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Research Article

Antioxidant activity and nutritional value of *Citrus limetta* and *Ananas comosus* pomace

Abstract

Pulp waste from two fruits, *Citrus limetta* and *Ananas comosus* were analysed for *in vitro* antioxidant activity, total carbohydrate and pectin content and ascorbic acid. Total polyphenols determined in terms of catechol equivalents per 100g of pomace were higher in *Citrus limetta* (63-112 mg of catechol equivalents/100g pomace) as compared to *Ananas comosus* (22-86 mg of catechol equivalents/100g pomace). Polyphenols were higher in methanol extract of both the fruit pomace as compared to other extracts. Antioxidant activity was determined in terms of reducing power and there was no significant difference in antioxidant activity of the two fruits. Total carbohydrate content determined was 14-70 mg glucose equivalents/100g pomace for *Citrus limetta* and 42-71 mg glucose equivalents/100g pomace for *Ananas comosus* pulp waste. *Citrus limetta* pomace was relatively rich in ascorbic acid (16mg/100g pomace) and pectin (213 mg/g pomace) as compared to *Ananas comosus*. Thus, this study reveals biowastes of these two fruits from the food industry can be utilized further.

Introduction

Free radicals cause oxidative damage to various biomolecules viz. lipids, proteins and nucleic acids and thus initiate onset of degenerative diseases. Dietary intake of fruits and vegetables is also associated with decreased risk of diseases like cardiovascular diseases and certain forms of cancer [1,2]. This might be due to phenolic and other phytochemical antioxidants found in fruits and vegetables which neutralize free radicals [3]. Defensive effects of natural antioxidants of fruits and vegetables are vitamins, phenolics and carotenoids. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely being used in food industry to preserve and stabilize the freshness, nutritive value, flavor and colour of foods and animal feed products. However study has revealed that BHT could be toxic, especially at high doses [4]. Therefore, natural antioxidants need to be used as functional food ingredients to replace their synthetic equivalents that experience growing rejection.

Residues from the processing of fruits and vegetables, are being increasingly recognized as inexpensive source of high-phenolic products. These are also available in large quantities. Some of the agricultural byproducts of apples, citrus fruits and Brassica vegetables have already been used in the production of dietary fibre [5,6]. Pomace is the residue after fruits processing for juice, wine or other products. Fruit pomace reportedly

contain abundant phenolic compounds [7], and products with high phenolics from various pomace such as grape [8], and apple [9], have been recovered.

Ananas comosus is a highly nutritious fruit having high concentration of vitamin C [10], phenolic compounds and β -carotene; all are natural antioxidants. Citrus fruits are also rich in antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins. Citrus byproducts also represent a rich source of naturally occurring flavonoids. Both *Ananas comosus* and *Citrus limetta* have been widely used in juice as well as food industry. A considerable amount of pulp is left after filtration of juice and is available as a byproduct. Therefore, the study was undertaken with the objective of analyzing the pulp waste of *Ananas comosus* and *Citrus limetta* for its chemical composition. These byproducts might be useful raw material for producing value added products.

Materials and Methods

Chemicals

Methanol, Acetone, Folin and Ciocalteu's (Fisher Scientific, India), Ethyl Acetate, Ascorbic Acid, Ferric Chloride (Rankem India), Catechol, Sodium Carbonate, Anthrone (S.D. Fine Chemical Ltd. India), Trichloroacetic acid (Himedia, India), Sulphuric Acid (Ranbaxy, India). All chemicals were of analytical grade.

Plant material

Ananas comosus and *Citrus limetta* were obtained from local market, cleaned, peeled, grated, homogenized and filtered for separating juice. The pulp left after juice extraction was dried in oven at 45°C and powdered using an electrical grinder and stored in air-tight jars under refrigeration at 4°C till further use.

Preparation of sample extracts in solvents

After selection, fruits were washed and pressed and remaining pomace was dried for 48 hours at 45°C in oven. Dried pomace of each fruit was ground with electrical grinder. This dried powder was used for extract preparation.

Methanol extract: 25 g dry powder of fruit pomace was taken. Extraction was carried out in a soxhlet extractor for 4 hours using 95% methanol.

Acetone extract: 25 g dry powder of fruit pomace was taken. Extraction was carried in soxhlet extractor for 4 hours by using 80% acetone.

Ethyl acetate extract: 25 g dry powder of fruit pomace was taken. Extraction was carried by soxhlet extractor for 4 hours by using 250 ml ethyl acetate.

Aqueous extract: 25 g dry powder of fruit pomace was taken in 250 ml of water. Extraction was carried out by shaking for 24 hours at room temperature. Residue was separated by filtration. Residue was discarded and use supernatant as extract.

Determination of total polyphenol content

Total polyphenols were estimated by Folin-Ciocalteu method [11], with slight modifications. A known volume of extract was dissolved in water and 0.25 ml of Folin-Ciocalteu reagent (1M) was added. After one minute, 0.75ml of 20% Na₂CO₃ was added and the volume was made up to 5.0 ml with water. After 2 h of incubation at 25°C, the absorbance was recorded at 760 nm and compared to catechol calibration curve. Total polyphenols were determined as mg of catechol equivalents per 100 g of pomace and then values are presented as mean of triplicate analysis. The experiments were performed in triplicates and average value was determined.

Determination of reducing power

The reducing power of the extracts was determined by the method of Oyaizu [12]. In this assay, Fe³⁺/ferricyanide complex was reduced to ferrous form by antioxidants and the Fe²⁺ formed was monitored by measuring the formation of Perl's Prussian blue at 700 nm. One ml of each extract, dissolved in water, was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of a 1% aqueous potassium hexacyanoferrate [K₃Fe (CN)₆] solution and incubated at 50°C for 30 min. Then 2.5 ml of 10% trichloroacetic acid was added and centrifuged for 10 min at 1000 rpm. Upper layer 2.5 ml was mixed with 2.5 ml water and 0.5 ml of 0.1% aqueous FeCl₃, and the absorbance was recorded at 700 nm. Iron (III) reducing activity was determined by using ascorbic acid as standard. The values are presented as mean of triplicate analysis.

Pectin extraction

Pectin extraction was carried out by the method of Canteri-Schemin et al. [13], with minor modifications. Aqueous extract was concentrated under vacuum and residues were extracted with 5% w/v citric acid at temperature 65°C for 60 min. The sample was cooled and filtered. The supernatant was precipitated with ethanol (2 vol. of supernatant). The resulting solution was incubated for 12 h at 4°C and pectin was separated by filtration and dried at 35 to 40°C.

Estimation of total carbohydrates

Total carbohydrates were estimated by Anthrone method. 4ml of anthrone reagent (0.2% w/v in conc. H₂SO₄), was added in each test tube and tubes were chilled in ice for 5 min. Then 0.05ml of different extracts were diluted to 1 ml with distilled water. The resulting solution was mixed with chilled anthrone. The tubes were boiled for 10 min and then cooled. The blue green product was read at 625nm. Total carbohydrates were determined as equivalents of glucose. The experiments were performed in triplicates and average value was determined.

Estimation of ascorbic acid content

Ascorbic acid was quantified by volumetric method [14]. In a conical flask, 10 ml of 4% oxalic acid and 3.5 ml of standard ascorbic acid (0.1 mg/ml) were added and titrated against 2,6-dichlorophenol indophenol dye. The appearance of pink colour was taken as end point. The amount of dye consumed is equivalent to the amount of ascorbic acid. For test 5 ml of sample was taken in conical flask having 10 ml of 4% oxalic acid and titrated against the dye. The experiments were performed in triplicates and average value was determined.

Results and Discussion

Total polyphenolics and antioxidant activity

Polyphenolic compounds confer significant antioxidant activity and are widely distributed in fruits and vegetables [15]. Many plants are source of potentially safe natural antioxidants for the food industry; various compounds have been isolated with many of them being polyphenols. In this study total polyphenolic contents of *Citrus limetta* and *Ananas comosus* pomace were estimated (Table 1) and methanol extract had the highest polyphenol content in both *Citrus limetta* (115mg) and *Ananas comosus* (86mg) per 100 g of pomace as compared to the other extracts. The difference between the extracts of two fruits is marginally significant with P value of 0.02. The antioxidant activity was higher in aqueous extract of *Citrus limetta* pomace and in methanol extract for *Ananas comosus* pomace. P>0.05 indicates no significant difference in antioxidant potential of two studied fruit pomace.

Plant extracts have been shown to possess health-promoting properties. The hydrodistilled extracts from basil, laurel, parsley, juniper, aniseed, fennel, cumin, cardamom, and ginger were assessed for their total phenol content and antioxidant activities by iron(III) reduction, inhibition of linoleic acid peroxidation, iron(II) chelation, 1,1-diphenyl-2-

Table 1: Total polyphenols and antioxidant activity of the studied fruit pomace.

Extracts	<i>Citrus limetta</i>	<i>Ananas comosus</i>	P-value
Total polyphenols (mg of catechol equivalents/100g of pomace)			
Aqueous	63±3.0	24±0.0	0.020*
Methanol	115±0.0	86±2.0	
Acetone	99±1.0	29±0.0	
Ethyl acetate	112±0.0	22±0.0	
Antioxidant activity (µg of ascorbic acid equivalents/ml extract)			
Aqueous	0.387±0.02	0.053±0.001	P-value 2.36 ^{ns}
Methanol	0.273±0.01	0.260±0.01	
Acetone	0.340±0.01	0.245±0.02	
Ethyl acetate	0.238±0.02	0.120±0.01	

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*P value ≤ 0.05 = marginally significant

ns Pvalue > 0.05= not significant

picrylhydrazyl radical-scavenging and inhibition of hydroxyl radical-mediated 2-deoxy-D-ribose degradation. The extracts from basil and laurel possessed the highest antioxidant activities except for iron chelation. Although parsley showed the best performance in the iron chelation assay, it was less effective at retarding the oxidation of linoleic acid [16]. Bluecrop (*Vaccinium corymbosum* L.) was also evaluated from random samples of commercial late harvest fields in New Jersey. Results from this study showed that blueberry fruit grown from organic culture yielded significantly higher sugars (fructose and glucose), malic acid, total phenolics, total anthocyanins, and antioxidant activity (ORAC) than fruit from the conventional culture [17]. The antioxidant activity was also determined by DPPH method using methanolic extract of 13 commercially available citrus sp. peel and tissues. From this study it was concluded that there is no correlation between the total phenolic and/ or flavonoids contents and antioxidant activity in tissues and/or peels [18]. The extraction, isolation and identification methods of polyphenols from grape as well as their bioavailability and potential toxicity was also determined [19].

Total Carbohydrate content

Total carbohydrate content of the two fruit pomace was estimated by anthrone method in mg of glucose equivalents per 100g of pomace and results are summarized in table 2. Both fruit pomaces were rich in carbohydrate contents but there was no significant difference between the two. There are no previous reports on total carbohydrate content of fruit pomace. It represents total dietary fibres and sugars.

Ascorbic acid and pectin estimation

Pectin producers mainly utilize citrus peel or apple pomace (byproduct of juice (or cider) industry). In food industry, pectin has widely been applied as thickening, gelling and emulsifying agents for jams, soft drinks, fish and meat products, fruit juice, desserts and dairy products. Pectin also finds place in

therapeutic industry wherein it helps in lowering serum cholesterol level, removing heavy metal ions from the body, stabilizing blood pressure and restoring intestinal functions and weight reduction. Pectin yield from *Citrus limetta* and *Ananas comosus* pomace is 213 and 28 mg per gram of fruit pomace respectively (Table 3), which is 2.8% and 21% of dry weight of respective fruit pomace. *Ananas comosus* pomace does not contain significant amount of pectin but *Citrus limetta* pomace is equivalent with citrus peel pectin. In fact, *Citrus limetta* pomace contains higher amount of pectin than other food industry by-products such as peach pomace [20], and sunflower head residues [21]. Apple pomace contains 10 to 15% of pectin on dry matter basis while citrus peel contains of 20 to 30 % [22].

The ascorbic acid (Vitamin C) content was 8.4 and 16.0 mg per 100 g of pomace for *Ananas comosus* and *Citrus limetta* respectively (Table 3). Ascorbic acid is a natural antioxidant which may inhibit the development of major clinical conditions including heart diseases and certain cancers. Ascorbic acid content of *Citrus limetta* was higher than *Ananas comosus* pomace. Ascorbic acid content of *Citrus limetta* was less as compared to that of juice of citrus fruits [23], but it is significant when compared to other pomace studied such as *Daucus carota* and *Beta vulgaris* pulp waste [24]. Leaves of *Tephrosia purpurea* Linn (sarpankh), belonging to the family Leguminosae being used for the investigation of the *in-vitro* antioxidant activity of aqueous and ethanolic extracts. All the methods are based on UV-Spectrophotometric determination was used. Then investigation was carried out for *in-vitro* antioxidant activity and radical scavenging assay by calculating its % inhibition by means of IC₅₀ values, all the extracts concentration has been adjusted to come under the linearity range and here many reference standards like tannic acid, gallic acid, quercetin, ascorbic acid have been taken for the method suitability [25].

Conclusion

This study indicates that the extracts obtained from *Ananas comosus* and *Citrus limetta* pomace possess considerable amounts of phenolic compounds and carbohydrates. Among the two pulp wastes *Citrus limetta* possesses good amount of pectin and ascorbic acid. Thus it can be concluded that these pulp wastes could be utilized as a source of supplement or further exploited for values addition as these were rich in antioxidants.

Table 2: Total carbohydrate content of the studied fruit pomace.

Extracts	<i>Citrus limetta</i>	<i>Ananas comosus</i>	P-value
Total carbohydrates (mg of glucose equivalents/100g of pomace)			
Aqueous	70±1.0	71±0.0	0.349 ^{ns}
Methanol	69±0.0	64±1.0	
Acetone	14±1.0	42±2.0	
Ethylacetate	66±2.0	63±0.0	

^{ns} P value > 0.05 not significant**Table 3:** Ascorbic acid and Pectin content of the studied fruit pomace.

Parameters	<i>Ananas comosus</i>	<i>Citrus limetta</i>
Ascorbic acid (mg/100g of pomace)	8.4±0.1	16.0±0.0
Pectin (mg/g of pomace)	28	213

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