Antioxidants From Pomegranate Peels

Enrichment of Commercially-Prepared Juice With Pomegranate (*Punica granatum* L.) Peel Extract as a Source of Antioxidants

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Abstract

Ready-to eat foods meet the demands of a modern lifestyle and the number of people seeking food that is convenient and safe is increasing. The extracts of peels from four different fruits were tested as potential value-added foods to offer to consumers. Physical and chemical analyses of the peel extracts were conducted to measure total phenolic compounds, tannins, phytic acid and antioxidant activity using the 1'-1'Diphenyl-2'picrylhydrazyl, and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid methods. The result of screening the antioxidant activity showed that the pomegranate peel had higher activity than the other peels (p<0.05). In addition, flavonoids and vitamin C were measured in the pomegranate peel, and low amounts of these components were found. The pomegranate peel had a high amount of phenolic compounds and high levels of antioxidants, and this peel was used to enrich a commercially-available juice. Furthermore, the sensory evaluation showed no difference between the control and enriched juice. The product was well accepted and feasible from a technological standpoint. Because the waste is rich in bioactive compounds, value is added to the final product, as these antioxidant compounds are known to protect health and improve the quality of life of the consumers.

Keywords: antioxidant; pomegranate, peel, enriched juice; sensorial evaluation

Abbreviations:

ABTS - 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ANOVA - Analysis of Variance Technique DEE - Dried Ethanol Extract DHAA - Dehydroascorbic Acid DPPH - 1'-1'Diphenyl-2'picrylhydrazyl FB – Fresh Basis FD – Dried Basis FRAP - Ferric ion reducing antioxidant power CEAGESP - General Warehouse Company of São Paulo LDL - Low-Density Lipoprotein) PA - Apple Peels PG - Grape Peels PM - Moriche Palm Peels PP - Pomegranate Peels

1. Introduction

Brazil is a major producer of fruits, both in quantity and in diversity. In Brazil, excellent quality fruits are produced to supply the market continuously in all seasons.

Fruit peels are byproducts of the juice processing industry and are excellent sources of antioxidants. The extracts of these peels (wastes) may be used in the form of food additives. In fact, the vegetable peels are richer in bioactive compounds than the pulp (Guimarães et al., 2010). Although the peels are a byproduct and considered waste, they are sources of various chemical compounds with biological activities, such as antioxidants. Antioxidants present in fruits have a beneficial effect because of their ability to sequester free radicals (Vinson et al., 2001).

The natural compounds with antioxidant activities include phenolic acids and their derivatives, such as flavonoids, tocopherols, phospholipids, amino acids, phytic acid, ascorbic acid, and pigments (Jayaprakasha & Jaganmohan, 2000). The phenolic compounds present in plants are defined as secondary metabolites, derived from the shikimic acid pathway and phenylpropanoid. The main groups are phenolic flavonoids, phenolic acids, and polyphenols (tannins) (Singh et al., 2002).

Antioxidants prevent the oxidation of LDL (low-density lipoprotein) cholesterol, thus delaying the onset and/or progression of diseases such as atherosclerosis, stroke, immune disorders, and inflammatory processes, such as platelet adhesion (Jayaprakasha & Jaganmohan, 2000). Functional foods have nutritional properties with inherent health benefits. Regular consumption of these foods reduces the risk of chronic diseases, including cardiovascular disease, cancer and diabetes (Roberfroid, 2007).

Parts of fruits such as the Moriche palm (*Mauritia flexuosa* L. f.), pomegranate (*Punica granatum* L.), apple (*Malus domestica Borkh*) and grape (*Vitis vinifera* L.) that are not typically consumed (such as the peels) have great antioxidant potential; these parts represent waste to the agribusiness sector, even though some agro-industrial residues are noteworthy, such as that from apples and grapes, because they are rich sources of phenolic compounds with strong antioxidant potential (Dimitrios, 2006; Ruberto et al., 2007).

This study aimed to evaluate the antioxidant activity of the peels of the Moriche palm (*Mauritia flexuosa* L. f.), pomegranates (*Punica granatum* L.), apples (*Malus domestica Borkh*) and grapes (*Vitis vinifera* L.) and to evaluate a juice enriched with the peel extract that which demonstrated the highest antioxidant activity (Johanningsmeier & Harris, 2011; Salgado, Ferreira, Biazotto, & Dias, 2012).

2. Materials and Methods

2.1 Fruit Collection and Samples Preparation

Peels of fresh grapes (*Vitis vinifera* L., dark purple Niagara variety), pomegranates (*Punica granatum* L.), apples (*Malus domestica Borkh*), Moriche palms (*Mauritia flexuosa* L. f.) and guava juice. were used. Fresh fruits were purchased from the General Warehouse Company of São Paulo (CEAGESP) in São Paulo, Brazil. The fruits were transported and kept under refridgeration at 4 °C. The fresh fruits were manually selected, washed for 15 minutes in a solution of 200 ppm sodium hypochlorite and rinsed with distilled water and weighed (FB). The process began with a manual separation of the peels, which were then placed in stainless steel trays, frozen in an Ultrafreezer at -80 °C, and dried by lyophilization for 24 hours (using equipment from Modulyo and EC Apparatus Corp.). After drying, the samples were weighed, ground in a knife mill (*Marconi*® MA475) and stored in plastic polyethylene under refrigeration at 4 °C (samples in FD). The peels of four fruits used were abbreviated as PM (Moriche palm peels), PA (apple peels), PP (pomegranate peels) and PG (grape peels). This dried ethanol extract (DEE) was used in the enrichment of a commercially-avaiable fruit juice.

Each sample of dried peel was weighed and then dissolved in ethanol-water (80:20) at a concentration of 1 g/20 mL. The solvent is polar and able to dissolve the compounds studied. The extraction was conducted in a shaker, at room temperature, for 15 minutes, and the extract was centrifuged at 5000 rpm (model NT 825, Novatecnica). The physical and chemical properties of the four different peels were analyzed using the supernatant.

2.2 Chemical Analysis

The following reagents were used: 1'-1'Diphenyl-2'picrylhydrazyl (DPPH), ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid), Trolox, Folin-Ciocalteu reagent, ascorbic acid, catechin, gallic acid, aluminum nitrate, potassium acetate, quercetin and 2,6-dichlorophenolindophenol were purchased from Sigma Aldrich (Germany). Other reagents obtained from Sigma Aldrich Germany, were of analytical grade. Analyses were performed in quadruplicate. All reagents used were of high purity.

Physical and chemical analyses performed on the samples of PM, PA, PP and PG and included measurements of tannins, phytic acid, total phenolic compounds and the antioxidant capacity for scavenging free radicals using DPPH and ABTS methods. PP was determined to be the peel with the highest antioxidant activity and thus the PP was further analyzed for flavonoids and vitamin C.

2.3 Determination of Tannins and Phytic Acid

Tannins were quantified using a method described previously by Price, Hagerman, and Butler (1980). The samples were read at at 500 nm. A catechin standard curve was used, and the results were expressed as mg of catechin equivalent per gram of freeze-dried peel. Phytic acid was performed according to a method previously described by Grynspan and Cheryan (1989). The absorbance reading was in a spectrophotometer at 500 nm. The values were expressed as mg of phytic acid equivalent per gram of freeze-dried peel.

2.4 Content of Total Phenolics

The analysis of the content of total phenolic compounds in the samples was conducted using the method using a Folin-Ciocalteu reagent, and gallic acid was used as the standard (Swain & Hillis, 1959). The absorbance was measured at 660 nm in a spectrophotometer (model 2800 Unico ® UV/VIS, Interprise Brazil

2.5 Screening of Antioxidant Activity of the PM, PA, PP and PG

The antioxidant activity of the samples (peels) was determined by analyzing the ability to scavenge the free radical DPPH (Brand-Williams, Cuvelier, & Berset, 1995). The absorbance was read in a spectrophotometer (model 2800 Unico ® UV/VIS, Interprise Brazil) at 517 nm. The values were expressed as mg of Trolox equivalent per gram of freeze-dried peel. The antioxidant activity was also determined using the ABTS method (Re et al., 1999). The absorbance was read in a spectrophotometer (model 2800 Unico ® UV/VIS, Interprise Brazil) at 517 nm. The values were expressed as mg of Trolox equivalent per gram of freeze-dried peel. The antioxidant activity was also determined using the ABTS method (Re et al., 1999). The absorbance was read in a spectrophotometer (model 2800 Unico ® UV/VIS, Interprise Brazil) at 734 nm using ethanol as the blank. The results of the antioxidant activity assay were expressed as mg of Trolox equivalent per gram of the freeze-dried peel.

2.6 Determination of Flavonoids and Ascorbic Acid

The quantification of flavonoids was determined as previously described (Jurd & Geissman, 1956). The values were expressed as mg of quercetin equivalent per gram of freeze-dried peel. The determination of ascorbic acid was performed as described (Benassi & Antunes, 1988). Vitamin C used as standard. The values were expressed as mg ascorbic equivalent /gram of freeze-dried peel.

2.7 Enrichment of Commercially-Prepared Juice

Enrichment was carried out with commercially-prepared guava juice in tetrapack packaging (1 L). The juice was enriched with the DEE-PP; the decision to enrich the juice with the pomegranate rind was determined after the antioxidant activity assay had been performed on the PM, PA, PP and PG samples. The following percentages were used for the pre-enrichment of the commercially-prepared juice: 0.0% (control), 0.3, 0.5 and 0.7%. Concentrations above these values compromised organoleptic properties of the enriched juice.

2.8 Antioxidant Activity of the Commercially-Prepared Juice Enriched With Different Concentrations of DEE-PP

The antioxidant activity of juice enriched with different concentrations was determined by DPPH free radical sequestration using the methodology described previously (Brand-Williams et al., 1995).

2.9 Test of the Acceptability of Guava Juice Fortified With DEE-PP

The acceptability of the samples of guava juice alone and that enriched with different concentrations of DEE-PP was tested at the Laboratory of Bromatology in the Department of Agribusiness, Food and Nutrition, ESALQ/USP. The test used a hedonic scale with nine points, ranging from "1" (disliked extremely) to "9" (liked extremely). The attributes evaluated were taste, aroma and overall appearance. The participants were 56 randomly selected untrained subjects of both sexes who reported no health problems that could interfere with their sensory organs. Approximately 10 ml of juice from each of the formulations (0.0, 0.3, 0.5 and 0.7%) of DEE-PP was given to each participant. Samples (triplicates) of the juice enriched with different concentrations (0.0, 0.3, 0.5 and 0.7%) were analyzed for microbes before the acceptability test to ensure the safety of the sample for consumption. The acceptability tests followed the regulations of the Research Ethics Committee of the "Luiz de Queiroz" Higher School of Agriculture/USP (Piracicaba/SP-Brazil) following analysis and approval by this committee.

3. Statistical Analysis

The data are reported as the averages of four replicates, which were analyzed using the analysis of variance technique (ANOVA) and Tukey's studentized range test (statistical significance was determined at p < 0.05). All analyses were carried out using the Statistical Analysis System software (SAS v.9.2- Institute Inc., Cary, NC). The statistical analysis of sensory analysis results relied on a randomized trial with 4 treatments (4 juices with different dried pomegranate extract concentrations).

4. Results and Discussion

4.1 Tannins, Phytic Acid, Phenolic Compound Content and Antioxidant Activity

The highest value of tannins was found in the fresh PM samples (17.57 mg g^{-1}), followed by the PP, PA and PG values, which were statistically equal. The PP contained the highest value of phytic acid among the four peels (14.71 mg of catechin equivalent per gram of freeze-dried peel). This value is similar to the value of 13 mg ml⁻¹ found in pomegranate juice obtained from the whole fruit (pulp, seeds and rind) (González-Molina, Moreno, & García-Viguera, 2009). The other peels contained values of approximately 1.5 mg (Table 1).

Table 1. Tannins and phytic acid of dried peels of Moriche palm (PM), apples (PA), pomegranates (PP) and grapes (PG)

Tannins ¹		Phytic Acid ²		
Samples	* FD	FB **	* FD	FB **
PM	$148.5 \pm 7.55a$	$17.57 \pm 0.90a$	$1.6 \pm 0.53b$	$0.19 \pm 0.06b$
PA	$42 \pm 1.73c$	$9.75\pm0.41b$	$1.38 \pm 0.60b$	$0.32\pm0.14b$
PP	$38.25 \pm 2.87c$	$9.88\pm0.71b$	$14.71 \pm 0.73a$	$3.82\pm0.19a$
PG	$55.75 \pm 1.89b$	$8.75\pm0.32b$	$1.54\pm0.33b$	$0.24\pm0.05b$

¹ expressed as mg catechin equivalent per gram of freeze-dried peel

² expressed as mg of phytic acid equivalent per gram of freeze-dried peel

* FD - value of dehydrated and freeze-dried sample expressed on a dry basis

** FB - value of freeze-dried and dried sample, expressed relative to the fresh basis

The results are presented as the mean \pm standard deviation.

Different letters indicate vertical significant differences between treatments (p<0.05).

Some studies have shown the tannin content to be low, in the range of 0.5 to 5.4 mg catechin equivalent g^{-1} sample examined (Ross, Zhang, & Arntfield, 2010). Oomah et al. (2011) found values in the range of 5.9 to 15.1 g of sodium phytate equivalent, although different methodologies were used in these studies. The value corresponding to PP was also similar to the average content of phytic acid found in traditional beans (Oomah et al., 2011). This suggests that the use of pomegranate peel as studied in our research is viable human consumption, given its level tannins and phytic acid compounds.

	Phenolic compounds ¹		DPPH ²		ABTS ²	
Peel	* FD	FB **	* FD	FB **	* FD	FB **
PM	$4.75\pm0.5b$	$0.55\pm0.4b$	$24\pm0.0b$	$2.88\pm0.03b$	$62.67 \pm 1.53b$	$16.3 \pm 0.41b$
PA	$3.67\pm0.58b$	$0.83 \pm 0.08b$	18.67 ± 2.52b	$4.26\pm0.63b$	$30.67 \pm 1.15b$	$8.02\pm0.30b$
РР	$36 \pm 3.46a$	$9.35\pm0.9a$	644 ± 27.22a	$167.14 \pm 7.05a$	1219 ± 117.81a	$316.46 \pm 30.56a$
PG	$3.25\pm0.5b$	$0.51\pm0.07b$	$20 \pm 2.16b$	$3.11\pm0.33b$	$44\pm2.65b$	$11.37\pm0.62b$

Table 2. Total phenolic content and antioxidant activity assayed using the DPPH and ABTS free radical scavenging ability methods

¹ expressed in mg gallic acid equivalent per gram of freeze-dried peel

² expressed in mg of the Trolox equivalent per gram of freeze-dried peel

* FD - value of dehydrated and freeze-dried sample expressed on a dry basis

** FB - value of freeze-dried and dehydrated sample, expressed relative to the fresh basis

The results are presented as the mean \pm standard deviation. Different letters in the vertical columns indicate significant differences between treatments at a 5% significance level.

Analysis of total phenolic content showed values from 0.6 to 0.8 for PM, PA and PG, and 9 for PP expressed in mg gallic acid equivalent per gram of freeze-dried peel. The value found for PP was 36 mg, while for others this value was, on average, 4 mg (Table 2). Although the levels of phenolic compounds found in the peels analyzed in this study were lower than those reported in another study (Guimarães et al., 2010) that found values of 55.88 for grapefruit peel, 87.77 for lemon peel, 124.63 for lime peel, and 79.75 for orange peel, it is noteworthy that there are differences in the samples (peels were used in this study versus extracts in the previous study). A study of pomegranates found values of phenolic compounds in the juice of 243.89 mg gallic acid equivalent to 100 ml⁻¹ (González-Molina et al., 2009), whereas in our study, the value was 9.35 mg g⁻¹ for freeze-dried peel. The difference can be explained by variations in the extraction methods employed, solvents used, and the variety and seasonality of the samples studied.

The values for antioxidant activity determined using the DPPH free radical scavenging method for the PM, PA and PG were, on average, equivalent to 20 mg of Trolox g^{-1} peel, and there were no significant differences among the samples. The antioxidant activity of the PP was thirty times greater than the other peels (on a dry basis). Using the ABTS method, this trend continued as the PP showed a high antioxidant activity (1,219 mg equivalent of Trolox g^{-1} PP) that was different from the others (Table 2).

The high antioxidant activity of pomegranate peel measured by the ABTS and FRAP (Ferric ion reducing antioxidant power) methods correlates with the content of phenolic compounds present in the samples of the pomegranate (peel, mesocarp and arils) (Fischer, Carle, & Kammerer, 2011), a trend also observed in this study. The content of total phenolic compounds in the pomegranate peel was higher than that of its juice, demonstrating that, as in most vegetables consumed, the fraction with a higher plant bioactivity is discarded rather than consumed.

The predominant polyphenols in pomegranate peels are hydrolysable tannins (262.7 mg g⁻¹ of tannic acid equivalent), among which stands out punicalagin, suggesting that this compound is the main bioactive compound present in pomegranates (Çam & Hişil, 2010). For comparison, the antioxidant activity of PP in this study was superior to that found for the noni fruit (*Morinda citrifolia* L.), widely recognized for its antioxidant properties (Yang et al., 2010).

The values for phenolic compounds were between 3 and 4 for PM, PA and PG and 36 for PP on a dry basis. The values for antioxidant activity assessed by the DPPH free radical scavenging method were between 19 and 24 (PM, PA and PG) and 644 (PP), all on a dry basis (mg equivalent of Trolox per gram of freeze-dried peel, Table 2). Based on the results obtained in this stage of analysis, PP was selected to enrich the guava juice samples because of its high antioxidant power. Thus, the bioactive composition was further investigated by quantifying the total content of flavonoids and vitamin C, as these compounds are related to the antioxidant activity found in fruits (Yang, Gadi, Paulino, & Thomson, 2010).

4.2 Content of Flavonoids and Vitamin C From Freeze-Dried Pomegranate Peels (PP)

At this stage of the study, only pomegranate peel (*Punica granatum* L.) was studied because of its high antioxidant power. The value found in this study for the content of flavonoids as may be observed in Table 3.

Table 3. Fla	vonoids and	vitamin C	content from	1 the pom	legranate pee	l
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	Flavonoids	Vitamin C	
Sample	$FB ** (mg / g)^1$	FB ** $(mg / g)^2$	
РР	5.85 ± 0.02	0.21 ± 0.01	

¹ expressed in mg quercetin equivalent per gram of freeze-dried peel

² expressed in mg of ascorbic acid equivalent per gram of freeze-dried peel

** FB - value of freeze-dried and dried sample, expressed relative to the fresh basis

The results are presented as the mean \pm *standard deviation.*

Regarding the composition of flavonoids, a study by Guimarães et al. (2010) found values in the peels (polar fraction) of grapefruit, lemon, lime and orange of 2.29, 15.96, 13.61 and 3.97 mg catechin equivalent g^{-1} of the extract, respectively. Although the value for PP of 5.85 mg/g is whithin the range mentioned above, however, the methodology and type of peel used for determination of flavonoids was different (Jia, Tang, & Wu, 1999). The value found for the content of ascorbic acid, expressed in mg ascorbic acid equivalent per gram of freeze-dried peel, was 0.21 in fresh samples (Table 3). This is low compared with a study by González-Molina et al. (2009) which found no significant levels of vitamin C for pomegranate juice, which had less than 6 mg dehydroascorbic acid (DHAA) 100 ml⁻¹ compared to 36.14 mg 100 ml⁻¹ in lemon juice, which has been shown to have a high content of vitamin C (González-Molina et al., 2009; Del et al., 2004). A study on citrus fruits evaluated the ascorbic acid content of the peels (polar fraction) and found values in the range of 0.823 to 1.780 mg of ascorbic acid equivalent to g^{-1} extract. The difference may be due to method used in this study (Jayaprakasha & Jaganmohan, 2000).

4.3 Analysis of the Antioxidant Activity of Commercially-Prepared Juice Enriched With an Ethanol Extract of Dried Pomegranate Peel (DEE-PP)

A direct relationship was observed between the enrichment of guava juice with DEE-PP and an increase in antioxidant activity (Table 4). The lowest concentration tested (0.3%), however, did not show antioxidant activity that was significantly different from the control (0%). This result indicates that for the enrichment of guava juice with antioxidant properties, concentrations above 0.3% DEE-PP should be used. A study using the pulp of a pomegranate extract identified the enzyme chitinase (acidic endochitinase class III), which is thermostable at a pH between 3 and 9 and a temperature of 65 °C as responsible; an understanding of these properties aids in industrial processes and the development of biotechnology (Kopparapu, Liu, Yan, Jiang, & Zhang, 2011).

Concentration of PP-DEE (%)	Antioxidant activity Trolox eq mg ml ⁻¹ of enriched juice
0.00	$0.02 \pm 0.01c$
0.30	$0.29 \pm 0.00c$
0.50	$1.85 \pm 0.08b$
0.70	$2.28 \pm 0.11a$

Table 4. The antioxidant activity (DPPH method) of PP-DEE commercially-prepared guava juice enriched with different concentrations

The results are presented as the mean \pm *standard deviation.*

Different letters indicate significant differences between treatments at a 5% significance level.

4.4 Test Acceptability of Commercially-Prepared Guava Juice Fortified With DEE-PP

Acceptability tests were performed to evaluate the sensory characteristics of the enriched juice. There was no significant effect (p > 0.05) of the addition of DEE-PP to commercially-prepared guava juice on three sensory attributes (i.e., taste, aroma and overall appearance) and to measure the intention to purchase the product (Table 5).

Table 5. Mean (\pm standard deviation) of flavor, aroma, overall appearance and purchase intent of guava juice fortified with PP-DEE

PP-DEE concentration (%)	Flavor	Aroma	Global Aspect	Purchase Intent
0	7.46 ± 1.56a	$7.80 \pm 1.08a$	$7.60 \pm 1.27a$	$4.16 \pm 1.15a$
0.3	$7.51 \pm 1.29a$	$7.55 \pm 1.30a$	$7.35 \pm 1.55a$	$4.13\pm0.98a$
0.5	$7.33 \pm 1.45a$	$7.40 \pm 1.47a$	$7.45 \pm 1.46a$	$4.00\pm0.98a$
0.7	$7.38 \pm 1.48a$	$7.66 \pm 1.27a$	$7.58 \pm 1.18a$	$4.11 \pm 1.47a$

Means followed by the same letter do not differ according to the Tukey test (p < 0.05)*.*

The addition of the extracts in the three concentrations studied did not cause a reduction in product acceptance in relation to the attribute of flavor. The average of the four treatments showed values between 7 (like moderately) and 8 (really liked), indicating good acceptance by the tasters. In addition, treatment with the addition of 0.3% DEE-PP showed an average of 7.51 acceptance, higher than that recorded for the control treatment (without any extract) (Table 5).

Tannins are known to have an astringent taste (Wollgast & Anklan, 2000). In this study, although the PP had a tannin content of 9.88 mg of catechin equivalent per gram of freeze-dried peel, the addition of the DEE-PP to the commercially-prepared guava juice ready did not significantly affect the taste of the product.

With regard to the attribute of aroma and overall appearance, the testers again found no significant difference (p > 0.05) between treatments with the addition of DEE-PP and the control. Among the samples with the added extract, the treatment with the highest concentration (0.7%) had the highest averages of 7.66 and 7.58 for aroma and overall appearance, respectively. When asked about their intention to purchase the product, the response mean was between the terms "probably buy" and "definitely would buy". The commercially-prepared enriched guava juice was well accepted by the consumers, with average scores above 7 ("like moderately") for all treatments (Table 5).

The acceptance test results indicated that the enrichment of commercially-prepared guava juice with DEE-PP did not affect the sensory parameters of the product compared to a control sample (Table 5).

5. Conclusions

Pomegranate peel had a high amount of phytic acid and higher values for phenolic compounds and antioxidant activity. Conversely, this peel had low levels of flavonoids and vitamin C. The juice enriched with pomegranate peel extract showed an increase in antioxidant activity. The enrichment of commercially-prepared guava juice did not alter any of the sensory analyses.

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