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Jag Pal

Department of Fish Processing
Technology, KVAFSU, College
of Fisheries, Mangalore-,
Karnataka, India

CV Raju

Department of Fish Processing
Technology, KVAFSU, College
of Fisheries, Mangalore-,
Karnataka, India

Lakshmisha IP

Department of Fish Processing
Technology, KVAFSU, College
of Fisheries, Mangalore-,
Karnataka, India

Arun Kumar Prakash

Department of Fish Processing
Technology, KVAFSU, College
of Fisheries, Mangalore-,
Karnataka, India

Gayatri Pandey

Department of Fish Processing
Technology, KVAFSU, College
of Fisheries, Mangalore-,
Karnataka, India

Rajkumar Ratan Kumar Singh

Department of Fish Processing
Technology, KVAFSU, College
of Fisheries, Mangalore-,
Karnataka, India

Sarojini A

Department of Fish Processing
Technology, KVAFSU, College
of Fisheries, Mangalore-,
Karnataka, India

Amitha

Department of Fish Processing
Technology, KVAFSU, College
of Fisheries, Mangalore-,
Karnataka, India

Correspondence**Jag Pal**

Department of Fish Processing
Technology, KVAFSU, College
of Fisheries, Mangalore-,
Karnataka, India

Comparatives study of antioxidant activity and total phenolic contents of pomegranate and orange peels extracts

Jag Pal, CV Raju, Lakshmisha IP, Arun Kumar Prakash, Gayatri Pandey, Rajkumar Ratan Kumar Singh, Sarojini A and Amitha

Abstract

Antioxidants were extracted from the pomegranate and orange peels by ethanol and their activity were evaluated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging, Ferric-reducing antioxidant power assay. The total phenolic compound (TPC) was determined by the Folin-Ciocalteu method to quantify the amounts of Phenolic contents. The (Pomegranate Peel Extracts) PPE exhibited higher total phenolic content (TPC) 139.40 mg/g GAE while the orange peel extracts (OPE) showed three times lower TPC of 35.73 mg/g GAE. The result of antioxidants activity of PPE showed the radical scavenging activity of 92.38% at the concentration of 35µg/mL whereas the OPE showed the radical scavenging activity 85.68 % at a higher concentration of 700 µg/mL. The results of FRAP of the PPE showed the Abs range of 0.08-1.40Abs at700nm while the results of the OPE exhibited 0.08-0.24 Abs at 700nm. The PPE showed about six times higher reducing power compared to OPE, at the concentration of 700 µg/mL and exhibited the 1.40 Abs at 700nm while at the same concentration OPE showed only 0.24 Abs at 700nm. The PPE showed a higher reducing power of 1.43 optical densities. The above results indicate that the PPE is good source of the phenolic compounds and higher antioxidant activity as compared to OPE.

Keywords: Antioxidant activity, Phenolic compound, pomegranate peel and Orange peel

Introduction

Pomegranate (*Punica granatum*) is native of Iran and cultivated in various countries like Northern India and Mediterranean and South Asian region. Since ancient time, various parts of the plant have been used for medicinal purposes for human health. Several authors have reported use of pomegranate in Ayurvedic and Unani system of medicine as a therapeutic agent for the treatment of inflammatory diseases and disorders of the digestive tract (Lansky and Newman, 2007) [10]. Oranges (*Citrus sinensis*) belong to the family "Rutaceae" it is cultivated in many parts of the world like India, UK, France etc. In Asia, oranges originated thousands of years ago, in the region from southern China to Indonesia from which they were spread to the India (Arora and Parminder, 2003) [2]. It is one of the commercial fruit crops grown in the entire world. Very large amounts of by-product are formed as wastes during the production of citrus juices (Manthey and Gorthmann, 2001) [13]. The industrial transformation of fruits and vegetables generates large quantities of fruit waste, which has become an environmental problem. They are rich source of bioactive compounds may well be suitable for several purpose (Viuda-Martos *et al.*, 2009) [19]. Citrus by-products such as peels, seeds are useful sources of phenolic compounds and flavone glucosides mainly consisting of naringin, hesperidin, narirotin, and neohesperidin. Nowadays, citrus peels are used as the main source for natural antioxidants to be used in foodstuff for preventing rancidity, fostering stability, and inhibiting lipid oxidation, so it has attracted the attention of researchers (Rehman *et al.*, 2006) [16]. Phenolic compounds are the plant metabolites that are distributed throughout the plants kingdom and contribute to their antioxidant capacity. These compounds include phenolic acids, flavonoids, and tocopherols (Wang *et al.*, 1996) [20]. The present study under taken to the utilize fruit waste by extracting the natural antioxidant that can useful for retarding the lipid oxidation.

Material and methods**Preparation of pomegranate (PPE) and orange peel extracts (OPE)**

The pomegranate and orange peels were collected from different juice vendors in Mangalore city Karnataka India.

The pulp was separated manually from the peel and washed to remove the unwanted materials. The washed peels were cut into small pieces and both peels were dried in hot air oven at 60 °C for 12 h. The dried peels were ground in the kitchen grinder to make the fine powder to pass through 1 mm sieve. The extraction was carried out according to the methods described by Iqbal *et al.* (2008) [8] with slight modification. About 25 g of peel powder was mixed with 150 mL of ethanol. The mixture of peel powder and ethanol subjected to shaking at ambient temperature for 12 h at the speed of 190 rpm. The mixture was filtered and residue was re extracted with same solvent. The filtrates of the mixture were placed under a hood in the rotavapor to remove the residual ethanol under vacuum at 40 °C. The extract was obtained from both the peels powder were weighed to calculate the yield. The both the extracts were stored at -20 °C in a sample container for further analysis.

Total phenolic contents

The concentration of phenolics in the extracts was determined by following method of Singh *et al.* 2002 [18] and results were expressed as gallic acid equivalents. 12 mg of PPE and 33 mg OPE were dissolved in a 25 and 33 mL respectively of 10 % ethanol. Exactly dissolved samples (0.2 ml) were mixed with 1.0 mL of 10-fold diluted Folin–Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 765 nm using a VIS double beam spectrophotometer. The estimation of phenolic compounds in the extracts was carried out in triplicate. The total phenolic content of the peel extracts was calculated by using standard curve prepared from gallic acid at the concentration range of 20 to 100 µg/mL. The total phenolic content of the sample was expressed as mg/g of gallic acid equivalents.

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH free radical scavenging capacity of PPE and OPE was determined by using DPPH assay according to the method described by Abdulwahab *et al.* (2011) [1] with little modifications. The 1.5 mL extracts of different concentrations were added to 1.5 mL of freshly prepared DPPH solution (0.1 mM) in methanol in to different test tubes. The control was prepared as the same without any extract. These tubes were thoroughly mixed by using cyclomixer at high speed. The tubes were kept in the dark for 30 min and their absorbance was measured at 517 nm. Radical scavenging of both extracts was compared with the BHA and Ascorbic acid at different concentrations. The changes in the absorbance of the prepared samples were measured at 517 nm. Radical scavenging activity was estimated as the inhibition percentage and was calculated using the following formula,

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

Ferric reducing power (FRAP)

The total reducing power of samples PPE and OPE was determined according to the method described by Oyaizu (1986) [15]. Different concentrations of both the extracts were prepared and 1 mL of each extracts were added with the 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6), and 2.5 mL of 1% potassium ferricyanide were added to each test tube

and incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid (to stop the reaction) was added and centrifuged at 2000g for 10 min. The upper layer in each tube (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm against a blank. The reducing power increases with the increase of absorbance. The total reducing power ability of PPE and OPE at different concentrations was compared with the Ascorbic acid and BHA as a positive control.

Results and discussion

Total phenolic compounds

The total phenols are the compounds that have a hydroxyl group associated with an aromatic ring structure. These compounds are predominantly found in the plant kingdom and inhibit lipid oxidation through their ability to scavenge free radicals and convert the resulting phenolic radicals into a low-energy form that does not further promote oxidation (Decker, 2002) [5]. They are the class of antioxidant compounds, which can act as free radical terminators (Shahidi and Wanasundara, 1992) [17]. The pomegranate peel exhibited higher total phenolic content (TPC) of 139.40 mg/g GAE while the orange peel showed three times lower TPC of 35.73 mg/g GAE. The result of PPE was more similar to the result of TPC of 132 mg/g in pomegranate peel extract reported by Kanatt *et al.* (2009) [9]. Predominantly, fruit peel contains high amounts of phenolic compounds such as punicalagins, Gallic acids, ellagic acids and anthocyanins. The result of OPE were in agreement with the result of Hegaz and Ibrahim (2012) [7] who reported higher TPC of 169.5 mg/g. These higher contents of TPC in the PPE might be due to the variation in the method of extraction followed and the species of orange and pomegranate peel used.

DPPH radical scavenging activities

The DPPH is the stable free radical. This assay has been widely used to evaluate the free radical scavenging ability of various plant extracts. In the presence of a hydrogen/electron donor (antioxidant) the absorption intensity is decreased and the radical solution from purple chromogen of DPPH radicals is discolored to a pale yellow hydrazine according to the number of electrons captured (Locatelli *et al.*, 2009) [12]. The DPPH radical scavenging activity both the samples exhibited a dose dependent activity. The DPPH radical scavenging activity significantly ($p < 0.05$) increased with increase in the concentration of extract. The DPPH radical scavenging activity of both the extracts showed at different concentration range i.e. 5 µg/mL to 35 µg/mL in PPE while OPE showed at the concentration range of 100 µg/mL to 700 µg/mL (Fig. 1). The PPE extract showed 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. The OPE showed the radical scavenging activity 85.68 % at a higher concentration of 700 µg/mL. The PPE exhibited strong radical scavenging activity as compared to OPE. Similar results were reported by Okonogi *et al.* (2007) [14] that, among the eight fruit peel extracts; pomegranate peel had the highest DPPH radical scavenging activity. The higher radical scavenging activity of PPE could be due to its high amount of phenolic compounds, such as punicalagins, gallic and ellagic acids and anthocyanins as stated by Bingham *et al.* (2003) [4] and Fischer *et al.* (2011) [6].

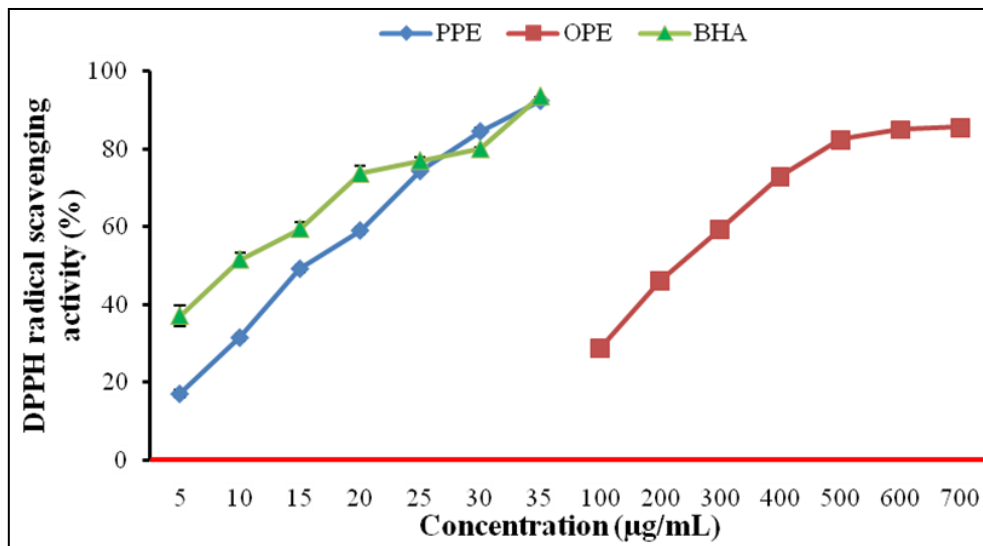


Fig 1: DPPH radical scavenging activity of PPE, BHA and OPE

Ferric-reducing antioxidant power assay

The FRAP assay measures the capability of any antioxidants to reduce yellow ferric 2, 4, 6- tripyridyl-s-triazine complex $[\text{Fe}^{3+}-(\text{TPTZ})_2]^{3+}$ to the blue-colored ferrous complex $[\text{Fe}^{2+}-(\text{TPTZ})_2]^{2+}$ by electron donation, that is, single electron transfer mode (Benzie and Strain 1996) [3]. The reducing powers of both the extracts were assessed in the concentration range of 0 to 700 $\mu\text{g/mL}$. The FRAP value significantly ($p < 0.05$) increased with increase in concentration of the extract. The results of the PPE exhibited the Abs range of 0.08-1.40 Abs at 700nm while the results of the OPE exhibited 0.08-0.24 Abs at 700nm (Fig. 2). The PPE showed about six times higher reducing power compared to OPE, at the concentration of 700 $\mu\text{g/mL}$ and exhibited the 1.40 Abs at 700nm while at the same concentration OPE showed only 0.24 Abs at 700nm. The PPE showed a higher reducing power of 1.43 optical densities. This high reducing power of PPE extract might be due to the response of some phytochemicals like ellagitannins, punicalin punicalagin and numerous piperidine alkaloids contained in PPE. The present investigation is also common with finding of Li *et al.* (2006) [11] reported that, the FRAP value of the pomegranate peel extract was much stronger than the pulp extract. The results of FRAP indicated that, the peel extract has more potential antioxidant activity compared to the seed extracts.

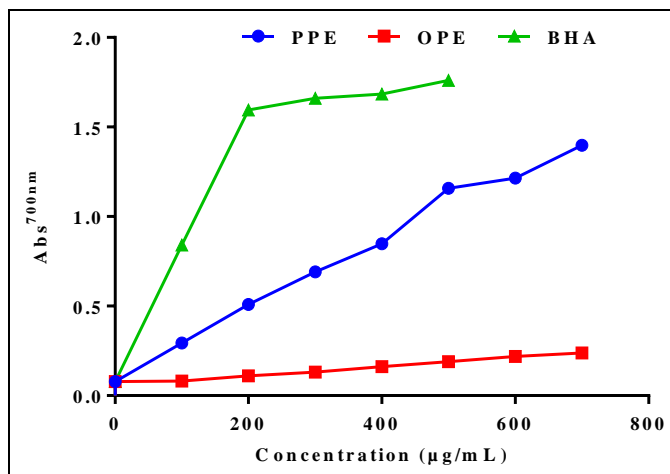


Fig 2: Ferric Reducing Antioxidant Power of PPE and OPE

Conclusion

The above results conclude that the both extracts showed antioxidant activity and phenolic compounds. This comparative study of pomegranate and orange peel fruit peel extracts indicated the presence of phenolic compounds with possible commercial applications like controlling lipid oxidation and enhancing the shelf life of food products.

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