Citrus peel extract incorporated ice cubes to protect the quality of common pandora

Fish storage in ice with citrus

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Abstract The objective of this study was to investigate the effects of ice with albedo and flavedo fragments of Citrus (Grapefruit (*Citrus paradisi*) and Bitter orange (*Citrus aurantium* L.)) extracts on the quality of common pandora (*Pagellus erythrinus*). Concentrated citrus extracts were diluted with distilled water (1/100 w/v) before making of ice. The ice cubes were spread on each layer of fishes and stored at 0 °C for 15 days. The pH value showed a regular increase in all samples. TVB-N levels of bitter orange treatment groups were recorded lower than the other groups reaching to 25.11 ± 0.02 mg/100 g at the end of the storage. The TMA-N values of bitter orange treatment groups were lower than that of control and grapefruit treatment groups. In terms of TBARS value, alteration was observed in the control samples and this value significantly ($p < 0.01$) increased from 0.101 ± 0.011 mg MA/kg to 0.495 ± 0.083 mg MA/kg, while remained lower in the citrus extracts treatment groups at the end of storage since their antioxidant capacity. The oxidation was suppressed in citrus extracts treatment groups, especially in bitter orange flavedo treatment. The results showed the bitter orange albedo and bitter orange flavedo extracts in combination with ice

storage have more effectiveness in controlling the biochemical indices in common pandora.

Keywords *Pagellus erythrinus* · Common pandora · Grapefruit · Bitter orange · Ice storage

Introduction

Marine fish are highly susceptible to rapid spoilage at ambient temperature. Storage time and storage conditions have also great effect on the quality of fish. Furthermore, the storage stability of fish depends on the composition. The rate of deterioration during ice storage of fish varies with species and depends on the concentrations of substrates and metabolites in the tissue, microbial contamination and conditions of storage after catching (Pacheco-Aguilar et al. 2000). Common pandora (*Pagellus erythrinus* L., 1758) is highly priced and promising new candidate species in Mediterranean aquaculture in recent years. It is crucial to keep the initial quality of the fish until consumption. The fish are generally transported and put up for sale in ice. The storage in ice provides direct contact with the fish and allows washing away the mucus layer and keeping the material cold. Preservation in ice is one of the most efficient ways of retarding spoilage. When evaluating the quality of fish and fishery products depending mainly on the procedures followed during handling, processing and storage, from the moment it is caught until it reaches the consumers, freshness is the most important and fundamental single criterion (Hernandez et al. 2009). Natural protective methods are popular with the increasing health concern of conscious consumer. Antioxidants are added to fish and fish products to maintain quality and to extend shelf life.

Turkey is the top ten countries in the production of citrus fruits in the world. About 80 % of the citrus harvest is used by

Highlights Citrus peel extracts are effective in controlling biochemical indices of common Pandora. Addition of citrus extracts into ice increased its protective effect on the quality. Oxidation was suppressed by bitter orange flavedo due to high antioxidant activity. Citrus peels can be replaced with synthetic protective agents in industry.

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the juice industry. However, during the citrus juice extraction process, large quantities of by-products are produced (55 % of the weight products) those of constituted by peels and seeds. These wasted by-products must be considered as natural protection material and hence, contribute to the prevention of environmental pollution. Thus new aspects concerning the use of these by-products for further utilization on the production of food additives or supplements with high nutritional value and economically attractive have gained increasingly interest. Recent studies showed that citrus by-products are a source of bioactive compounds; peels are rich on secondary metabolites such as phenolic compounds (Li et al. 2006; Khan et al. 2010; He et al. 2011). The content of total phenolic compounds and the antioxidant activity of various parts of citrus fruits such as fruit, fruit juice, seed, peel, pulp were studied by many researchers (Manthey and Grohmann 2001; Barreca et al. 2011). Citrus flavonoids exhibit antioxidant, antimicrobial, anticarcinogenic, antiviral, anti-allergic and anti-inflammatory activities (Madeira et al. 2014). Moreover, it has been reported that the peels have higher antioxidant activity than the seeds of citrus (Tripoli et al. 2007). There are many classes of flavonoids, flavanones being the most abundant group in citrus fruits (Ferreira et al. 2013). Grapefruit find its place with high bio-active compounds in citrus sp. The peel of grapefruit is a rich source of naringin and neohesperidine (Zhang et al. 2011). Polymethoxiflavons, which are in small amounts in other plants, are present in the flavedo fragments, whereas flavanons are in the albedo fragments (Ortuno et al. 2006). Bitter orange is demanded in recent years owing to its bioactive compounds such as synephrine, alkaloids, limonoids, phytosterols and flavonoids (Barreca et al. 2011). Treatment with fruit extracts, in combination with ice, has proven to be an effective preservation method for the extension of shelf-life of foods (Holley and Patel 2005). Many spices and plants have been extensively studied for their natural antioxidant characteristics. The principal objective of this research was to produce ice with citrus peel extracts and to determine its effects on the shelf-life and quality of common pandora (*Pagellus erythrinus*) during refrigerated storage. Moreover, the aspects of effective utilize of by-product of citrus tried to be revealed.

Materials and methods

Materials

Common pandora-bronze bream (*Pagellus erythrinus*) was caught using trawl nets in the Gulf of Antalya in April 2013. Akdeniz University research vessel was used for catching. The fish died naturally in trawl operation. The average weight and length of the fish were 65.7 ± 5.4 g and 15.2 ± 1.7 cm, respectively. The fish were transported within 1 h in ice boxes

to the laboratory. The grapefruit (*Citrus paradisi*) was obtained from local market and bitter orange (*Citrus aurantium*) was collected from the trees situated in the campus of Akdeniz University.

Extraction

The albedo and flavedo tissues of grapefruit and bitter orange were peeled manually. The lipid content of the flavedo layers were separated by diethyl ether using cold extraction. Plant tissues were dried at 40 °C for 17 h in an oven. Dried tissues were grinded into small pieces and were extracted in ethanol:water 1:4 v:v (1:10 w/v). The mixtures were first held in ultrasonic bath (Bandelin, Sonorex, Germany) for 15 min at 40 °C, and then placed in a water bath with shaker (GFL 1086, Germany) at 40 °C for 2 h. The extracts were filtered and concentrated in a rotary evaporator (Heidolph, HEIVAP, Germany) to get crude extracts.

Ice trays were used in order to obtain ice cubes having the same dimensions. Extract solutions were prepared by dissolving 1.0 g of each citrus tissue extract in 100 ml distilled water. The solutions were poured into ice trays and frozen.

Treatment

Total 180 fish were divided into five groups. Each group was put into polystyrene boxes and covered with ice cubes on each layer. Treatment groups were defined as grapefruit albedo (GA), grapefruit flavedo (GF), bitter orange albedo (BOA) and bitter orange flavedo (BOF) and traditional ice treatment was regarded as control. The bottoms of the boxes were drilled for drainage. The treatment groups were stored at 0 °C. Ice fortification and analyses were done on 3 day intervals. Six fish samples of each treatment group were used on the sampling days for the analyses. All assays were conducted on duplicate samples from the homogenate pool of the muscle and skin of the fish for each replication.

Analyses

Total phenolic compound contents of citrus extracts were determined by the Folin-Ciocalteu colorimetric method (Spanos and Wrolstad 1990). Results were expressed as g gallic acid equivalents (GAE) in 100 g extract as means of three consecutive measurements of the same sample.

The antioxidant capacity of citrus extracts was determined according to the photometric method (Re et al. 1999). The results were expressed in μ M Trolox equivalents (TEAC). Analyses were conducted on triplicate samples of citrus extracts.

The pH of the fish samples were measured by dipping the probe of the pH-meter (Hanna Instruments pH 211,

Microprocessor pH Meter) into the mixture of homogenized sample and water (1:1 w:v) (Manthey et al. 1988).

Total volatile basic nitrogen (TVB-N) was determined on 10 g of homogenized fish with MgO using distillation apparatus. The distillate was collected in 10 ml of 0.1 mol equi/L HCl solution and titrated with 0.1 mol equi/L NaOH with the presence of tashiro indicator (Schormuller 1968). TVB-N content was expressed as mg TVB-N/100 g fish muscle.

Trimethylamine nitrogen (TMA-N) analysis was performed as described as Schormuller (1968). A 10 g homogenized fish was blended with 90 ml of 5 g/100 ml trichloroacetic acid using an ultraturrax homogenizer and filtered. A 4 ml aliquot was transferred into test tubes and 1 ml formaldehyde (20 g/100 ml), 3 ml KOH (50 g/100 ml) solution and 10 ml toluene were added to each test tubes. The test tubes were stoppered and shaken vigorously. The toluene phase (5 ml) was then transferred into a tube and mixed with 5 ml picric acid (0.02 g/100 ml). The absorbance of the standards and samples was measured at 410 nm using a spectrophotometer (Thermo Scientific, Evolution 160 UV–VIS, Germany). TMA-N content was expressed as mg TMA-N/100 g fish muscle.

Thiobarbituric acid reactive substances (TBARS) analysis was performed according to Tarladgis et al. (1960). Fish sample (10 g) was steam distilled with 2.5 ml HCl: distilled water (1:2). A 5 ml of the distillate was mixed with 5 mL thiobarbituric acid (TBA) solution (0.288 g/100 ml). The mixture was left into the water bath at 110 °C for 35 min in order to result in color complex. The absorbance was measured with a UV–vis spectrophotometer at 538 nm against a blank containing distilled water and TBA solution. The results were expressed as mg malonaldehyde/kg fish flesh.

For the determination of total psychrophilic bacteria count, a 10 g sample was added to 90 ml of sterile 0.1 g/100 ml saline peptone water (0.1 g/100 ml peptone and 0.85 g/100 ml NaCl) and homogenized in a stomacher (Stomacher 80, Seward Medical, London, UK) for 2 min at low speed at room temperature. Serial decimal dilutions were made and plated onto agar (PCA—Oxoid CM0463B) and incubated at 8 °C for

7 days. The colonies from selected dilutions were enumerated as colony forming units.

All assays were conducted on duplicate samples for each replication. The data was subjected to analyses of variance using the SAS Software (Statistical Analysis System, Cary, NC, USA). When main effects or interactions were significant ($p < 0.01$), Duncan's multiple range test was used.

Results and discussion

The antioxidant activity does not always correlate with the quantities of total polyphenolic compounds due to the difference in their chemical structure. The phenolic compounds present in the extracts correspond to 7.93 ± 0.12 g GAE/100 g in grapefruit albedo, 3.85 ± 0.19 g GAE/100 g in grapefruit flavedo and 5.61 ± 0.03 g GAE/100 g in bitter orange albedo, 4.52 ± 0.02 g GAE/100 g in bitter orange flavedo fragments. Significant differences were observed among all groups with regard to total phenolic content ($p < 0.01$). Total phenolic content of bitter orange peel was found as 0.51 mg GAE/100 g dietary fiber (Garau et al. 2007). The antioxidant activities of GA and GF were found to be 0.103 ± 0.007 and 0.183 ± 0.009 μ M TEAC, respectively. These values were determined as 0.221 ± 0.016 μ M TEAC in BOA and 0.371 ± 0.036 μ M TEAC in BOF. The highest total phenolic content was found in GA, whereas the antioxidant activity of BOF was recorded higher ($p < 0.01$) than the other plant extracts. Abd el-aal and Halaweish (2010) determined the total phenolic content and antioxidant activity of the peels of two orange species. They found that these values were effective and higher in the peels after removal of the lipid content. Total eleven flavanons were determined in the flavedo part of bitter orange and their content was recorded as 8.540 g/kg (Zhang et al. 2011).

The pH changes during storage in ice are shown in Table 1. The pH value of the fresh fish is about 6.0–6.5, and it increases throughout the storage. The increases in the pH are related to the accumulation of alkaline compounds, such as ammonia

Table 1 pH values of the common pandora fish during the storage in ice produced with citrus peel extracts^a

Storage time (days) ^b	C ^c	GA ^c	GF ^c	BOA ^c	BOF ^c
0	6.51 ± 0.02 ^{eA}	6.51 ± 0.02 ^{eA}	6.51 ± 0.02 ^{eA}	6.51 ± 0.02 ^{eA}	6.51 ± 0.02 ^{dA}
3	6.59 ± 0.01 ^{dC}	6.59 ± 0.01 ^{dC}	6.67 ± 0.01 ^{bcB}	6.69 ± 0.01 ^{cAB}	6.71 ± 0.01 ^{BA}
6	6.64 ± 0.01 ^{cBC}	6.61 ± 0.01 ^{dC}	6.63 ± 0.01 ^{dB}	6.68 ± 0.01 ^{cAB}	6.67 ± 0.02 ^{CA}
9	6.64 ± 0.01 ^{cB}	6.77 ± 0.01 ^{CA}	6.64 ± 0.03 ^{cdB}	6.62 ± 0.01 ^{dB}	6.66 ± 0.01 ^{cB}
12	6.71 ± 0.01 ^{BC}	6.83 ± 0.01 ^{BA}	6.69 ± 0.01 ^{BC}	6.78 ± 0.01 ^{BB}	6.69 ± 0.01 ^{bcC}
15	6.87 ± 0.01 ^{aD}	6.90 ± 0.01 ^{AC}	7.03 ± 0.01 ^{AA}	6.93 ± 0.01 ^{AB}	6.83 ± 0.01 ^{aE}

^a Values are means $\pm$ standard deviation of the analysis ($n=6$)

^b Values within the same row (A,B,C) and the same column (a,b,c,d,e) with different letters are different ($p < 0.01$)

^c C control, GA grape fruit albedo, GF grapefruit flavedo, BOA bitter orange albedo, BOF bitter orange flavedo

Table 2 Total volatile basic nitrogen (tvb-n) values (mg/100 g) of the common pandora fish during the storage in ice produced with citrus peel extracts^a

Storage time (days) ^b	C ^c	GA ^c	GF ^c	BOA ^c	BOF ^c
0	21.00±0.98 ^{cA}	21.00±0.98 ^{bA}	21.00±0.98 ^{cA}	21.00±0.98 ^{bA}	21.00±0.98 ^{bcA}
3	22.40±0.00 ^{cA}	19.60±0.14 ^{bB}	20.30±1.01 ^{cAB}	20.50±1.41 ^{bAB}	19.60±1.97 ^{cAB}
6	30.63±1.59 ^{abA}	20.26±2.11 ^{bB}	19.43±1.10 ^{cB}	22.32±2.21 ^{bB}	20.15±1.76 ^{cB}
9	29.32±2.77 ^{bA}	20.90±2.04 ^{bBC}	31.30±2.87 ^{bA}	19.50±1.03 ^{cC}	23.70±1.34 ^{abB}
12	29.15±2.02 ^{bA}	22.22±2.97 ^{bB}	33.45±3.04 ^{bA}	21.01±1.46 ^{bB}	23.02±0.96 ^{abB}
15	34.93±1.95 ^{aB}	30.60±2.77 ^{aBC}	40.40±2.65 ^{aA}	28.83±0.82 ^{aCD}	25.11±0.02 ^{aD}

^a Values are means±standard deviation of the analysis ($n=6$)^b Values within the same row (A,B,C) and the same column (a,b,c,d,e) with different letters are different ($p<0.01$)^c C:control, GA grape fruit albedo, GF grapefruit flavedo, BOA bitter orange albedo, BOF bitter orange flavedo

mainly derived from microbial action during fish muscle spoilage. The initial pH value of common pandora was determined as 6.51 ± 0.02 and showed a similar increase in control, GA and GF samples according to time of storage, whereas this value indicated fluctuations in BOA and BOF. The increase in pH value was significant ($p<0.01$) in all samples. At the end of the storage, there were statistically significant ($p<0.01$) differences among all groups in which BOF remained at the lowest pH levels. The pH increases are in agreement with the findings of Hernandez et al. (2009) and Erkan et al. (2011) for other fish species stored in ice. pH value increased according to storage time, but pH value is not a criterion of spoilage. It has to be supported by other chemical analyses (Schormuller 1968; Scott et al. 1992).

TVB-N is widely used as an indicator for fish deterioration. TVB-N, which is mainly composed of ammonia and primary, secondary and tertiary amines, resulted from degradation of proteins and non-protein nitrogenous compounds, which is chiefly caused by microbial activity. Lang (1983) suggested that the quality classification of fish and fish products regarding TVB-N values would be ‘high quality’ up to 25 mg/100 g, ‘good quality’ up to 30 mg/100 g, ‘limit of acceptability’ up to 35 mg/100 g, and ‘spoilt’ above 35 mg/100 g.

TVB-N concentrations of the common pandora stored in ice are shown in Table 2. The initial TVB-N value was

determined as 21 ± 0.98 mg/100 g and increased up to unacceptable levels in control and GF samples. The highest ($p<0.01$) TVB-N value was obtained in GF treatment at the end of the storage whereas the lowest ($p<0.01$) value was observed in BOF treatment. Bitter orange treatment groups were still in good condition after the 15th day in terms of TVB-N value. A similar observation was found by Erkan et al. (2011) for bluefish combined with thyme and laurel essential oils and stored in ice for 13 days. They found TVB-N value as 28.14 and 31.17 mg/100 g in treatment groups at the end of the storage. On the other hand, a higher TVB-N value was reported for aquacultured sea bream (*Sparus aurata*) by Cakli et al. (2006). Sharp increase for ungutted seabass and seabream during the storage in ice for 14 days was determined. The increase in TVB-N and TMA-N is related to the activity of spoilage bacteria and endogenous enzymes (Kyrana et al. 1997). The antimicrobial activity of citrus fruits, peel and essential oil is reported by many researchers (Tripoli et al. 2007; Espina et al. 2011).

Trimethylamine oxide (TMAO), found in a large number of marine fish, is broken down to trimethylamine (TMA) by either endogenous enzymes or the bacterial enzyme trimethylamine oxidase. The quantitative level of TMA in fish is considered as a major index of the quality of marine fish. Initial average value was 0.422 ± 0.241 mg/100 g of muscle for

Table 3 Trimethylamine nitrogen (tma-n) values (mg/100 g) of the common pandora fish during the storage in ice produced with citrus peel extracts^a

Storage time (days) ^b	C ^c	GA ^c	GF ^c	BOA ^c	BOF ^c
0	0.42±0.24 ^{dA}	0.42±0.24 ^{dA}	0.42±0.24 ^{dA}	0.42±0.24 ^{eA}	0.42±0.24 ^{dA}
3	0.17±0.00 ^{dC}	0.19±0.01 ^{dC}	0.10±0.02 ^{dD}	0.85±0.00 ^{deA}	0.49±0.01 ^{dB}
6	1.75±0.02 ^{cB}	0.45±0.08 ^{dC}	0.43±0.01 ^{dC}	1.33±0.07 ^{cdA}	1.30±0.03 ^{cdA}
9	1.98±0.02 ^{cBC}	2.11±0.04 ^{cAB}	1.87±0.08 ^{cC}	1.64±0.06 ^{cD}	2.24±0.10 ^{bcA}
12	3.30±0.06 ^{bA}	3.69±0.39 ^{bA}	2.76±0.70 ^{bA}	3.70±0.25 ^{bA}	3.29±0.67 ^{bA}
15	5.71±0.01 ^{aBC}	6.36±0.21 ^{aAB}	7.16±0.09 ^{aA}	4.66±0.34 ^{aC}	4.67±0.78 ^{aC}

^a Values are means±standard deviation of the analysis ($n=6$)^b Values within the same row (A,B,C) and the same column (a,b,c,d,e) with different letters are different ($p<0.01$)^c C control, GA grape fruit albedo, GF grapefruit flavedo, BOA bitter orange albedo, BOF bitter orange flavedo

Table 4 Thiobarbituric acid reactive substances (tbars) values (mg ma/kg) of the common pandora fish during the storage in ice produced with citrus peel extracts^a

Storage time (days) ^b	C ^c	GA ^c	GF ^c	BOA ^c	BOF ^c
0	0.10±0.01 ^{cA}	0.10±0.01 ^{dA}	0.10±0.01 ^{cA}	0.10±0.01 ^{dA}	0.10±0.01 ^{cA}
3	0.10±0.01 ^{cB}	0.06±0.01 ^{cC}	0.09±0.01 ^{cB}	0.15±0.01 ^{cdA}	0.13±0.01 ^{dA}
6	0.11±0.01 ^{cC}	0.13±0.01 ^{cB}	0.18±0.01 ^{bcA}	0.19±0.01 ^{cdA}	0.12±0.01 ^{dB}
9	0.31±0.01 ^{bA}	0.23±0.02 ^{bB}	0.17±0.00 ^{bcCD}	0.16±0.01 ^{bcD}	0.19±0.01 ^{cC}
12	0.38±0.03 ^{bA}	0.24±0.01 ^{bB}	0.29±0.01 ^{abB}	0.24±0.05 ^{bB}	0.22±0.01 ^{bB}
15	0.49±0.08 ^{Aa}	0.34±0.01 ^{aAB}	0.37±0.09 ^{aAB}	0.32±0.00 ^{aAB}	0.26±0.01 ^{aB}

^a Values are means±standard deviation of the analysis ($n=6$)^b Values within the same row (A,B,C) and the same column (a,b,c,d,e) with different letters are different ($p<0.01$)^c C control, GA grape fruit albedo, GF grapefruit flavedo, BOA bitter orange albedo, BOF bitter orange flavedo

common Pandora (Table 3). The alteration of TMA-N values were statistically significant ($p<0.01$) during the storage period in all samples. The TMA-N value of the samples with bitter orange extracts was found to be lower than those of control and grapefruit extracts. The pungent odour of spoiled fish has been often related to TMA tissue levels, also with the number of spoiling organisms present in many fish species and the rejection limit is usually 5 to 10 mg TMA-N/100 g of muscle (El Marrackchi et al. 1990). On the other hand, higher values (14.9 mg N/100 g muscle) were reported by Hernandez et al. (2009) for meagre fillets stored in ice. In this study, bitter orange extract treated groups still showed lower TMA-N levels (under 5 mg TMA-N/100 g of muscle) comparing to the control and grapefruit extracts groups at the end of the storage period.

TBARS is a secondary lipid oxidation product and widely used as an indicator of the oxidation (Aubourg 1999). The TBARS results of treatment samples were given in Table 4. TBARS values, indicating rancidity development, remained low (<5 mg MA/kg) and below the limit levels in the all groups at which rancid flavors may become evident in fish (Nunes et al. 1992). An obvious alteration was observed in the control samples and this value significantly ($p<0.01$) increased from 0.101 ± 0.011 mg MA/kg to 0.495 ± 0.083 mg MA/kg. This value remained low at the end of storage in the groups treated with citrus extracts owing to their antioxidant

capacity. TBARS value obtained for BOF group slightly increased throughout the storage and reached the lowest ($p<0.01$) levels comparing to the other treatment groups. Similar results were reported by Ozyurt et al. (2009) for red mullet and goldband goatfish stored in ice for 11 days. TBARS values of goldband goatfish and red mullet at the end of the storage reached to 0.69 mgMA/kg and 0.74 mgMA/kg, respectively. Hernandez et al. (2009) found that TBARS value for meagre fillets during ice storage for 18 days increased from 0.10 mgMA/kg to 2.55 mgMA/kg. Our values are lower than those reported from fish with higher fat contents than common pandora stored under similar conditions, such as sea bass (Papadopoulos et al. 2003) and sardine (Erkan and Ozden 2007), indicating lower oxidative rancidity, probably due to the low fat content of common pandora.

The count of total psychrophilic bacteria of all samples increased significantly ($p<0.01$) during the storage (Table 5). The most common flavonoids found in Citrus sp. can be classified as flavanones, flavones, flavanols and anthocyanins (Salas et al. 2011). These compounds are reported to have antimicrobial activities (Cushnie and Lamb 2005). The antimicrobial activity of phenolic compounds is also derived from the impairment of a variety of enzyme systems and destruction of genetic material (Mahmoud et al. 2004). The lowest counts of total psychrophilic bacterial count were found for the sample treated with bitter orange extracts. These findings are in

Table 5 Total psychrophyl bacteria count (log cfu/g) of the common pandora fish during the storage in ice produced with citrus peel extracts^a

Storage time (days) ^b	C ^c	GA ^c	GF ^c	BOA ^c	BOF ^c
0	3.23±0.00 ^{Af}	3.23±0.00 ^{Af}	3.23±0.00 ^{Ae}	3.23±0.00 ^{Af}	3.23±0.00 ^{Af}
3	4.25±0.06 ^{Ae}	4.12±0.04 ^{ABe}	4.16±0.08 ^{ABd}	4.03±0.11 ^{Be}	4.09±0.01 ^{ABe}
6	4.75±0.06 ^{Ad}	4.68±0.06 ^{ABd}	4.32±0.05 ^{Ccd}	4.59±0.02 ^{Bd}	4.43±0.05 ^{Cd}
9	6.09±0.01 ^{Ac}	5.39±0.05 ^{Cc}	5.51±0.04 ^{Bc}	5.47±0.01 ^{BCc}	5.61±0.09 ^{Bc}
12	8.01±0.02 ^{Ab}	7.23±0.12 ^{Cb}	7.47±0.00 ^{Bb}	6.00±0.01 ^{Eb}	6.39±0.01 ^{Db}
15	8.12±0.02 ^{Aa}	7.69±0.14 ^{BCa}	7.87±0.14 ^{Ba}	7.29±0.06 ^{Da}	7.45±0.02 ^{CDa}

^a Values are means±standard deviation of the analysis ($n=6$)^b Values within the same row (A,B,C) and the same column (a,b,c,d,e) with different letters are different ($p<0.01$)^c C control, GA grape fruit albedo, GF grapefruit flavedo, BOA bitter orange albedo, BOF bitter orange flavedo

agreement with the findings of TVB-N and TMA-N values. The highest microbial counts were determined in control samples for all sampling days.

Conclusion

The color of the bitter orange and grapefruit peels were harmonized with common pandora. Tiny particles of albedo fragments remained after thawing, which were negligible.

Addition of citrus extracts into ice has increased its protective effect on the quality of fish. Flavedo and albedo tissues of citrus peels are good natural sources for protecting the quality of fish. The peels of bitter orange, especially the flavedo tissue is successful in retarding deterioration of fish in terms of pH, TVB-N, TMA-N and TBA values. It seems quite clear that, citrus plant extracts stabilized the rate of oxidation owing to their antioxidant activity. Citrus peels can be replaced with synthetic protective agents in industry. Use of citrus extracts will also provide utilization of by-products of citrus processing.

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