Journal of Tropical Biodiversity and Biotechnology
Volume 10, Issue 02 (2025): jtbb16490
DOI: 10.22146/jtbb.16490

Research Article

Investigation on Nutritional Values of Three Different *Ananas comosus* (L.) Merr. Species Planted in Vietnam

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Keywords:
Antioxidant
Bromelain
Nutrients
Pineapple
Phytochemicals
Submitted:
17 September 2024
Accepted:
06 March 2025
Published:
30 June 2025
Editors:
Miftahul Ilmi
Liya Audinah

ABSTRACT
This study aimed to evaluate the nutritional values, phytochemicals, antioxidant activity, and anti-nutritional factors of three different pineapple cultivars (Cayenne, Queen, and MD2) cultivated in various provinces of Vietnam. The pineapple samples were collected from many farms ranging from the Central to the South of Vietnam. The analyses were performed as per standard test methods (the European standard and Association of Official Agricultural Chemists - AOAC methods). In general, there were no significant differences in energy-yielding nutrients (carbohydrates, fats, and protein). However, significant differences were observed in vitamin and mineral content. The MD2 cultivar had a high level of vitamin B5 and a low concentration of calcium, whereas both Cayenne and Queen were rich in vitamin C and chlorine. All three studied pineapple varieties exhibited moderate levels of phenolics and flavonoids, low concentrations of bromelain, and weak antioxidants. All three pineapple species studied contained low concentrations of oxalates and a void of tannins. These findings scientifically contribute valuable information to the food database system and can be considered in diet planning.

How to cite:
Hong, S.L., Le, T.N.K. & Huynh, N.B., 2025. Investigation on Nutritional Values of Three Different *Ananas comosus* (L.) Merr. Species Planted in Vietnam. *Journal of Tropical Biodiversity and Biotechnology*, 10(2), jtbb16490. doi: 10.22146/jtbb.16490

INTRODUCTION

Ananas comosus (L.) Merr. (*A. comosus*), commonly known as pineapple, belongs to the genus Ananas of the Bromeliaceae family. The plant is native to the tropical and subtropical regions of South America, such as Brazil and Paraguay. However, this tropical plant is now extensively cultivated for its fruit in many regions worldwide, including Costa Rica, Hawaii, India, Philippines, Thailand, and Vietnam. Pineapple is locally known as paina (Hawaiian), abacaxi (Portuguese), dứa or thơm (Vietnamese) (Wali et al. 2019).

In Vietnam, pineapple is extensively planted on large scales in several provinces from the North to the South of Vietnam; however, approximately 90 % of output is in the South, provincially in Tien Giang, Kien Giang, Long An, Ca Mau, Binh Dinh, Dak Lak, Dong Thap, Quang Nam, and Phu Yen. The total pineapple cultivation area in Vietnam is estimated at approximately 40,000 hectares, producing around 500,000 tons annually for domestic and international markets.

Three different pineapple cultivars are commonly grown and consumed: MD2-Golden yellow, Smooth Cayenne, and Queen (Figure 1). These cultivars are distinguished based on the leaf and fruit characteristics. *Ananas comosus* var. queen (ACQ) is commonly known as Queen pineapple (Joy & Anjana 2017). This cultivar, best known as the world's sweetest pineapple, has dark yellow flesh, a crispy texture, distinct aroma and flavour. The leaf is recognizably characterized by a tiny spine on the edge of the leaf. *Ananas comosus* var. cayenne (CAC) is commonly known as Cayenne pineapple. The long, curved leaves have only a few spines at the tips. The fruit is large, dark green when unripe, and turns bronze when ripe. The fruit is juicy and has yellow flesh, large and shallow pineapple eyes, and thin skin. *Ananas comosus* var. MD2 (MD2), named after Millie Dillard, the wife of the former general manager of Del Monte, is a pineapple variety bred from two pineapple varieties Cayenne and Queen, with thin skin, succulent, bright yellow, fragrant, and extremely delicious flavour. The fruit is light green when unripe and bright yellow when fully ripe. The fruit's eyes are large and the eye pits are very shallow (Redwan et al. 2018).

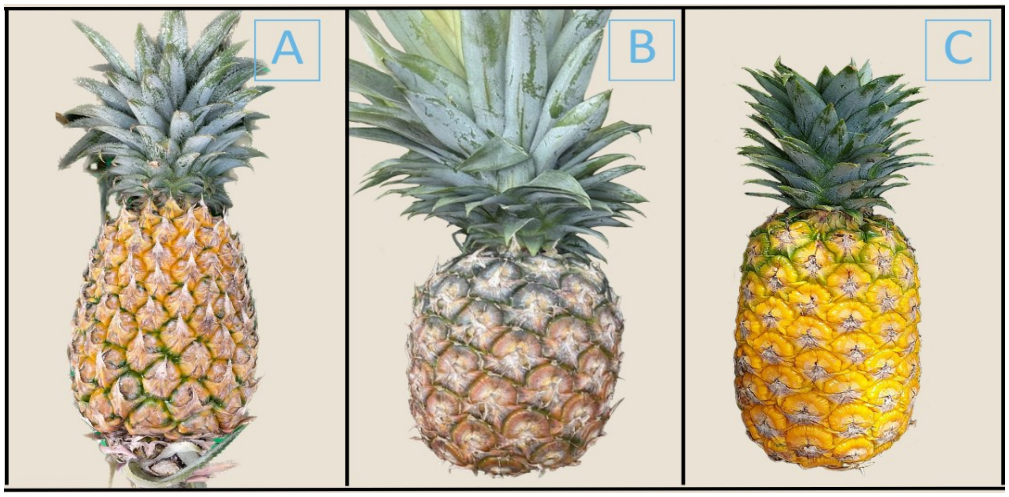

Figure 1. Three pineapple cultivars (A): *Ananas comosus var. queen*; (B): *Ananas comosus var. cayenne*; (C): *Ananas comosus var. MD2.*

Pineapple is commonly used as a popular food in Vietnamese people's daily meals. Pineapple is an ideal fruit for weight loss because it is a great source of fiber and low in calories and carbohydrates. Bromelain, a cysteine protease found in pineapple flesh, is probably the main ingredient contributing to medical applications. Pineapple has numerous medicinal properties, including the ability to aid digestion, heal wounds, and prevent platelet ag-

gregation. Pineapple exhibits anti-inflammatory and fibrinolytic effects, regulating cytokinin activity and immune response (Joy 2010). The nutritional values and phytochemicals of different pineapple cultivars may vary significantly depending on various factors, including species, genotypes, climatic conditions, geographical origin, cultivation practices, and harvesting periods (De Ancos et al. 2017). Furthermore, the biological activities of pineapple are not always correlated with the variation of components present in pineapple. Therefore, this study focused on investigating the variation of nutrients and phytochemical components across pineapple of different cultivars, namely Queen pineapple, Cayenne pineapple, and MD2, to empower consumers to make informed dietary choices and support further development in nutraceuticals and cosmeceuticals. Additionally, this study aims to provide data with significant potential to inform evidence-based policy formulation for governmental entities involved in sustainable agricultural development. Specifically, these findings offer actionable insights into enhancing production efficiency in targeted material sectors, facilitating the fulfilment of domestic demand, and the expansion of export markets.

MATERIALS AND METHODS

Materials

All chemicals and reagents were purchased from Sigma-Aldrich and provided by the Pharmaceutical Chemistry Laboratory and Applied Biochemistry Laboratory of the Applied Biochemistry Department at International University HCMC.

Selection and preparation of samples

Fresh pineapples of three varieties were randomly selected from various farms in Kien Giang (10°0′N 105°10′E), Dak Lak (12°40′N 108°3′E), Phu Yen (13°10′N 109°10′E), Tien Giang (10°25′N 106°10′E), and Dong Thap provinces (10°40′N 105°40′E) (Figure 2). Transportation was carried out in ice boxes and stored at 4 °C. The fruits were washed with distilled water, and the pineapple flesh was continuously dried at 48 °C for 18 hours (Ojike et al. 2020). The dried samples were then ground into a fine powder and stored in a sealed jar at 4 °C prior to further analysis.

Figure 2. Sampling sites as highlighted.

Nutritional values

Total carbohydrate content

The total carbohydrate content of the DFS sample was determined using a phenol-sulfuric acid colorimetric assay(AOAC 988.12 1990); (Nielsen 2010). Briefly, 5 g of the pineapple sample was extracted with 45 mL of 80 % (v v^{-1}) ethanol under gentle agitation for 15 minutes. The resulting suspension was quantitatively transferred to a 50 mL volumetric flask and brought to volume with 80 % (v v^{-1}) ethanol. The mixture was subsequently filtered, and 10 mL of the filtrate was retained for analysis. For the colorimetric assay, 1 mL of the diluted filtrate was combined with 1 mL of 5 % (w v^{-1}) phenol solution and 5 mL of concentrated sulfuric acid. The reaction mixture was incubated in a water bath for 10 minutes, followed by a 5-minute cooling period at room temperature. The absorbance of the resulting coloured complex was measured spectrophotometrically at 490 nm against a reagent blank. Total carbohydrate content was quantified using a glucose standard curve and expressed as milligrams of glucose equivalents per gram of dry sample (mg GE/g) and as a percentage of the sample's dry weight.

Protein content

The determination of the protein content of pineapple samples was carried out using Kjeldahl method (AOAC 2001.11 2005; AOAC 991.20 2005). A Kjeldahl digestion and titration procedure was implemented. One gram of the sample was subjected to acid digestion within a Kjeldahl flask, utilizing 10 mL of concentrated sulfuric acid and 1 gram of a catalyst mixture. This catalyst was formulated from potassium sulfate and copper sulfate in a 9:1 mass ratio. The digestion process was carried out under heat until a clear solution was achieved. The resulting digestate was then diluted to a final volume of 100 mL using deionized water in a volumetric flask. Subsequently, the diluted digestate was subjected to steam distillation following the addition of 25 mL of 45 % sodium hydroxide solution to the distillation apparatus. The distillation continued until 100 mL of distillate was collected. This distillate was collected in a conical flask containing 10 mL of 4 % boric acid solution, along with a few drops of bromocresol green indicator. The nitrogen content was determined by titrating the distillate with 0.1 N hydrochloric acid until a pink endpoint was observed. The protein content of the sample was then calculated as a percentage using a standard nitrogen-to-protein conversion factor of 6.25.

Total crude fat content

The total crude fat content of the pineapple samples was determined using the Randall extraction-submersion method (AOAC 2003.05 2005; AOAC 2003.06 2005). A 4.5 g sample was precisely measured and transferred into a cellulose thimble suitable for Soxhlet extraction. This thimble was subsequently installed within the Soxhlet apparatus system (TT-SOX606), and 50 mL of hexane was introduced as the extraction solvent. The system was then activated, initiating a continuous reflux of the solvent through the sample material. After approximately 6 hours, ensuring comprehensive lipid removal, the heating element was deactivated, and the solvent was allowed to cool to ambient temperature. The collection flask, containing the extracted lipids, was then removed from the apparatus. To eliminate residual moisture, the flask was subjected to a drying process, followed by cooling in a desiccator, prior to final gravimetric analysis.

Crude fibre content

The Weeden method (AOAC 978.10 2000) was used to determine crude fibre content. A 2 g portion of each dried sample was weighed into a conical flask with 200 mL sulfuric acid (0.128 M) and incubated for 30 minutes in

periodically shaking. The filtrate was then washed with hot water to remove acid residues. The filtrate was then boiled and washed again with 200 mL of sodium hydroxide (0.313 M) in a separate conical flask. The filtrate was collected in a dried crucible, and the excess water was evaporated in the oven at 105 °C for 2 hours and cooled down in the desiccator for 20 minutes. The crucible containing fibre was weighed as W1. The crucible set was then placed in a muffle furnace and heated up to 500 °C for 2 hours. Once the crucible was removed from the furnace and cooled in a desiccator for 20 minutes, it was reweighed as W2. The crude fibre percentage was calculated by the formula:

$$\% \text{ crude fibre} = \frac{W1-W2}{2} \times 100\%$$

Moisture content

The experiment was performed using the method of measuring the loss of volatile substances under temperature (AOAC 931.04 2000). A 10 g of samples were weighed in dried petri dishes and recorded as W_0. The sample was kept in a 70 °C-oven overnight until its mass no longer changed as W_1. The moisture formula was calculated as follows:

$$\% \text{ Moisture} = \frac{W_0-W_1}{W_0} \times 100\%$$

Ash content

The ash content was determined using the gravimetric method AOAC 940.26, 2000 (Thiex et al. 2012). The empty crucibles were dried by the muffle furnace (Model LEF-230P) at 100 °C for 20 minutes and weighed to be W1. A 20 g of each wet sample was placed into those dried crucibles and weighed to obtain W2. The crucibles holding samples were kept in the furnace at 500 °C for 2 hours and then weighed for the W3 values. The percentage of ash content was calculated by the formula:

$$\% \text{ Ash content} = \frac{W3-W1}{W2} \times 100\%$$

Vitamin and mineral contents

The vitamin contents in pineapple samples were quantified primarily based on various AOAC methods. Vitamin A, E, D, K1, C, and vitamins group B including B1, B2, B3, B6, and B12 were analyzed by the high-performance liquid chromatography (HPLC) method (Vries et al. 1979; AOAC 961.14 1989; European Standard EN 14122-2003 2003; Mann et al. 2005; AOAC 2001.13 2011; AOAC 2011.10, 2011; AOAC 2012.21 2013; Delmonte et al. 2013; Hossain et al. 2019). The levels of vitamins B5 and B9 within the pineapple samples were quantified using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), following previously established protocols (AOAC 2011.06 2011; Andrieux et al. 2013). Vitamin content was expressed as per 100 grams of the pineapple sample.

Pineapple samples underwent analysis to determine their mineral composition, following established analytical procedures. Specifically, sodium and potassium levels were ascertained through flame photometry, adhering to AOAC 969.23, 2005. The calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), and iron (Fe) were achieved using atomic absorption spectrophotometry (AOAC 968.08 2000). For manganese (Mn) and selenium (Se), inductively coupled plasma-mass spectrometry (ICP-MS) was employed, in accordance with AOAC 2015.01 (2015) and Nelson et al. (2019). Phosphorus content was determined via spectrophotometric analysis (AOAC 995.11 2000). The chlo-

rine concentrations were measured using the Volhard titration technique, as per the Volhard method (Nordtest method 1996).

Phytochemicals analysis and antioxidant activity

Determination of total phenolic content

According to the Folin-Ciocalteu method (Siddiqui et al. 2017), the total phenolic content (TPC) was measured and expressed as mg GAE/g, relative to gallic acid. The assay involved combining 150 µL of the diluted supernatant with 375 µL of Folin-Ciocalteu reagent and 375 µL of a 7.5 % sodium carbonate (Na_2CO_3) solution. After a 5-minute vortex, the mixture was incubated in darkness at ambient temperature for 30 minutes. Absorbance was then recorded at 765 nm using a Biotek Synergy HT 96-well plate reader (USA).

Determination of total flavonoid content

The aluminium chloride method, as described in previous studies (Chang et al. 2020; Shraim et al. 2021), was used to determine the total flavonoid content (TFC) of pineapple samples. The supernatant, resulting from sample preparation, served as the basis for the TFC analysis. Specifically, 100 µL of diluted supernatant was combined with 560 µL of distilled water, 300 µL of 80 % methanol, 20 µL of 10 % $AlCl_3$, and 20 µL of 1M CH_3COOK. This mixture was then vortexed for 5 minutes and incubated for 30 minutes in the dark at room temperature. Quercetin (QE) was used as a standard to generate a calibration curve. Absorbance was measured at 430 nm using a Biotek Synergy HT 96-well plate reader (USA), with a blank as the reference. The TFC was reported as mg QE/g.

Estimation of bromelain

100 g of pineapple flesh was blended with 100 mL of phosphate buffer (0.05 M, pH = 7.0) prior to filtering. The yellowish precipitate was suspended in phosphate buffer (0.2 M, pH = 7.0) followed by re-fractionating with ethanol. The filtrate was centrifuged at 10,000 rpm at 4 °C for 20 minutes, and the supernatant was collected as "crude extract". The crude extract was then subjected to ammonium sulfate precipitation by adding ammonium sulfate (60 %) (More et al. 2019) with gentle stirring on the ice for 30 minutes and then centrifuged at 10,000 rpm at 4 °C for 20 minutes. The pellets of their fractions were then collected and resolubilized with a phosphate buffer of pH 8.0 (Sari et al. 2018).

A 100 µL aliquot of the soluble pellet was combined with 1.8 mL of 1 % casein and 2 mL of phosphate buffer (pH 8) in a test tube. Following a 5-minute incubation at 37 °C, the reaction was terminated by the addition of 10 % trichloroacetic acid (TCA), and the resulting mixture was centrifuged at 6000 rpm for 5 to 10 minutes. Subsequently, 2 to 3 mL of the supernatant was transferred to a tube containing 5 mL of 37 % formaldehyde solution. After thorough mixing and incubation at room temperature for 10 minutes, 1 mL of Folin reagent was added. Finally, absorbance was a 30-minute dark incubation prior to spectrophotometrically determined at 600 nm.

Determination of antioxidant activity

The ability to scavenge free radicals was evaluated using the DPPH assay, as previously described (Baliyan et al. 2022). The prepared supernatant served as the source material for antioxidant activity analysis. First, 750 µL of a 0.1 mM DPPH solution was mixed with 250 µL of the diluted sample or standard. After thorough agitation, the reaction mixtures were held in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a Biotek Synergy HT 96-well plate reader (USA) in a 96 well plate. The IC_{50}, defined as the sample concentration needed to achieve 50 % DPPH free radical inhibition, was used to quantify antioxidant activity.

Determination of anti-nutritional factors

Determination of oxalate content

The oxalate content of the pineapple samples was quantitatively evaluated by titration method (Karamad et al. 2019). An amount of 0.25 g of each pineapple sample was placed into a 250 mL Erlenmeyer flask containing 50 mL of de-ionized water. Subsequently, 5 mL of concentrated sulfuric acid was introduced, and the resulting mixture was heated in an 80 °C water bath. Before titrating the samples, a 0.1 N potassium permanganate ($KMNO_4$) solution was standardized against a 0.1 N oxalic acid standard. Following filtration of the treated sample through Whatman No. 1 filter paper, the filtrate was titrated with the standardized 0.1 N $KMnO_4$ solution until a persistent pale pink endpoint was observed, lasting a minimum of 30 seconds.

Determination of total tannin content

The determination of total tannin content was conducted using the colorimetric method (Siqueira et al. 2012). A calibration curve was generated using serial dilutions of a 0.1 mg mL^{-1} tannic acid stock. The analytical procedure involved combining 0.1 mL of the pineapple sample with 500 µL of Folin-Ciocalteu reagent and 1 mL of 35 % sodium carbonate (Na_2CO_3) solution. Subsequently, deionized water was added to achieve a final volume of 10 mL. After a 5-minute vortex, the mixture was held in the dark at ambient temperature for 30 minutes. Absorbance was then recorded spectrophotometrically at 725 nm against a blank using a Biotek Synergy HT 96-well plate reader (USA). The total tannin content was reported as milligrams of tannic acid equivalent (TAE) per gram of sample.

Statistical analysis

All experiments were conducted in triplicate for statistical analysis. The data were statistically analysed using SPSS (version 22.0) software by the Kruskal-Wallis test and Dunn's test with the threshold of statistical significance at $\rho < 0.05$ and expressed as the MEAN ± STDEV.

RESULTS AND DISCUSSION

Essential nutrients

Carbohydrate, fibre, and moisture indices of pineapple varieties are presented in Table 1 whereas fat was absent in all studied species. The analyses of variance revealed significant differences ($\rho < 0.05$) in all nutrient values, except for carbohydrate and protein contents ($\rho = 0.72$).

The carbohydrate values were derived from the calibration curve of glucose, $y = 0.008x + 0.129$ ($R^2 = 0.997$), with the highest content in QAC, fol-

Table 1. Nutritional values of three different pineapple varieties.

Parameters	**Quantity** (g/100 g)		
	Cayenne pineapple	**Queen pineapple**	**MD2 pineapple**
Carbohydrate	9.96 ± 0.67[a]	11.02 ± 2.56[a]	10.74 ± 0.87[a]
Fat	-ND-	-ND-	-ND-
Protein	0.86 ± 0.21[a]	0.72 ± 0.03[a]	0.64 ± 0.07[a]
Fiber	1.05 ± 0.08[ab]	1.20 ± 0.05[a]	0.91 ± 0.05[b]
Moisture	86.26 ± 0.40[a]	87.60 ± 0.61[ab]	93.15 ± 0.93[b]
Energy (kcal/100 g)	44.00	47.00	48.50

-ND-: not detected.

[a,b] Different letters in the same row indicate significant differences in means ($\rho \leq 0.05$)

lowed by MD2 and CAC. The amount of carbohydrates found in pineapples from Bangladesh was about 13.7 g/100 g in a previous study (Farid Hossain 2015), slightly higher than the carbohydrate content in the three pineapple species planted in Vietnam.

There was no significant difference in protein among the tested samples. It should be noted that the pineapples are generally not rich in protein, with a typical content of approximately 0.54 g/100 g as reported by the US Department of Agriculture (USDA). However, the lowest protein index found in MD2 was greater than that of the USDA document. The highest protein content was recorded in CAC (0.86 ± 0.21 g/100g).

The fibre content in all three pineapple species was significantly different ($\rho \geq 0.005$). The USDA reported that pineapple has a dietary fibre content of 1.4 g/100 g, slightly higher than that of QAC- the highest fibre content in this study (1.20 ± 0.05 g/100 g), followed by CAC and MD2.

The moisture content was higher than 80 % in all studied samples, while non-fat and low-calorie (44.00 - 48.50 kcal/100 g) were recorded, which is suitable for people aiming to control weight.

In a previous study A P & P M 2020, the MD2 pineapple species from Kerala revealed that those samples contained approximately equal amounts of carbohydrate and protein as this study at 10.873 ± 0.194 g/100g and 0.66 ± 0.02 g/100g, respectively, while indicating much higher fibre content and additionally documented the presence of crude fat content.

Vitamins and minerals

Overall, all fat-soluble vitamins and certain B-group vitamins, including B2, B3, B9, and B12, were not detected in all tested species (Table 2). These results were relatively similar to the data of the USDA pineapple record.

Vitamins B1 and B6 were only found in MD2 sample with the values of 0.20 ± (1 x 10^{-3}) mg/100 g and 0.19 ± 0.06 mg/100 g, which respectively were 2.5 and 1.5 times higher than that of the USDA recorded (0.079 and 0.112 mg/100 g, respectively). Various studies have indicated that these vita-

Table 2. Vitamin contents of three different pineapple species.

Vitamins	Quantity (mg/100 g)		
	Cayenne pineapple	Queen pineapple	MD2 pineapple
A	-ND-	-ND-	-ND-
B1	-ND-	-ND-	0.20 ± (1 x 10^{-3})
B2	-ND-	-ND-	-ND-
B3	-ND-	-ND-	-ND-
B5	0.24 ± 0.08^{a}	0.23 ± 0.05^{a}	0.60 ± 0.04^{b}
B6	-ND-	-ND-	0.19 ± 0.06
B9	-ND-	-ND-	-ND-
B12	-ND-	-ND-	-ND-
C	31.10 ± 0.20^{a}	30.20 ± 0.20^{b}	12.56 ± 0.30^{c}
D	-ND-	-ND-	-ND-
E	-ND-	-ND-	-ND-
K	-ND-	-ND-	-ND-

-ND-: not detected (LOD ≤ 0.01)

^{a,b,c} Different letters in the same row indicate significant differences in means ($\rho \leq 0.05$)

mins play essential roles in maintaining the central nervous system (CNS) and the peripheral nervous system (PNS). Notably, the combination of vitamins B1, B6, and B12 synergistically improves neuropathy, nociceptive, and neuropathic pain (Calderón-Ospina & Nava-Mesa 2020).

Vitamin B5, found in all three pineapple varieties, ranged from 0.23 mg per 100 g (QAC) to 0.60 mg per 100 g (MD2), which were nearly thrice as much as that of the USDA documented (0.21 mg/100 g). Vitamin B5 is a precursor of coenzyme A (CoA), which plays a crucial role in energy metabolism and fatty acid oxidation (Bourgin et al. 2022).

Vitamin C in MD2 (12.56 ± 0.30 mg/100 g) was recorded at the lowest value, which was less than a half of CAC (31.10 ± 0.20 mg/100 g) and QAC (30.20 ± 0.20 mg/100 g). These documented vitamin C contents were generally lower than that of the USDA recorded (average valued at 47.80 mg/100 g). Nevertheless, these results were significantly higher than the data conducted by previous research on two species of Bangladesh pineapple, which reported the vitamin C contents ranged from 9.62 ± 0.50 to 12.69 ± 0.20 mg/100 g (Kader et al. 2013); and from 6.45 ± 0.68 to 18.88 ± 0.03 mg/100 g for two pineapple species from Bangkok, Thailand (Kongsuwan et al. 2009).

Half of the studied essential minerals were detected (Table 3). The analysis of variance revealed significant differences ($\rho < 0.05$) among the three pineapple varieties. Three minerals (including Ca, K, and Mg) were identified in all pineapple varieties. QAC was found to be rich in calcium, with a value of 37.90 ± 0.20 mg/100 g, followed by CAC (36.30 ± 0.20 mg/100 g), significantly greater than the USDA record (6-19 mg/100 g). These species potentially provide rich sources of calcium compared to some other well-known calcium-rich fruits, such as tangerines (37 mg/100 g), oranges (40 mg/100 g), and pears (56 mg/100 g). Calcium is essential for healthy bones, blood clotting, nerve signalling, muscle contraction, and hormone release. Additionally, it lowers the risk of some chronic diseases and helps control blood pressure

Table 3. Mineral contents in three different pineapple species.

Minerals	Quantity (mg/100 g)		
	Cayenne pineapple	**Queen pineapple**	**MD2 pineapple**
Calcium (Ca)	36.30 ± 0.20[a]	37.90 ± 0.20[b]	4.87 ± 0.40[c]
Chlorine (Cl)	340.00 ± 3.27	300.00 ± 3.72	-ND-
Potassium (K)	126.00 ± 0.20[a]	120.00 ± 0.40[b]	128.00 ± 0.20[c]
Magnesium (Mg)	13.50 ± 0.01[a]	12.80 ± 0.03[b]	11.80 ± 0.01[c]
Manganese (Mn)	-ND-	-ND-	-ND-
Sodium (Na)	1.06 ± 0.02	1.53 ± 0.04	-ND-
Phosphorus (P)	-ND-	-ND-	-ND-
Selenium (Se)	-ND-	-ND-	-ND-
Zinc (Zn)	-ND-	-ND-	-ND-
Copper (Cu)	-ND-	-ND-	-ND-
Iron (Fe)	0.24 ± 0.01	-ND-	0.26 ± 0.01
Iodine (I)	-ND-	-ND-	-ND-

-ND-: not detected (LOD ≤ 0.01)

[a,b,c] *Different letters in the same row indicate significant differences in means ($\rho \leq 0.05$)*

(Institute of Medicine 1998).

Both chlorine and sodium were found in QAC and CAC, but not MD2. Notably, both these species had low quantities of sodium, but extremely high concentrations of chlorine (300.00 ± 3.72 and 340.00 ± 3.27 mg/100 g, respectively). In addition to sodium's functions, chlorine, the most abundant electrolyte in blood serum, plays a vital role in the regulation of body fluids, electrolytes, electrical neutrality, acid-base balance, and participation in many other pathways (Berend et al. 2012). Meanwhile, the analysis of three tested pineapple species revealed moderate concentrations of potassium with the value of 120.00 ± 0.40 mg/100 g for QAC to 128.00 ± 0.20 mg/100 g for MD2 which is consistent with the USDA record ranging from 81- 162 mg/100 g.

Phytochemicals and antioxidant activity

Regarding their anti-inflammatory and antioxidant properties, polyphenols and flavonoids play a crucial role in mitigating the risk of inflammation and chronic diseases, including cardiovascular disorders. Using gallic acid and quercetin as standards for calibration in TPC and TFC quantification, the result was observed that the pineapple varieties exhibited moderate levels of both phenolic and flavonoid content. However, significant differences in concentration were noted for TPC ($\rho = 0.002$) but not TFC ($\rho = 0.012$), typically CAC pineapple had the highest value of TPC (21.93 ± 2.72 mg GEA/g) while MD2 showed the highest TFC (6.15 ± 0.77 mg QE/g) whereas QAC exerted the lowest amount of both TPC (12.75 ± 1.08 mg GEA/g) and TFC (3.26 ± 0.71 mg QE/g) (Table 4).

Bromelain is a natural enzyme that is believed to have many health benefits, including the ability to help digest protein, reduce inflammation and pain, and aid in the body's cleansing process and digestive issues (Rathnavelu et al. 2016). Studies in Vietnam and around the world have also shown that the enzyme bromelain has anti-inflammatory properties and is used effectively to reduce symptoms of arthritis, including rheumatoid arthritis. In this study, the QAC sample possessed the highest bromelain value of 1.56 ± 0.03 UI/g, whereas MD2 had the lowest bromelain concentration with a value of 0.92 ± 0.14 UI/g. In general, a person can consume up to 12 g of bromelain daily without any major side effects (Pavan et al. 2012).

The IC_{50} value, indicative of the sample concentration required to inhibit 50 % of DPPH free radicals, was used to quantify the antioxidant activity of three pineapple species. Consistent with published literature, which indicates a relationship between phenolic and flavonoid content and antioxidant activity, the tested species demonstrated a moderate level of antioxidant activity when compared to ascorbic acid (Table 5). However, there was a significant difference in antioxidant activity among the three pineapple species ($\rho < 0.05$). MD2 sample had the strongest antioxidant activity (IC_{50} = 99.17 ± 0.44 µg mL^{-1}) whereas the QAC sample exerted the weakest IC_{50} of 162.70 ± 0.24 µg mL^{-1}.

Table 4. Phytochemicals and antioxidant activity of three different pineapple species.

	Calibration curve (R^2)	Cayenne pineapple	Queen pineapple	MD2 pineapple
Total phenolic (mg GEA/g)	y = 0.003x + 0.194 R^2 = 0.971	21.93 ± 2.72	12.75 ± 1.08	16.96 ± 0.94
Total flavonoid (mg QE/g)	y = 0.004x + 0.067 R^2 = 0.998	5.05 ± 0.91	3.26 ± 0.71	6.15 ± 0.77
Bromelain (UI/g) ($\rho = 0.0004$)		1.56 ± 0.03	1.36 ± 0.07	0.92 ± 0.14

Anti-nutritional factors

There were extremely low amounts of oxalate and no signals showing the presence of tannin in all tested species (Table 6). The American Academy of Nutrition and Dietetics advises patients with kidney stones to limit their daily oxalate intake to below 50 mg (Bernardino & Parmar 2017). The oxalate values recorded in the three species were approximately 130-206 times lower than the standard and also confirmed no significant differences among them ($\rho = 0.165$). These pineapple species are generally considered safe for human consumption, even for people with kidney problems or abnormal iron absorption.

CONCLUSIONS

In conclusion, no significant differences in energy-yielding nutrients were observed among the three pineapple species; however, significant variations were recorded in their vitamin and mineral content. All three studied pineapple species generally possessed moderate values of phenolics, flavonoids, low concentrations of bromelain, and weak antioxidants. The contents of tannin and oxalate were not significant and thus generally considered safe for daily consumption. These findings provide well-informed options for nutritionists/ dietitians, food manufacturers, and consumers. Moreover, this study aligns with a strategic focus on maximizing the utility of pineapple resources through a two-pronged approach. By simultaneously increasing production for broader market penetration and meticulously analysing bromelain enzyme content for innovative product development, this research contributes to both economic expansion and biotechnological advancement. The integration of agricultural intensification with targeted biochemical research exemplifies a commitment to value-added processing, ultimately enhancing the overall contribution of pineapples to both domestic and international markets within the food and pharmaceutical sectors.

Table 5. Antioxidant activity in three pineapple species.

Sample/ Reference	Linear regression equation	IC_{50} (µg mL^{-1})
Ascorbic acid	$y = 9.677x + 26.578$ ($R^2 = 0.995$)	2.45 ± 0.04
Cayenne pineapple	$y = 0.280x + 12.547$ ($R^2 = 0.995$)	133.40 ± 0.46
Queen pineapple	$y = 0.259x + 7.742$ ($R^2 = 0.994$)	162.70 ± 0.24
MD2 pineapple	$y = 0.217x + 28.420$ ($R^2 = 0.996$)	99.17 ± 0.44

Table 6. Anti-nutrient contents in three pineapple species.

	Quantity (g/100 g)		
	Cayenne pineapple	**Queen pineapple**	**MD2 pineapple**
Oxalate	0.24 ± 0.09	0.32 ± 0.09	0.38 ± 0.05
Tannin	-ND-	-ND-	-ND-

-ND-: not detected.

AUTHOR CONTRIBUTION

SLH and TNKL conceived, formulated, supervised, designed the research, as well as wrote the manuscript. TNKL and NBH performed and analysed the data. All authors have read and approved this manuscript.

ACKNOWLEDGMENTS

This study was supported by the Department of Applied Biochemistry, Faculty of Biotechnology- International University- National University, Ho Chi Minh City, Viet Nam.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest, and this study received no funding from any individuals or organizations.

REFERENCES

A.P., N., & P.M., R., 2020. Evaluation of Proximate and Mineral Constituents in Different Commercial Cultivars and Local Varieties of Ananas comosus (L.) Merr. From Kerala. *International Journal of Fruit Science*, 20(3), pp.620–634. doi: 10.1080/15538362.2019.1628683

Andrieux, P., et al.,2013. Pantothenic Acid (Vitamin B5) in Infant Formula and Adult/Pediatric Nutritional Formula: First Action 2012.16. *Journal of AOAC INTERNATIONAL*, 96(3), pp.497–499. doi: 10.5740/jaoacint.13-054

AOAC 978.10, 2000. *Fiber (Crude) in Animal Feed and Pet Food.* Official Methods of Analysis of AOAC International.

AOAC 931.04, 2000. *Moisture in Cacao Products.* Official Methods of Analysis of AOAC International.

AOAC 940.26, 2000. *Ash of Fruits and Fruit Products.* Official Methods of Analysis of AOAC International.

AOAC 961.14, 1989. *Niacin and Niacinamide in Drugs, Foods, and Feed- Colorimetric Method.* Official Methods of Analysis of AOAC International.

AOAC 968.08, 2000. *Minerals in Animal Feed and Pet Food.* Official Methods of Analysis of AOAC International.

AOAC 969.23, 2005. *Sodium and Potassium in Seafood.* Official Methods of Analysis of AOAC International.

AOAC 988.12, 1990. *Phenol–Sulfuric Acid Assay for Total Carbohydrate Determination.* Official Methods of Analysis of AOAC International.

AOAC 991.20, 2005. *Nitrogen (Total) in Milk.* Official Methods of Analysis of AOAC International.

AOAC 995.11, 2000. *Determination of phosphorus content—Spectrophotometric method.* Official Methods of Analysis of AOAC International.

AOAC 2001.11., 2005. *Protein (Crude) in Animal Feed, Forage (Plant Tissue), Grain, and Oilseeds.* Official Methods of Analysis of AOAC International.

AOAC 2001.13, 2011. *Vitamin A (Retinol) in Foods.* Official Methods of Analysis of AOAC International.

AOAC 2003.05, 2005. *Crude Fat in Feeds, Cereal Grains, and Forages.* Official Methods of Analysis of AOAC International.

AOAC 2003.06, 2005. *Crude Fat in Feeds, Cereal Grains, and Forages.* Official Methods of Analysis of AOAC International.

AOAC 2011.06, 2011. *Folate in Infant Formula and Adult/Pediatric Nutritional Formula.* Official Methods of Analysis of AOAC International.

AOAC 2011.10, 2011. *Vitamin B12 in Infant Formula and Adult Nutritionals.* Official Methods of Analysis of AOAC International.

AOAC 2012.21, 2013. *Vitamin C in Infant Formula and Adult/Pediatric Nutritional Formula.* Official Methods of Analysis of AOAC International.

AOAC 2015.01, 2015. *Heavy Metals in Food*. Official Methods of Analysis of AOAC International.

Baliyan, S. et al., 2022. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. *Molecules*, 27(4), 1326. doi: 10.3390/molecules27041326

Berend, K., Van Hulsteijn, L.H., & Gans, R.O.B., 2012. Chloride: The queen of electrolytes. *European Journal of Internal Medicine*, 23(3), pp.203–211. doi: 10.1016/j.ejim.2011.11.013

Bernardino, M. & Parmar, M. S., 2017. Oxalate nephropathy from cashew nut intake. *Canadian Medical Association Journal*, 189(10), pp.e405–408. doi: 10.1503/cmaj.151327

Bourgin, M., Kepp, O. & Kroemer, G., 2022. Immunostimulatory effects of vitamin B5 improve anticancer immunotherapy. *OncoImmunology*, 11(1), 2031500. doi: 10.1080/2162402X.2022.2031500

Calderón-Ospina, C.A. & Nava-Mesa, M.O., 2020. B Vitamins in the nervous system: Current knowledge of the biochemical modes of action and synergies of thiamine, pyridoxine, and cobalamin. *CNS Neuroscience & Therapeutics*, 26(1), pp.5–13. doi: 10.1111/cns.13207

Chang, C.-C., et al., 2002. Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, 10(3), Article 3. doi: 10.38212/2224-6614.2748

De Ancos, B., Sánchez-Moreno, C. & González-Aguilar, G.A., 2017. Pineapple composition and nutrition. In *Handbook of Pineapple Technology, 1st ed.* Wiley, pp.221–239. doi: 10.1002/9781118967355.ch12

Delmonte, P., Barrientos, S. & Rader, J.I., 2013. Modifications of AOAC Official Method 999.15 to Improve the Quantitation of Vitamin K1 in Complex Formulated Nutritional Products. *Journal of AOAC INTERNATIONAL*, 96(1), pp.91–101. doi: 10.5740/jaoacint.12-191

EN 14122-2003, 2003. *Determination of Vitamin B1 by HPLC*. European Standard.

Farid Hossain, Md., 2015. Nutritional Value and Medicinal Benefits of Pineapple. *International Journal of Nutrition and Food Sciences*, 4(1), 84. doi: 10.11648/j.ijnfs.20150401.22

Hossain, M.F. et al., 2019. A Simplified, Specific HPLC Method of Assaying Thiamine and Riboflavin in Mushrooms. *International Journal of Food Science*, 2019(1), 8716986. doi: 10.1155/2019/8716986

Institute of Medicine, 1998. *Dietary Reference Intakes: A Risk Assessment Model for Establishing Upper Intake Levels for Nutrients*. Washington, D.C.: National Academies Press. doi: 10.17226/6432

Joy, P.P., 2010. *Benefits and uses of pineapple*. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam District, Kerala, India. doi: 10.13140/RG.2.1.2782.4888

Joy, P.P. & Anjana R., 2017. Chapter 16: Evolution of Pineapple. In *Genesis and Evolution of Horticultural Crops, Ed. 1*. Kruger Brentt, pp.263-295.

Kader, A. et al., 2013. A comparative analysis on the nutritional contents of two varieties of pineapple of Chittagong region. *Chittagong University Journal of Science*, 5(1), pp.105–112. doi: 10.3329/cujbs.v5i1.13375

Karamad, D. et al., 2019. Analytical procedures and methods validation for oxalate content estimation. *Biointerface Research in Applied Chemistry*, 9 (5), pp.4305–4310. doi: 10.33263/BRIAC95.305310

Kongsuwan, A., et al., 2009. Bioactive compounds and antioxidant capacities of phulae and nanglae pineapple. *Journal of Agricultural and Food Industrial Organization*, Special issue, pp.44–50.

Mann, D. L., et al., 2005. Liquid Chromatographic Analysis of Vitamin B6 in Reconstituted Infant Formula: Collaborative Study. *Journal of AOAC INTERNATIONAL*, 88(1), pp.30–37. doi: 10.1093/jaoac/88.1.30

More, K. et al., 2019. Extraction and Partial Purification of Bromelain from Fruit and Crown of Pineapple (Ananas comosus) and It's Application as a Meat Tenderizer. *International Journal of Pharmacy and Biological Sciences*, 9(3), pp.1361–1367. doi: 10.21276/ijpbs.2019.9.3.1

Nelson, J., et al., 2019. Simultaneous Analysis of Iodine and Bromine Species in Infant Formula using HPLC-ICP-MS. *Journal of AOAC INTERNATIONAL*, 102(4), pp.1199–1204. doi: 10.5740/jaoacint.18-0352

Nielsen, S. S., 2010. Phenol-Sulfuric Acid Method for Total Carbohydrates (S. S. Nielsen, Ed.). Springer, Boston, MA: Springer International Publishing.

Nordtest method, 1996, 'Concrete, Hardened: Chloride Content by Volhard Titration (NT BUILD 208)', in *NORDTEST*, viewed November 27 2023, from https://www.nordtest.info/wp/1996/11/28/concrete-hardened-chloride-content-by-volhard-titration-nt-build-208/

Ojike, O., Okonkwo, W.I. & Ime, C., 2020. Effect of Drying Temperatures on the Vitamin C Content of Pineapple Fruit (Ananas comosus). *Proceedings of the 2020 Sustainable Engineering & Industrial Technology Conference*, 12, pp.1–4.

Pavan, R. et al., 2012. Properties and Therapeutic Application of Bromelain: A Review. *Biotechnology Research International*, 2012(1), 976203. doi: 10.1155/2012/976203

Rathnavelu, V. et al., 2016. Potential role of bromelain in clinical and therapeutic applications. *Biomedical Reports*, 5(3), pp.283–288. doi: 10.3892/br.2016.720

Redwan, R.M., Saidin, A. & Kumar, S.V., 2018. The Draft Genome of the MD-2 Pineapple. In *Genetics and Genomics of Pineapple*. Springer Switzerland, pp.109–129. doi: 10.1007/978-3-030-00614-3_9

Sari, A.A. et al., 2018. Isolation and purification of bromelain from pineapple core (Ananas comosus L. Merr) by ammonium sulfate and ethanol precipitation. *AIP Conference Proceedings*, 2023, 020076. doi: 10.1063/1.5064073

Shraim, A.M., et al., 2021. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT*, 150, 111932. doi: 10.1016/j.lwt.2021.111932

Siddiqui, N. et al., 2017. Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). *Journal of Taibah University Medical Sciences*, 12(4), pp.360–363. doi: 10.1016/j.jtumed.2016.11.006

Siqueira, C.F.D.Q. et al., 2012. Levels of Tannins and Flavonoids in Medicinal Plants: Evaluating Bioprospecting Strategies. *Evidence-Based Complementary and Alternative Medicine*, 2012(1), 434782. doi: 10.1155/2012/434782

Thiex, N., Novotny, L. & Crawford, A., 2012. Determination of Ash in Animal Feed: AOAC Official Method 942.05 Revisited. *Journal of AOAC INTERNATIONAL*, 95(5), pp.1392–1397. doi: 10.5740/jaoacint.12-129

Vries, E.J.D. et al., 1979. Analysis of Fat-Soluble Vitamins. XXIII. High Performance Liquid Chromatographic Assay for Vitamin D in Vitamin D3 and Multivitamin Preparations. *Journal of AOAC INTERNATIONAL*, 62(6), pp.1285–1291. doi: 10.1093/jaoac/62.6.1285

Wali, N., 2019. Pineapple (Ananas comosus). In *Nonvitamin and Nonmineral Nutritional Supplements*. Academic Press, pp.367–373. doi: 10.1016/B978-0-12-812491-8.00050-3.