indian mackerel.

Conclusion

Potential risks and concern over the use of synthetic antioxidants and antimicrobials have renewed the interest of consumers towards natural and safe alternatives in seafood products. To address the need, several plant extracts are being used by researchers. In the present study, grape and papaya seeds were screened for antioxidant and antimicrobial properties. The study revealed that, the GSE possess higher phenolic and flavonoid content in comparison to PSE, highlighting its contribution to the antioxidative activity in the array of in-vitro assays. In addition, GSE showed higher inhibitory activity against lipid oxidation in linoleic acid model system and a promising antimicrobial action towards *Staphylococcus aureus* and *Bacillus subtilis*. Hence the extracts were used to enhance the keeping quality of Indian mackerel and found to be effective in reducing the oxidative spoilage considerably. The natural extract treatment maintained a good sensory properties for extended period, increased the lag phase of bacterial population and reduced the formation of volatile bases and other products. A shelf life of 15 and 12 days was observed in GSE and PSE treated mackerel respectively in comparison to control for only 9 days. The present study indicated that the preservative effect of GSE was comparable to that of BHT and controlled the growth of microorganisms. Therefore it can be recommended that the GSE can be used as an promising alternative to that of synthetic counterparts in the industry.

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