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Comparative Assessment of Phytochemicals in Four (4) Varieties of Ananas Comosus (l.) Merr Peels

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ABSTRACT

The paper reports comparative assessment of some phytochemicals in four (4) varieties of pineapple (*Ananas comosus* (*L.*) *merr*) peels / rinds. These varieties were Native Pineapple (A), Queens Pineapple (B), MD-2 Pineapple (C), and Smooth Cayene pineapple (D). The pineapples were collected, properly washed, and the peels were processed. After that, the peels were pulverized and oven-dried at 50 ^{0}C for 48 h. Chefman 500 W Blender was used to blend these dry samples into 0.5 µm sizes. The resulting peels were used for the analyses of alkaloid, tannin, phytate, oxalate, flavonoid, cardiac glycoside, total phenolic, and βeta- carotene using standard reported procedures. The results implied higher levels of flavonoid and total phenol in the varieties C and D than for the varieties A and B. The levels of β-carotene and cardiac glycoside were found to be significantly different ($P<0.05$) amongst the different peels. However, it was observed that there was no significant difference ($P<0.05$) in tannin and phytate contents of these rinds. There was also no significant difference in the oxalate contents of varieties A and B; but difference in the oxalate levels of varieties C and D was significant ($p<0.05$). Like the varieties A and B, the difference in alkaloids content of varieties C and D was not significant ($p<0.05$). These peels can be blended into our diets because of the presence of these phytochemicals.

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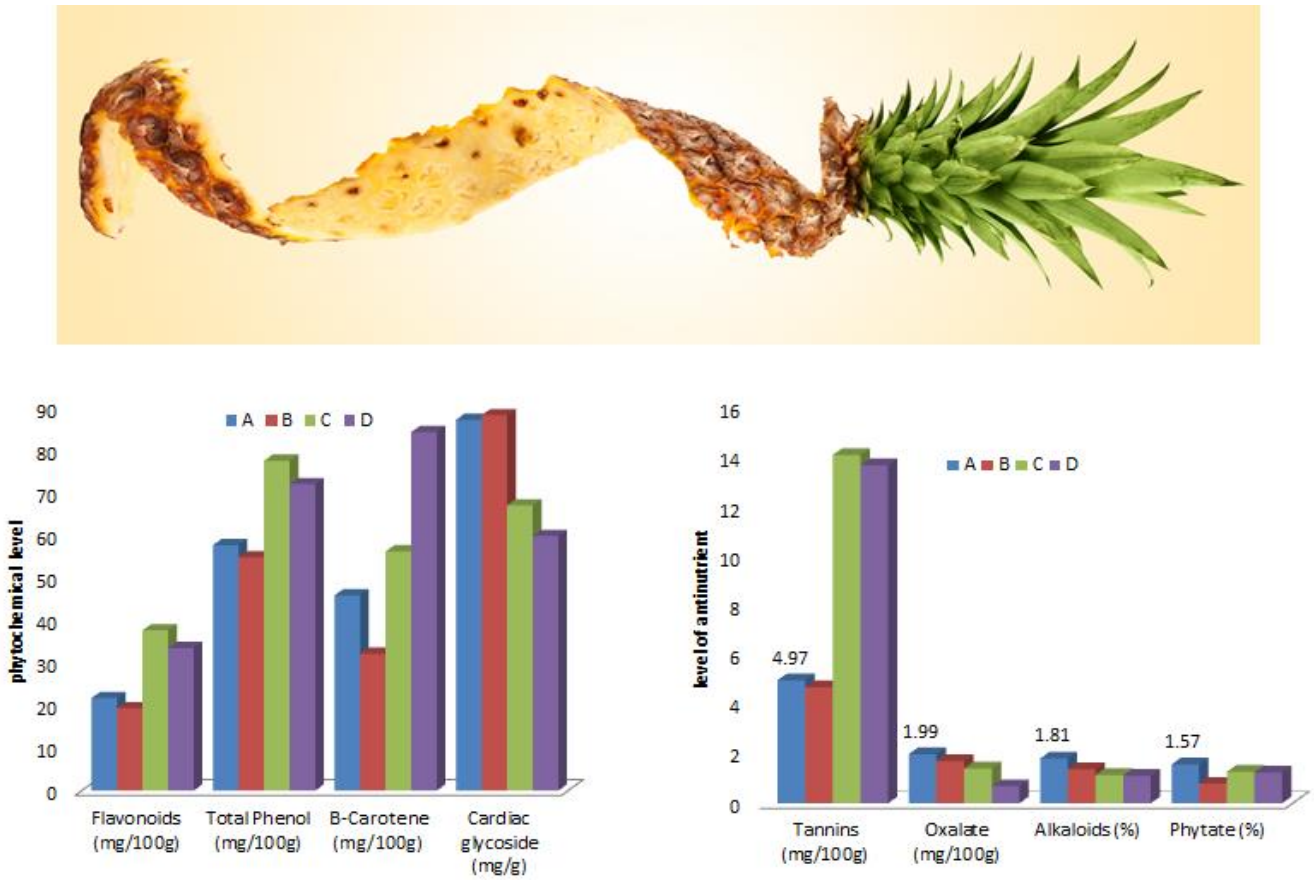
GRAPHICAL ABSTRACT



Introduction

Fruits and vegetables are known to contain variety of natural bioactive compounds such as flavonoids, anthocyanins, vitamin C, B and E, phenolic compounds, dietary fibers and carotenoids[1]. The consumption of fruits has increased due to their medicinal and nutritional values[2]. Pineapple (*Ananas comosus (L) merr*) is a perennial herbaceous plant with shallow roots[3]. Pineapple is of the bromeliad family, having 50 genera and about 2,500 known species[4]. The pineapple cultivars are classified into Smooth Cayenne, Red Spanish, Queen and Abacaxi[5], where Smooth Cayenne is the predominant variety[5]. According to Joy and Anjana [6], the general varieties of pineapple include Smooth Cayenne, Mauritius, Amritha**,** and MD-2. Furthermore, Kader *et al.* [7] reported about Giant Kew and Honey Queen varieties of pineapple from Chittagong region. Importantly, MD-2 pineapple is the standard for the international market because of its colour, flavour, shape, lifespan and ripeness. Ornamental and baby pineapples are also very popular among the gardeners and landscapers [6]. Omotoyinbo and Sanni in their work mentioned *Ananas fitzmuelleri, Ananas comosus,* and *Anana erectifolius* varieties of pineapples. Pineapple is widely consumed fruits and the major producing areas are Southeast Asia and Latin America[8]. With its excellent flavor and taste, pineapple is considered as the queen of fruits[9]. It is the third most important tropical fruit in the world after banana and citrus[9]. Pineapple fruits have a range of shapes, round, oval, and conical [10]. 16 - 18 million tons of pineapple are produced worldwide. Thailand, Brazil, India, Philippines

and China are major producers [5]. The pineapple is consumed not only in raw form but also in many processed forms like juice jams, dehydrated, canned or even frozen etc. Other products of pineapple include alcoholic beverages, organic acids, and the enzyme bromelain. This excellent fruit was probably indigenous of Brazil (South America) [11]. It grows worldwide and best in moderately warm climate (16- 33 ^{O}C) [12].

Nigeria is currently ranked 7th in the world as the producer of pineapple with a total production in 2011 as 14,000 metric tons [11]. Pineapple rinds are commonly found as waste industrially and domestically. However, the rinds are not well utilized. Almost 100% of the rinds are discarded indiscriminately thereby becoming potential environmental hazard[5]. Though the peels can be used for various culinary purposes such as tea, juice and even candy preparation [13], a great deal of them are still wasted in several other places and situations. The rinds account for 34.7% of the whole fruit [14]. Pineapple rinds contains vitamin C which is a powerful antioxidant against free radicals [14]. Free radicals have been shown to promote the diabetic heart disease, damage the cells of the colon, and contribute to the joint pain, and disability [15]. Indeed there is the need to enhance and popularize the benefits and hence utilization of the pineapple peels[16]. This will also fulfil the principles of Green Chemistry of waste reduction and use of renewable materials [17]. The study will further enlighten, support and lend credence on the utilization of pineapple rinds/peels.

Furthermore, phytochemicals are plant chemicals that possess protective or disease preventive properties [18-20]. About 20,000 phytochemicals are found from fruits, vegetables and grains [21]. Phytochemicals are used as pharmaceuticals, agrochemicals, flavors, fragrances, coloring agents, biopesticides and food additives[21]. Similarly, phytochemicals play a vital role in health as antioxidants against many diseases or antibacterial, antifungal, antiviral, cholesterol- lowering, antithrombotic or anti- inflammatory effects[18], [21, 22]. Anti-nutrients are also secondary metabolites with biological activities[23]. They are used by plants to defend against fungi, insects and predators[23]. Anti-nutrients reduce the availability of minerals, vitamins and even proteins, and interfere with the metabolic processes such as growth[23]. Though there are different varieties of pineapples, there is scarce comparative analysis of nutritional profile of them. Hence this research reports comparative assessment of phytochemicals in four (4) varieties of *Ananas comosus (L.) merr* peels.

Materials and Apparatus

Sample collection/identification

The pineapple rinds were of Native Pineapple (A), Queens Pineapple coded (B), MD-2 Pineapple coded (C) and Smooth Cayene coded (D). Varieties A and B were purchased at Railway Market, Makurdi, Benue state -Nigeria, Variety C was purchased in Mile 2 Fruits Market, Lagos – Nigeria, while Variety D was purchased in Edo State -Nigeria. All chemicals and reagents were of analytical grade. Identification of the pineapples was done at Biology Department, Benue State University Makurdi - Nigeria.

Sample preparation

The pineapples were then properly washed and the peels were processed. The rinds were pulverized and oven-dried at 50 ^{O}C for 48 h. Chefman 500 W Blender was used to blend these dry samples into 0.5 µm sizes. The blended samples were used for the following analyses.

Determination of Total Flavonoid Content:

Total flavonoid content was measured by the standard aluminium chloride colorimetric assay as described by AOAC[24] and Snell and Snell[25]. The reaction mixture consisted of 1mL of extract and 4 mL of distilled water was taken

in a 10 mL volumetric flask. To the flask, 0.30 mL 0f 5% sodium nitrite was treated and after 5 minutes, 0.3 mL of 10% aluminium chloride was mixed. After 5 min, 2 mL of 1M NaOH was treated and diluted to 10 mL with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the blank at 510 nm with Jenway 6405 UV/visible spectrophotometer. The Total flavonoid content was expressed as mg of QE/g of extract using the following based on calibration curve. Y= 0.09x- 0.006; where X= Concentration and Y= Absorbance.

Determination of Alkaloids

Alkaloids were determined by using standard procedure as described by in AOAC [24]. About 10 g of the sample was taken to 250 mL separating funnel, 5 mL of dilute H_2SO_4 and 5 mL of distilled water were added. The mixture was shaken twice with 10 mL of $CHCl_3$ and the combined $CHCl_3$ extract was transferred to the second separating funnel containing 5 mL of dilute H_2SO_4 and 10 mL of distilled water. The $CHCl_3$ was discarded and the aqueous acidic layer was transferred to the contents of the first separating funnel. The extract was made alkaline with ammonia and shaken for about 30 seconds. The extract alkaloids were extracted completely by successive portion of $CHCl_3$ each of 20 mL (complete extraction was tested using Meyer's reagent). The combined $CHCl_3$ extract was shaken with 5 mL of distilled water. The extract then ran through a plug of cotton wool previously muster with $CHCl_3$ and covered with little anhydrous sodium sulphate; the sodium sulphate was washed with 5 mL of $CHCl_3$. The combined $CHCl_3$ was inserted into a 250 mL dry conical flask. The $CHCl_3$ was completely distilled and 5 mL neutral alcohol was added and evaporated on a boiling water bath. The residue was further heated on the boiling water bath for about 10- 15 min to remove the volatile bases. The residue was then dissolved in 2 mL of $CHCl_3$ and 20 mL of N/50 H_2SO_4 was added. The sample was then placed in the water bath to remove the $CHCl_3$ completely and cooled down. It was titrated with N/50 NaOH using methyl red as an indicator till the first drop of N/50 NaOH caused color change from pink to yellow. For calculation: 1 mL of N/50 NaOH ≡ 0.005787g alkaloids. Hence, % alkaloids content = (20 mL of N/50 H_2SO_4) x (0.005787 x 100/10).

Determination of Cardiac glycosides

Cardiac glycoside was determined by using standard procedure as described in AOAC [24]. 8 g of the sample was transferred to 100 mL volumetric flask and 60 mL of H_2O and 8 mL of 12.5% lead acetate was added, mixed and filtered. 50 mL of the filtrate was transferred into another 100 mL of volumetric flask and 8 mL of 47% Na_2HPO_4 were added to precipitate excess Pb^{2+} ion. This was mixed and completed to volume with water. The mixture was then filtered twice with the same filter paper to remove excess lead phosphate. 10 mL of purified filtrate was transferred into clean Erlyn- Meyer flask and treated with 10 mL Baljet reagent. A blank titration was carried out using 10 mL distilled water and 10 mL Baljet reagent. This was allowed to stand for 1 h for complete color development. The color intensity was measured colorimetrically at 495 nm. The % glycosides = A x 100/77 g %; where A= Absorbance.

Determination of Total Phenolic Content: Total Phenolic content was determined by the Follin-ciocalteu method as described by Muller [26] and Signleton *et al*. [27]. A 0.02 mL aliquot of extract solution was mixed with 1.16 mL distilled water and 0.1 mL of Follin-ciocalteu reagent followed by addition of 0.3 mL of Na_2CO_3 solution (20%). Subsequently, the mixture was incubated in a shaking incubator at 40 ^{0}C for 30 minutes and its absorbance was measured at 760 nm using Jenway 6405 UV/Visible spectrophotometer. Gallic acid was used as a standard for calibration

curve. Total phenolic content was expressed as gallic acid equivalent (GAE) and calculated using the following linear equation based on the calibration curve. $A = 0.98C + 9.25\times10^{-3}$; A = absorbance, C = concentration in mg GAE/ g dry weight.

Determination of βeta- carotene

Beta- carotene was determined as described in Rodrigue-Amaya *et al*. [28], though with slight modification. About 5 g of the sample was poured into the separating funnel and a solution containing 140 mL ethanol: instead of petroleum ether and acetone hexane (4:3) was added. The formation of emulsion was prevented by adding 2 mL of 2% sodium chloride (NaCl) solution. The mixture was vigorously shaken for about 3 min to stand for 30 min and the upper layer ran off. The absorbance of the top layer was determined at the wavelength of 425 nm using a spectrophotometer and the concentration of Beta- carotene was calculated using Beer-Lambert law as Beta- carotene (µg/g) = a*V*106/A %. Where: A= Absorbance, V= Total volume of the extract, W= Wavelength of the sample, A= 2590 (Absorbance coefficient of Beta-carotene in hexane).

Determination of Tannin Content

The tannin content was determined by Follin-coicalteu method[26]. About 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Follin-ciocalteu reagent. 1 mL of 35% Na_2CO_3 solution and dilute to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. Absorbance for the test and standard solution were measured against the blank at 725 nm with Jenway 6405 UV/visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/g of extract.

Determination of Phytate Content

Phytate content was determined as described by Loewus[29]. Phytic acid was determined as described in [36]. 2.0 g of the sample was weighed into a 250 mL conical flask. One hundred mL 2% concentrated HCl was used to soak sample for 3 h and then filtered with Whatman No. 1 filter paper. 50 mL of the filtrate and 10 mL of distilled water were added in each case to give proper acidity. 10 mL of 0.3% ammonium thiocyanate solution was added into the solution as indicated and titrated with standard iron (II) chloride solution containing 0.00195 g iron/ mL, end point observed to be yellow which persisted for 5 min. % Phytic acid = y x 1.19 x 100; where, y = titre value x 0.00195 g.

Determination of Oxalate Content

Oxalate was determined as described in[30][31]. About 1 g sample was placed in 250 mL volumetric flask, 190 mL of distilled water and 10 mL of 6 M HCl were poured. The mixture was warmed up to 90 ^{0}C for 4 h and centrifuged at 2000 rpm for 5 min using Centurion Scientific Centrifuge (Model K241). The supernatant was evaporated to 250 mL, plus three 50 mL aliqouts of the supernatant was evaporated to 25 mL. The resultant brown precipitates were filtered and washed. The combined solution and the washings were then titrated with conc. NH_3 solution under methyl orange indicator to yellow colour. The oxalate was precipitated with 5% $CaCl_2$ solution, kept overnight and then centrifuged. This precipitate was washed with hot 25% H_2SO_4, diluted to 125 mL and titrated against 0.05M $KMnO_4$.

Calculation: 1 mL 0.05M $KMnO_4$ = 2.2 mg oxalate.

Results and Discussion

Results of phytochemical content of the pineapple peels:

Some phytochemical contents of the peels of these pineapples are presented in Table 1. In this report the flavonoid content was within the

range of 19.28 – 37.56 mg/100g which was higher than those found in conventionally produced and organic potherbs and fruits (2.8 – 14.3 mg/100 g) [32]. Flavonoids have beneficial health values[33]. Manzoor *et al*. [34] reported that 150 mg/100g of flavonoid is sufficient to lower the blood pressure. More so, 150 mg/100g of flavonoid was found to exert anti-inflammatory and anti-tumor effect [35].

Table 1: Some Phytochemical Content of the Pineapple Rinds

Varieties	Flavonoid (mg/100g)	Total Phenol (mg/100g)	B-Carotene (mg/100g)	Cardiac glycoside (mg/g)
A	21.67±0.28^{c}	57.49±0.01^{c}	45.72±0.13^{c}	86.83±0.21^{b}
B	19.28±0.08^{d}	54.64±0.01^{d}	32.04±0.02^{d}	87.90±0.10^{a}
C	37.56±0.01^{a}	77.25±0.01^{a}	56.02±0.01^{a}	66.80±0.10^{c}
D	33.39±0.01^{b}	71.74±0.01^{b}	83.95±0.01^{b}	59.62±0.10^{d}
LSD	**0.01**	**1.26**	**0.15**	**0.5**

Values are means ± standard deviation from triplicate determinations. Means within the same column with same superscript are not significantly different ($p<0.05$). A = Native Pineapple variety, B = Queen Pineapple variety, C = MD-2 Pineapple Variety, D = Smooth Cayene Pineapple Variety

The total phenolic contents (TPC) of these peels were 57.49 mg/100g, 54.64 mg/100g, 77.25 mg/100g, and 71.74 mg/100g for the varieties A, B, C, and D, respectively. From literature the values of TPC of garlic, ducanut, lemon, garden egg, grape, and carrot were found as 5.75 - 98.77 µg/g [36]. Han *et al.* [33] informed that TPC of 152.09 mg/100g can trigger antioxidant effect.

The β-carotene contents of the pineapple rinds were obtained as 45.72 mg/100g in variety A and 32.04 mg/100g in variety B. Variety C and D had 56.95 mg/100g and 83.02 mg/100g, respectively. B-carotene is a vitamin A precursor that is important in human health. It is an antioxidant that plays a crucial role in the body's fight against free radicals[37]. Moon and Shibamoto[37] reported 30 mg/100g of β-carotene required to improve vision in human. Again, recommended dietary allowance (RDA) of β-carotene is 300 mg/100g [38]. Cardiac glycoside levels for these peels were within 59.62 – 86.83 mg/g. Tofighi *et al.* [39] reported cardiac glycosides of 176.47 - 181.40 mg/g in different generations of suspension culture of *Securigera securidaca.* Cardiac glycosides are secondary metabolites used in the treatment of congestive heart failure by inhibiting the amount of $Na^{+}$and K^{+} pump thus making way for the increase in the level of Ca^{2+} available for the contraction of heart muscles. Cardiac glycoside values below 100 mg/100g have been reported to be healthy in the body. The flavonoid and total phenolic content were higher in varieties C and D than in A and B.

Table 2: Anti-nutrients content of Varieties of Pineapple Rinds

	Antinutrients			
Varieties	**Tannins (mg/100g)**	**Oxalate(mg/100g)**	**Alkaloids (%)**	**Phytate (%)**
A	4.97±0.07^{c}	1.99±002^{a}	1.81±0.01^{a}	1.57±0.01^{a}
B	4.69±0.03^{c}	1.70±0.01^{a}	1.38±0.03^{a}	0.79±0.13^{b}
C	14.07±0.01^{a}	1.41±0.01^{b}	1.14±0.01^{b}	1.27±0.01^{a}
D	13.65±0.01^{b}	0.69±0.01^{c}	1.11±0.01^{b}	1.24±0.01^{a}
LSD	**0.32**	**0.50**	**0.50**	**0.50**

Differences in flavonoid and total phenolic content of the pineapple varieties were observed to be significant ($p<0.05$) across all the varieties studied. The levels of β-carotene and cardiac glycoside were also found to be significantly different across the four (4) varieties.

Anti-nutrient content of pineapple rinds

Table 2 contains the levels of antinutrients for these rinds. Values are means ± standard deviation from triplicate determinations. Means within the same row with same superscript are not significantly different ($p<0.05$). A = Native Pineapple variety, B = Queen Pineapple variety, C = MD-2 Pineapple Variety, D = Smooth Cayene Pineapple Variety. Though antinutrients can exert some beneficial health effects, their negative impact seems to overshadow [40]. The tannin contents of the pineapple rinds in varieties A and B were 4.97 mg/100g and 4.69 mg/100g, respectively, while varieties C (14.07 mg/100g) and D (13.65 mg/100g) were higher in tannin. These are comparable to those of 18.16 mg/ 100 g in *Corchorus oliterius* plant [41]; and 3.580 mg/ 100 g and 1.820 mg/ 100 g for *S. nigrum* and *C. gynandra* [42]. Excess amounts of tannins are known to limit the bioavailability of iron. As presented in Table 2, the concentrations of alkaloid in the peels A, B, C and D were 1.81%, 1.38%, 1.14% and 1.11%, respectively. Literature shows that 15.160% and 3.44% of alkaloid in *S. nigrum* and *C. gynandra,* were found respectively [42]. These are all higher than 0.53% level of alkaloid of *C. oliterius* plant [41].

The oxalate contents were 1.99 mg/100g, 1.70 mg/100g, 1.41 mg/100g, and 0.69 mg/100g for the rinds of varieties A, B, C and D, respectively. All these values are lesser than those of 241.96 mg/100 g for *C. oliterius* [41]. It has also been observed that 21.560 g/100g and 24.97 g/100g of oxalate were contained in *S. nigrum* and *C. gynandra,* respectively [42]. Excess oxalate content of food indicates low bioavailability of calcium in the food [43]. The rinds also contained 1.57% of phytate in the Variety A and 0.79% in B. The C variety contained 1.27% whereas variety D had 1.24%. High phytates limit the bioavailability of minerals like zinc and iron [44]. Phytate level of 0.80% in *C. oliterius* has been previously reported [41]. Furthermore, 0.099% and 0.250% phytate were observed in *S. nigrum* and *C. gynandra,* respectively[42]. In this study, the differences between the tannin contents of varieties A, B, C and D were insignificant ($p<0.05$). There was no significant difference in the oxalate contents of varieties A and B but the difference in the oxalate levels of varieties C and D was significant ($p<0.05$). Like in varieties A and B, the difference in alkaloids content of varieties C and D was not significant ($p<0.05$). Phytate content of varieties A, C and D were not significantly ($p<0.05$) different from each other but variety B contained a lower amount of Phytate that was significantly different from all the other varieties. Differences in phytochemical concentrations could be due to various effects: soil, harvest and postharvest handling and storage conditions [36][42][45]. Ndie and Okaka [46] commented that levels of phytate (23.5-130.65 mg/ kg), oxalate (4.7-95.6 mg/kg) and tannin (108.3 mg/kg) are high enough to be associated with health risk.

Conclusion

This paper reports comparative assessment of alkaloid, tannin, phytate, oxalate, flavonoid, cardiac glycosides, total phenolic, and βeta-carotene contents from the peels of four (4) pineapple (*Ananas comosus (L.) merr*) varieties. These varieties were Native Pineapple (A), Queens Pineapple (B), MD-2 Pineapple (C), and Smooth Cayene pineapple (D). The results implied higher levels of flavonoid and total phenol in the varieties C and D than for the varieties A and B. The levels of β-carotene and cardiac glycoside were found to be significantly different ($P<0.05$) amongst the different peels.

However, it was observed that there was no significant difference ($P<0.05$) in tannin and phytate contents of these rinds. There was also no significant difference in the oxalate contents of varieties A and B, but the difference in the oxalate levels of varieties C and D was significant ($p<0.05$). Like in varieties A and B, the difference in alkaloids content of varieties C and D was not significant ($p<0.05$). These peels can be blended into our diets because of the presence of these phytochemicals.

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Conflict of Interest

The authors declare no conflict.

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