Antioxidant activity and physicochemical properties of fresh, dried and infused herbal extract of Feijoa Fruit

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Abstract: In the present study fresh, dried and herbal infusion of feijo fruit (Feijoa sellowiana) were compared to their total phenol content, reducing power, DPPH and total antioxidant activity as well as different physicochemical characteristics. The study reveals that the physical characteristics of fruit, that is, color, texture and density were significantly affected by hot air drying. The chemical parameters of fruit determined, total soluble solid (TSS), acidity, pH, moisture, ash content, carbohydrate, protein, fat, ascorbic acid, total phenol, flavonoids and iodine content also evaluated as chemical characteristics. The results showed that the chemical and physicochemical characteristics are little affected by drying so with the various nutritional benefits, the fresh and dried fruits could be recommended for commercial exploitation and preparation of different value added products. Fresh fruit were found to be a good source of antioxidants and had the highest total phenol content, reducing power, DPPH and total antioxidant activity. A significant ($p< 0.05$) amount of antioxidants was lost after sun-drying of feijoa, whereas the herbal infusion of feijoa had the same total antioxidant activity with fresh fruit. These results suggest that all feijoa types serve as a good source of natural antioxidants and could potentially be considered as a functional food or functional food.
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1. Introduction

The feijoa (*Acca sellowiana*) also known as the Pineapple Guava and Guavasteen is an evergreen bush or small tree that originates from the highlands regions of South America but nowadays is widely distributed and cultivated in many countries. The fruit of feijoa has a smooth and soft green skin, the flesh is juicy and is divided into a clear, gelatinous seed pulp and a firmer, slightly granular, opaque flesh nearer the skin. Additionally, approximately thirty edible seeds are present within the flesh. Feijoa fruit usually picked fully mature, but not fully ripe and before falling to the ground, in order to assure good appearance and flavor. Many volatile compounds, including terpenes, tannins, quinones, steroidal saponins, flavonoids and both methyl and ethyl benzoate, which account for approximately 90% of the volatile fraction, are responsible for the strong feijoa-like character of the fruit (1-4). The fruits are rich in vitamin C, polyphenols, terpenes, tannins, steroidal, saponins, flavonoids hydrocarbons, minerals, iodine and both methyl and ethyl benzoate (4, 5). The optimum storage temperature and relative humidity for feijoa is 5 ± 1°C (41 ± 2°F), RH 90% to 95% and the fruit has the potential to be stored for four to five weeks depending on the cultivar and ripeness stage (6). Feijoa has shown potent antimicrobial and antifungal activities and anti-cancer activities so used for medical and pharmaceutical purposes (7-10) Moreover, an antioxidant activity of feijoa plant has been described (Vuotto et al., 2000). According to (11), plant-derived antioxidants are molecules, which donate electrons or hydrogen atoms. These compounds are able to form less reactive antioxidant-derived radicals, which are efficiently quenched by other electron or hydrogen sources to prevent cellular damage therefore, they help delay and inhibit lipid oxidation, protect human cells against oxidative damage, leading to a reduced risk of several oxidative-stress associated degenerative diseases, such as cancer, cardiovascular or neurodegenerative diseases (12) and when added to foods tend to minimize rancidity, retard the formation of toxic oxidation products, help to maintain the nutritional quality and increase their shelf life (13). Since there are few studies on the antioxidant activity of feijoas, the general objective of this study was to evaluate antioxidant activity tests such as, the total antioxidant activity of fresh, dried and traditionally consumed (aqueous) herbal infusions, instead of plant extracts.

There has also been a major effort to commercialize the feijoa. Both domestic and imported fruit can often be found in the markets, but the demand does not seem to be great due to of lack of clear information regarding nutritional and other characteristic of feijoa fruit. The review of literature showed that very limited studies have been conducted using this fruit in terms of physical and chemical characteristics (18, 14, 10, and 6). In view of this, comparison of antioxidant and physicochemical methods of fresh, died and infusion of this fruit is carried out in this work.

2. Materials and methods

2-1-Materials

Feijoa fruits were collected from botanical gardens of experimental institute in Ramsar, Mazandaran province, Iran. All the chemicals and solvents used in this study were supplied by Sigma, Merck and Aplichem.

2-2- Sample preparation

Fresh fruit peels were coarsely grounded before extraction. A known amount of each part was extracted at room temperature, using the by percolation method, with methanol and water; methanol/ water (80:20, 400 mL × 3 times) as the extraction solvent. The resulting extract was concentrated in a rotary vacuum, until a crude solid extract was obtained. The extract was freeze-dried for complete solvent removal. Air dried feijoa was conducted in an oven (DK63, Yamato, Japan) at 80 °C for 2 h and then shifted to 60 °C for 6 h. the products were then ground to powder and extraction process was done as above. to prepare infusions, the friut (1.5 g) was mixed with 100 ml of boiling water for 5 min, with constant shaking and the samples were then filtered through Whatman No. 1 filter paper and freeze-dried for further experiments.

2-3-antioxidant activity

2-3-1- Total phenol content

Determination of total phenolic content in was carried out using a Folin-Ciocalteau colorimetric method, calibrating against gallic acid as the reference standard and expressing the results as gallic acid equivalents (GAE) using the following linear equation based on the calibration curve: (19).

$A = 0.023\ C + 0:109, \quad R^2 = 0.99$ [1]

Where A is absorbance at 760 nm and C is concentrations of gallic acid equivalents (μg/ml).

2-3-2- Reducing power

The ability of extracts to reduce iron (III) was evaluated using the method of Yildirim *et al* (20) Samples (1 ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10 g l^{-1}) and incubated for 30 min at 50 °C. Then 2.5 ml of trichloroacetic acid (100 g l^{-1}) were added to the solution and centrifuged for 10 min. Finally, 2.5 ml of supernatant was combined with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1 g l^{-1}). The absorbance of samples was measured at 700 nm. Higher absorbance means higher reducing power.

2-3-3- DPPH radical scavenging activity

The radical scavenging ability was determined as described by Mensor *et al* with slight modification(21). Briefly, one ml from 0.3 mM alcohol solution of DPPH was added to 2.5 ml from the samples with. The samples were kept at room temperature in the dark and after 30 min the absorbance of each sample was measured at 517 nm and the percentage of scavenging activity calculated from the following equation:

$$DPPH\ scavenging\ activity\ (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

2-3-4- Total antioxidant capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (22). The assay was done using the method of Prieto et al. (1999). 0.1 ml of sample was mixed with 1 ml of reaction solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 m Mammonium molybdate) and incubated for 90 min at 95 °C and the absorbance of samples was measured at 695 nm.

2-4-Chemical characteristics fresh, dried and infusion of feijoa

Analysis of the moisture, pH, ash, titratable acidity و protein, total fat, crude fiber and carbohydrate contents were carried out according to the Association of Official Analytical Chemist (AOAC) (2003).

2-4-1-Determination of ascorbic acid

The ascorbic acid (vitamin C) content of the fruit was determined following the method of Malik and Singh (2005) with some modifications(23, 24). The ascorbic acid concentration was calculated against a 100% (w/v) ascorbic acid standard curve and was expressed as mg as milligrams of ascorbic acid (AA) per 100 g of fresh weight (FW).

2-4-2-Determination of iodine

Iodine was determined by the method of (25). The principle involves alkaline incineration of the sample at 600°C to remove all organic material, followed by determination of iodine by measuring the rate of catalytic destruction of iron thiocyanate by nitrite in the presence of iodine

2-5-Physical characteristics

The fruit was randomly selected for physical measurement which includes the fruit weight, with an electronic balance with 0.01 g sensitivity; the fruit length and diameter, using a digital caliber with 0.1-mm sensitivity; the fruit transverse and longitudinal shape; skin and flesh color using a Minolta colorimeter (CR5, Minolta Camera Co., Japan) determining the chromaticity values L* (Lightness), a* (green to red) and b* (blue to yellow); total soluble solids content (TSS, ◦Brix) by digital refractometer (Sinergica Soluzioni, DBR35, Pescara, Italy); percent edible portion; percent waste; percent of juice, Texture profile analysis (TPA) was performed using a Texture Analyzer (model TA.XT. Plus). The texture profile analysis was carried out by two compression cycles between parallel plates performed on cylindrical samples (diameter 10 mm, height 3 mm) using a flat 75 mm diameter plunger, with a 5 s of time between cycles. The parameters that have been used were the following: 5 kg force load cell and 0.5 mm s−1 test speed. All the measurements were done in triplicate and the average of the reading was reported.

2-6-Statistical Analysis

All data are reported as mean±standard deviation of three replicates. One-way analysis of variance (ANOVA) was used to compare the means of all evaluated parameters. Differences were considered significant at P<0.05. Calculations were done by SAS 9.1.3 Portable software.

3. Results and discussion

3-1- Antioxidant activity

Phenols are important components due to their hydroxyl groups and scavenging properties and may have a direct relation with antioxidant activity(26). according to Table 1 a significant (P<0.05) difference between total phenol contents of these three extracts were observed. The highest amount of total phenols was given by fresh fruit, dried fruit the least total phenolic content. This result concurred with the reports of Rhim et al. who reported that most drying methods have an undesired effect on antioxidant activity(27). Shahidi and Naczk also reported that drying, in general, is regarded as unfavorable due to the possibility of inducing oxidative decomposition either enzymatically by polyphenol oxidase and glycosidase or by thermal degradation of phenolic compounds (28).

Table 1- Content of total phenolics (by the Folin–Ciocalteu method), l Reducing power, DPPH and total antioxidant capacity of feijoa (fresh, dried and infusion) (means±SD).

	Total phenol content[1]	Reducing power[2]	DPPH (%)	Total antioxidant activity[3]
Fresh fruit	76.15±4.20[a]	0.91±0.34[a]	83.87±0.12[a]	0.83±0.23[a]
Dried fruit	46.76±0.91[b]	0.79±1.3[b]	82.88±0.33[a]	0.68±0.11[b]
Infusion extract	59.74±0.20[c]	0.73±2.0[b]	81.39±1.82[a]	0.79±0.22[a]

Values in the same column followed by different letters are significantly different (P<0.05)

1. g of gallic acid per 100 g (dry weight) of extract
2. absorbance at 700 nm
3. absorbance at 695 nm

Reducing properties are generally related to the ability of reductants to donate a hydrogen atom and thereby break a radical chain. Furthermore, reductants react with peroxide precursors and prevent the formation of peroxides (26). Thus, samples with higher reducing powers are more able to donate electrons. Fresh fruit extract showed significantly (P<0.05) higher reducing power (Table 1). This result was in agreement with some previous studies (29-34).

DPPH scavenging activity assay is widely used to evaluate the ability of compounds to scavenge free radicals or donate hydrogen, and determine the antioxidant activity in foods (26). There were no significant differences between scavenging activity of all treatments.

Extracts with higher electron donating activity can terminate the radical chain and turn free radicals into more stable products (Pan et al. 2011). The extracts in the present study were shown to have considerable amounts of phenolic compounds that can donate electrons. However, the highest antioxidant activity was found for extracts from both fresh and infusion on feijoa.

3-2- Physical characteristics of feijoa fruit

Physical characteristics of fresh feijoa which include weight, diameter, shape and length are shown in Table 2. Fresh feijoa fruit is oval in shape. It measures about 4.63 in length, 4.83 inches in diameter, and weigh about 20 g. Although its skin is edible as in guava, it generally discarded and edible index was 76.36%. Several studies, on different fruit species, demonstrated that variations in the fruit weight were mainly influenced by the genotype but also by the soil, the climatic conditions and by the fruit maturity stage(24, 35).

The influence of drying on physical characteristics of feijoa was shown in table 3. Fresh feijoa with higher moisture contents tend to have a higher bulking weight, because of the presence of water, which is considerably denser than the dry solid so, density was affected by the drying process. The bulk density of hot air dried samples increased as drying proceeded while it decreased with reduction in the moisture content.

Figure 1- longitudinal and transverse diameters of fresh feijoa fruit

Table 2- Physical characteristics of fresh feijoa fruit

Physical characteristic	Parameter
Weight of fruit (g)	20.31±1.04
Length (cm)	4.63±1.34
Diameter (cm)	4.81±0.98
Transverse shape	Ovate
Longitudinal shape	Oblong with moderately pointed apex
Peel weight (g)	4.80±1.24
Edible index (%)	76.36
Waste index (%)	23.63

The parameter hardness can be related to the force performed by mastication that takes part during eating. With respect to the results obtained, it was possible to see that rupture of the skin from the flesh side required a lower force (10.9 N) when compared with the same action from the skin side (13.8 N) which means that drying makes the product softer. From the results presented in Table 3, it can be observed that hot air drying had a small effect on cohesiveness compared with the fresh fruit. Regarding chewiness, it diminished greatly with drying, as a result of the variation observed previously in hardness. Many studies indicate that this firming effect of precooking can be attributed to the action of pectin-esterase on the cell-wall materials, particularly pectic substances, thereby resulting in desertification of pectin molecules and the subsequent formation of calcium bridges between free carboxyl groups of adjacent molecules(36-38).

The average values of the color parameters for feijoa in fresh, and after air drying are presented in Table 3 for *L** (brightness), *a** (redness), *b** (yellowness). In general, the air drying at 60 ◦C produced no remarkable changes in the color parameters of feijoa, as compared with the fresh feijoa. However, *L** (lightness) of dried fruit was significantly reduced.

Table 3- comparison of some physical characteristics of fresh and dried feijoa fruit

	Physical characteristics									
	Density Kg/m^3	texture			Color parameters					
		Firmness (N)	Cohesiveness (N)	Chewiness (N)	Skin color			Flesh color		
					L*	a*	b*	L*	a*	b*
Fresh fruits	1.001±0.004^a	6.2±0.1^a	1.4±0.12^a	-0.13±0.02^a	35.34±0.11^a	-12.46±0.18^a	17.27±1.02^a	21.32±0.53^a	-13.43±0.23^a	29.43±0.04^a
Dried fruits	2.004±0.11^b	0.024±1.01^b	1.212±0.73^a	-2.34±0.39^a	25.45±0.03^b	-11.73±0.02^a	29.73±0.11^a	11.23±0.11^b	-12.77±0.666^a	30.01±0.33^a

Values in the same column followed by different letters are significantly different (P<0.05)

Similar results were observed by (39-41) who studied on physicochemical characteristic of fruits.

3-2- Chemical characteristics

Table 4 summarized chemical characteristics of fresh and dried feijoa. It is evident from these finding that Iodine contents of both fresh and dried fruit was high (181-183 ppm) which means feija fruit is an excellent source of iodine. Carbohydrate content increased during drying because of condensation. Changes in ash content, fat and protein were not significant (P>0.05) showing relatively good thermal stability of these compounds.

Figure 2- Cut overripe feijoa fruit showing browning.

Table 4- chemical characteristics of fresh and dried feijoa fruit

Characteristics	Fresh feijoa	Dried feijoa
pH	3.03±0.02[a]	3.06±0.06[a]
°Brix	12.83±0.04[a]	13.03±0.04[a]
Moisture (%)	87.05±0.11[a]	14.11±0.43[b]
Ash (%)	0.33±0.29[a]	0.33±0.24[a]
Acidity (°D)	1.09±0.007[a]	1.1±0.04[a]
Protein (g)	6.01±0.25[a]	6.22±1.29[a]
Fat (g)	0.87±0.11[a]	0.91±0.23[a]
Total fiber (g)	4.10±0.40[a]	2.09±1.05[b]
Total carbohydrate (dry basis) (g)	19.20±2.07[a]	23.87±1.87[b]
Ascorbic acid (pm)	920±1.98[a]	521±3.02[b]
Iodine (ppm)	188.34±3.44[a]	181.04±2.78[a]
Total phenol (mg GAE/100 g FW)	76.15±3.98[a]	46.76±0.44[b]
Flavonoids (mg CE/100 g FW)	16.09±1.85[a]	15.81±3.06[a]

Values in the same rows followed by different letters are significantly different (P<0.05)

The average ascorbic acid content of fresh, whole feijoa 920 ppm whereas after drying, this content felt to 521 ppm, representing a decrease of about 43.3% in the nutrient content. As seen, the ascorbic acid content is considerably affected from the drying. In fact, ascorbic acid highly sensitive to heat. Similar results were reported for several fruits and vegetables like guava (42), pear(43), pineapple (44), tomato (45), pepper(46) and kiwifruit (47). The recommended daily intake (RDI) of ascorbic acid is about 30 mg/day for adults and 17 mg/day for children (48). With these fruits therefore, Tanzanian could be considered as good sources of ascorbic acid for purposes of human nutrition.

The concentration of total fiber was decreased by drying processing. Previous studies showed that heat stability of fibers varies in different food, times, temperatures and pressures. The fluctuations of each component of dietary fiber contents (total, soluble and insoluble) can be different, as well(49). For example, (50) reported that 5 min boiling of treated white cabbage with acetic acid decreased 9.7-11, 4.9-14 and 6-19% of total, insoluble and soluble fiber, respectively. Whereas, (51) observed that 10 minutes cooking of commercial mushrooms resulted in 42, 29 and 46% increases in the total, insoluble and soluble fiber, respectively.

4- Conclusion

Present data showed that *feojoa* fruit extracts possessed high phenolic content, and exhibited strong free radical scavenging activity indicates that the extract has good potential as a source for natural antioxidants to prevent free radical mediated oxidative damage. Antioxidant activities of natural antioxidants in this study were related to their total phenols. Fresh fruit had the highest antioxidant activity. Moreover, many physicochemical characteristics of dried feijoa fruit remained practically constant after drying. Finally, the results of the present study can be seen as a valuable tool to evaluate drying process of feijoa, in order to retain most of health promoting characteristics. Results from present study indicate that feijoa whether fresh, dried or herbal infusion can be used as appropriate substitutes for the synthetic antioxidant.

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