Journal of Food Technology Research

2025 Vol. 12, No. 4, pp. 266-277 ISSN(e): 2312-3796 ISSN(p): 2312-6426 DOI: 10.18488/jftr.v12i4.4602 © 2025 Conscientia Beam. All Rights Reserved.



Effect of fermentation on the nutritional composition antioxidant properties and antinutritional content of pineapple ananas comosus fruit processing waste

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ABSTRACT

Article History Received: 30 May 2025 Revised: 15 August 2025 Accepted: 1 December 2025 Published: 23 December 2025

Keywords

Anti-nutritional content Antioxidant activity Beneficial compounds DPPH assay Molasses concentration Phytochemical content Proximate composition. The Philippines is among the top global producers of pineapple (Ananas comosus), selling it as fresh fruit and canned products in local and international markets. Escalating demand for these products has resulted in the accumulation of processing waste in pineapple plantations and nearby landfills, leading to environmental hazards. This pressing scenario calls for the value addition of pineapple fruit processing waste (PFPW) as a sustainable waste management strategy. The study assessed the viability of using molasses to ferment PFPW. Molasses concentrations of 0%, 10%, 20%, and 30% were tested to enhance the proximate composition, phytochemical content, antioxidant activity (using DPPH assay), and to reduce the anti-nutritional content of PFPW. Significant results indicated a positive effect of fermentation on the proximate composition of PFPW, resulting in increased carbohydrates (3-15%), crude protein (7-45%), crude fat (6-52%), ash (93-238%), and reduced fiber (9-50%) compared to unfermented PFPW. Additionally, there was a very significant increase in phytochemical content: total phenolic content (TPC) by 105-395% and total flavonoid content (TFC) by 306-744%, leading to increased free radical scavenging activity of the fermented samples by 67-192%. Furthermore, reductions in anti-nutritional content, such as tannin by 45-56% and phytate by 10-40%, were also observed. These findings suggest that fermented PFPW can serve as a potential low-cost aquaculture feed additive, contributing to sustainable waste management and resource utilization.

Contribution/Originality: This study employs spontaneous fermentation, a natural fermentation process. This type of fermentation depends entirely on the indigenous microbiota present in pineapple fruit processing waste to initiate and sustain fermentation. Molasses, a by-product of sugar production, serves as the source of fermentable sugars for microbial growth and energy.

1. INTRODUCTION

Pineapple (Ananas comosus) is a perennial plant classified within the Bromeliaceae family. It has been ranked as the third most widely consumed fruit globally, following bananas and citrus fruits (FAOSTAT, 2022). Global annual pineapple production approaches 28 million tons, with the Philippines, Brazil, Costa Rica, China, and Indonesia identified as major producing countries. Specifically, pineapple production in the Philippines for the second quarter of 2023 was approximately 762.55 thousand metric tons. The province of Bukidnon, situated in Northern Mindanao (Region X), was the top contributor, yielding 391.16 thousand metric tons, which constituted 51.3% of the total production for that quarter (Philippine Statistics Authority, 2023). South Cotabato in the SOCCSKSARGEN region (Region XII) ranks second with a production of 189.29 thousand metric tons (24.8%), and the Bicol Region, contributing 62.13 thousand metric tons (8.1%), thus completing the top three pineapple-producing regions in the Philippines.

The global demand for fresh and processed pineapple products has consistently increased, leading to a significant annual rise in waste generated, including discarded fruits and residues from harvesting and processing (Roda & Lambri, 2019; Sarangi et al., 2022). Difonzo et al. (2019) illustrate this trend, indicating that approximately 60% of pineapple fruit is edible, with processing residuals constituting 45% to 65% of the fruit, which includes pulp, peels, stems, pomace, and crowns. These underutilized raw materials are frequently disposed of in landfills, contributing to significant environmental challenges, particularly concerning greenhouse gas emissions (Gomez-Garcia, Campos, Aguilar, Madureira, & Pintado, 2021). The disposal of fruit processing waste represents a considerable loss of valuable biomass and nutrients within processing industries (Khedkar & Singh, 2018).

Accordingly, pineapple processing by-products are enriched raw materials containing protein, pectin, insoluble fiber, and simple sugars as their main compounds, with considerable levels of vitamins, minerals, and phenolic compounds that can be exploited for various uses by different sectors (Banerjee, Ranganathan, Patti, & Arora, 2018). The leaves have been noted to contain citric and ferulic acid; peels, leaves, and stems contain bromelain, and the core contains ascorbic acid (Banerjee et al., 2022; FAO-FAOSTAT, 2019). Moreover, fresh pineapple peels are characterized by their composition of soluble and insoluble dietary fibers, predominantly cellulose and hemicellulose (Campos, Ribeiro, Teixeira, Pastrana, & Pintado, 2020). Additionally, pineapple by-products contain proteins, vitamins, minerals, pectins, sugars, phenolic compounds (gallic acid, epicatechin, catechin, ferulic acid), bioactive compounds (phenols and flavonoids), and polyphenols (syringic and ferulic acid) that exhibit potent antioxidant and antimicrobial activities (Banerjee et al., 2017; Diaz-Vela, Totosaus, Cruz-Guerrero, & De Lourdes Pérez-Chabela, 2013; Li et al., 2014; Mirabella, Castellani, & Sala, 2014; Rivera et al., 2023; Upadhyay, Lama, & Tawata, 2010).

Addressing the issue of fruit waste management is of critical importance, as fruit processing waste represents a significant environmental pollutant (Mo, Choi, Man, & Wong, 2020). One promising approach to mitigate this concern is to optimize the utilization of processing by-products into value-added products through fermentation. Fermentation is a natural biochemical process that can enhance both the nutritional and functional properties of organic substrates while concurrently reducing waste volume. Common farm practice for fermentation is through the addition of molasses, a by-product of sugar production. It is considered a cost-effective and nutrient-rich additive due to its high content of sugars, vitamins, and minerals that can stimulate microbial growth and activity. Thus, it facilitates the conversion of pineapple fruit processing waste (PFPW) into a bioactive-rich fermented product.

This study focused on the natural fermentation of PFPW utilizing molasses as a carbon source, with a focus on molasses concentration as a factor affecting fermentation efficiency. This parameter has a direct influence on microbial activity and the biochemical transformation of the substrates. The conversion of PFPW into a value-added product has significance, as it enhances the economic value of these materials while serving as an effective waste management strategy. Consequently, we characterized the fermented pineapple fruit processing waste (PFPW), examining its dry matter proximate composition specifically carbohydrates, crude protein, crude fiber, crude fat, and ash as well as its phytochemical composition: total phenolic content (TPC) and total flavonoid content (TFC). Furthermore, we

assessed the antioxidant activity using the DPPH assay and determined the presence of anti-nutritional factors, specifically tannin and phytate.

2. MATERIALS AND METHODS

2.1. Pineapple fruit processing waste collection and fermentation preparation

Pineapple fruit processing waste (PFPW) was obtained from a local pineapple fruit processing plant in Cagayan de Oro City, Misamis Oriental, Philippines. PFPW was subjected to thorough washing to remove adhering dirt and possible contaminants and was slightly drained after washing, just enough to remove excess water. The cleaned PFPW was ground, weighed, and fermented, adopting the fermentation procedure of Syahirah and Nazaitulshila (2018) with modifications. The molasses concentration used to ferment per kilogram of PFPW was 0% (control), 10%, 20%, and 30%, at days of fermentation: Day 0 (control), Day 15, Day 30, and Day 60. Clear airtight 6-L capacity plastic containers were used as fermentation vessels. Manual shaking of each fermentation container was done daily throughout the fermentation duration.

2.2. Preparation of fermented PFPW powder for analyses

Fermented PFPW samples were subjected to sun drying after each fermentation period. The dried fermented PFPW was then ground into a fine powder and sieved using a 45-µm mesh stainless steel sieve. Powdered samples of fermented PFPW were weighed and kept in properly sealed containers, labeled with codes, and stored at room temperature until further analysis and use.

2.3. Proximate composition analyses

Representative samples of fermented PFPW from various days of fermentation and different molasses concentrations were subjected to analyses of their proximate composition: crude protein (Kjeldahl method), crude fat (modified Randall; AOAC 945.16 method), crude fiber (filter bag technology; AOCS Procedure Ba 6a-05 method), ash (ignition; AOAC 942.05 method), and moisture (using moisture analyzer; MOC63u) at the Integrated Laboratory Division, Feed Chemical Laboratory Analysis Section of the Department of Agriculture, Regional Field Office XI, Bago Oshiro, Mintal, Davao City, Philippines.

2.4. Determination of phytochemical composition and antioxidant activity

2.4.1. Preparation of Extract

Ultrasound-assisted extraction method, adopted from the study of Mahmood et al. (2019) with slight modifications, was the extraction process used in the study. Five grams of pulverized fermented pineapple fruit processing waste (PFPW) samples were added to 100 ml of 80% ethanol and placed in a sonicator powered at 20 kHz for 15 minutes at room temperature. The mixture was filtered after sonication and subjected to analyses to determine its phytochemical composition and antioxidant activity.

2.4.2. Antioxidant Activity

The free radical scavenging activity was measured in the study through the DPPH assay, employing the techniques outlined by Hossain and Rahman (2011) with modifications. In this methodology, approximately 1.6 mL of ethanolic extract of the fermented PFPW samples and 2.4 mL of 0.1 mM ethanolic solution of DPPH were mixed thoroughly. The control was prepared by mixing 1.6 mL of 80% ethanol and 2.4 mL of 0.1 mM ethanolic solution of DPPH. The mixture was incubated for 20 minutes in the dark at room temperature. Changes in absorbance were determined at 517 nm using a UV-VIS spectrophotometer. The scavenging activity was calculated using the appropriate formula.

% Radical Scavenging Activity = (Control OD - sample OD/ control OD) x 100

2.4.3. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Folin-Ciocalteu reagent at a concentration of 0.50 mg/mL in distilled water was used in the determination of total polyphenol content. A 40- μ L extract was mixed with 200- μ L Folin-Ciocalteu's phenol reagent and 600- μ L of 20% sodium carbonate, then diluted with water to a total volume of 5 mL. Following a 2-hour incubation in the dark at room temperature, the absorbance of the blue-colored solution was measured using a UV-VIS spectrophotometer at 765 nm. The TPC quantification was performed using linear regression analysis, with gallic acid as the standard reference (0-1000 mg L⁻¹). The analytical results were expressed as milligrams of gallic acid equivalents (GAE/g) per gram of sample.

The determination of total flavonoid content (TFC) was measured using the reaction of the aluminum trichloride reagent with catechin as the reference compound as described (Zhishen, Mengcheng, & Jianming, 1999). A volume of 1000 μ L fermented PFPW extract was added to 300 μ L of 5% NaNO₂ solution and allowed to stand for 6 minutes; then, it was added with 300 μ L of 10% aluminum trichloride and left to incubate for 5 minutes. After the incubation period, 2 mL of 1 M NaOH was added to the solution, thereby adjusting with distilled water to achieve a total volume of 5 mL. Samples were further incubated for 15 minutes, after which the color of the solution was observed, and the absorbance was measured using a UV-VIS spectrophotometer at 510 nm. TFC concentration was evaluated using catechin as a standard ranging from 0-500 mg L⁻¹. Total flavonoid content was expressed as mg CE/100 g⁻¹ dry sample.

2.5. Determination of anti-nutritional contents

2.5.1. Total tannin content

The tannin content of each sample was determined using modified Folin and Ciocalteu colorimetric method as described by Islam et al. (2015). A standard (tannic acid) solution of six different concentrations, namely: 6.25, 12.50, 25.0, 50.0, 100.0, and 200.0 µg/ml, and a sample extract of 0.1 ml were taken from the different labeled tubes. Then, 7.5 ml distilled water, 0.5 ml Folin–Ciocalteu reagent, and 1 ml of 35% Na₂CO₃ solution were added, thereby the volume was finally adjusted up to 10 ml with distilled water. The mixture was well shaken and kept at room temperature for 30 minutes. Using a UV-Vis spectrophotometer, absorbance was determined at 725 nm against a blank. Total tannin content was expressed as mg Tannic Acid Equivalent (mg TAE/100 g sample).

2.5.2. Phytate content

The phytate content determination of each sample was performed using the Young and Greaves method (1940) as adopted by Lucas and Markakis (1975). Following the method, a 0.2g sample was weighed into a conical flask with a 250-ml capacity. Weighed samples were soaked in 100-ml of 20% concentrated hydrochloric acid (HCl) for 3 hours. After soaking, the samples were then filtered. A 50-ml filtrate of each sample was placed in a 250-ml beaker and added with 100-ml distilled water. A 10-ml portion of 0.3% ammonium thiocyanate solution was then added as an indicator and titrated with a standard iron (III) chloride solution containing 0.00195g iron/ml. Calculation of phytate content was done using the formula:

Phytic acid = $\underline{\text{Titre value x } 0.00195 \text{ x } 1.19 \text{ x } 100}$

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2.6. Statistical Analysis

Obtained data from different laboratory analyses were analyzed first by homogeneity test of variances. Data accorded to have homogeneity of variance were analyzed using one-way analysis of variance (ANOVA) and Kruskal-Wallis test for data having non-homogeneous variances using JASP software version 0.18.3 (copyright 2013-2024 University of Amsterdam). The statistical difference was evaluated using Tukey's post hoc test to compare significant differences between groups at 5% level of significance ($P \le 0.05$).

3. RESULTS AND DISCUSSION

Fermentation is an anaerobic biochemical process with the fundamental concept of introducing beneficial microorganisms (bacteria, yeast, or fungi) into a substrate to convert sugars and other organic compounds into various products. Like yeast, which converts sugars and starches into alcohol, proteins are converted into peptides and amino acids through enzymatic hydrolysis. Several studies have mentioned using starter cultures of fungi and bacteria in the fermentation process, such as the bioconversion of cocoa by-products to obtain enzymes, polysaccharides, beverages, and nutraceuticals, as concluded in the review conducted by Vásquez et al. (2019); enhancement of the biochemical composition of pineapple wastes (Aruna, 2019; Chaudhary et al., 2019; Rashad, Mahmoud, Ali, Nooman, & Al-Kashef, 2015) feed silage fermentation (Ávila & Carvalho, 2020) and production of high protein fungal biomass from solid state fermentation (SSF) of pineapple peels (Aruna, 2019). However, in the current study, we opted to use spontaneous fermentation, a natural fermentation process. This type of fermentation relies only on the autochthonous or naturally occurring microbiota, present in the processing waste/by-products to initiate and carry out fermentation (Verni, Rizzello, & Coda, 2019). Molasses, a by-product of sugar production, was then used as a source of fermentable sugars for growth and as an energy source for the indigenous microbiota present in pineapple fruit processing waste. Notably, molasses is an inexpensive, sugar-rich feedstock compared to glucose and other carbon sources, making it an ideal raw material for producing value-added products through fermentation. Moreover, molasses increased microbial activity and the bioavailability of nutrients, thereby boosting the fermentation process.

3.1. Proximate Composition

Findings of the article review by Rico, Gullón, Alonso, and Yáñez (2020) showed that the composition of pineapple processing by-products is 29–42% peel, 9.4–20% core, 2.4–6.8% stem, and 2.7–5.9% crown. These by-products are known as a rich source of beneficial compounds, including enzymes, dietary fiber, and antioxidants. Utilization of these by-products, specifically fresh and dried peel, as an ingredient in human food production has been reported in several studies (Chalchisa & Dereje, 2021; Roda et al., 2017; Singh, Dhar, Bhagya Raj, & Deka, 2022; Zhang et al., 2020).

In aquaculture, dried and powdered pineapple waste (core, leaves, and peel) and peel extract were used as alternative feed ingredients and functional feed additives to enhance the growth performance and health of fish species (Rahman et al., 2023; Sukri et al., 2022; Van Doan et al., 2021; Yuangsoi, Klahan, Charoenwattanasak, & Lin, 2018). In this study, pineapple fruit processing waste (mixed peel, core, and leaves) underwent fermentation with molasses (10%, 20%, 30%) at room temperature to enhance its nutritional components and bioactive compounds. The results of the present study showed a significant effect of fermentation on the proximate composition of pineapple fruit processing waste (PFPW), as presented in Table 1.

Carbohydrates increase by 3–15%; crude protein by 7–45%; crude fat by 6–52%; and ash by 93–238%, while fiber is reduced by 9–50% when compared to unfermented PFPW. The highest proximate composition values are recorded as follows: 70.23±2.52 g carbohydrates (with 30% molasses at fermentation period 30 days), 1.09±0.05 g crude fat (with 0% molasses at fermentation period 60 days), 6.65±0.28 g crude protein, and 8.11±0.11 g ash (with 30% molasses at fermentation period 15 days), while the lowest fiber content is 15.84±0.03 g (with 30% molasses at fermentation period 30 days).

Table 1. Proximate composition (g/100g) dry matter (D.M.) sample of fermented pineapple fruit processing waste (PFPW).

Molasses (%)	Fermentation period (Days)	Carbohydrates	Crude protein	Crude fat	Ash	Fiber
0	0	$60.82 \pm 2.52^{\mathrm{ab}}$	4.58±0.33°	0.63 ± 0.02^{ef}	2.40±0.23 ^h	31.57 ± 0.80^{b}
0	15	52.45 ± 1.42^{b}	5.16 ± 0.80^{abc}	0.99 ± 0.01^{ab}	2.02 ± 0.03^{h}	39.38±0.01ª
0	30	52.01±2.01 ^b	5.51 ± 0.4^{abc}	1.08 ± 0.04^{a}	2.26±0.10 ^h	39.14±0.01ª
0	60	$55.24 \pm 5.77^{\mathrm{ab}}$	$5.34 \pm 0.20^{ m abc}$	1.09±0.05a	2.23 ± 0.20^{h}	36.10 ± 0.00^{a}
10	15	$58.58 \pm 5.00^{\mathrm{ab}}$	$6.20\pm0.50^{\mathrm{ab}}$	$0.96\pm0.05^{\mathrm{ab}}$	5.62 ± 0.30^{ef}	$28.64 \pm 0.05^{\mathrm{bc}}$
10	30	63.30 ± 1.91^{ab}	5.51 ± 0.29^{abc}	0.86 ± 0.05^{bc}	4.95 ± 0.14^{fg}	$25.38 \pm 0.02^{\rm cd}$
10	60	66.37 ± 1.73^{ab}	$4.88\pm0.31^{\rm bc}$	$0.85 \pm 0.02^{\text{bcd}}$	4.64 ± 0.02^{g}	$23.25 \pm 0.13^{\text{de}}$
20	15	$62.80\pm1.16^{\mathrm{ab}}$	6.08 ± 0.01^{abc}	0.88 ± 0.01^{b}	$6.75\pm0.02^{\rm cd}$	$23.48 \pm 0.11^{\text{de}}$
20	30	$66.55 \pm 1.53^{\mathrm{ab}}$	5.64 ± 0.04^{abc}	$0.69 \pm 0.05^{\text{cde}}$	$7.20\pm0.05^{ m abc}$	19.93 ± 0.05 ^{ef}
20	60	68.72±3.51ª	5.05 ± 0.50^{bc}	$0.34\pm0.01g$	6.23 ± 0.10^{de}	$19.67 \pm 0.02^{\rm efg}$
30	15	63.40±0.8ab	6.65 ± 0.28^{a}	$0.67\pm0.04^{\text{def}}$	8.11±0.11 ^a	21.16 ± 0.05^{ef}
30	30	70.23±2.52a	$5.44 \pm 0.25^{\mathrm{abc}}$	0.51 ± 0.01^{fg}	7.97 ± 0.13^{ab}	15.84±0.03g
30	60	69.94±4.51 ^a	5.44 ± 0.00^{abc}	0.55 ± 0.03^{ef}	$7.05 \pm 0.05^{\text{bcd}}$	17.01±0.00 ^{fg}

Note: Values are mean ± SE of triplicate dry matter weight. Means having a common letter superscript in the same column are not significantly different at p>0.05.

Similar findings have been reported by several authors, indicating significant effects of fermentation on proximate composition, resulting in an increase in crude protein, crude fiber, crude fat, and ash; and a reduction in carbohydrates of pineapple peels after 72 hours of fermentation (Aruna, 2019). Fermentation also increased protein and reduced ash and carbohydrates in cashew apple residue (Akinnibosun & Oyetayo, 2018), increased crude protein and crude fiber; decreased crude fat and carbohydrates in ripe and unripe plantain flour after 72-hour fermentation (Eromosele, Ojokoh, Ekundayo, Ezem, & Chukwudum, 2017), increased crude protein and ash in rapeseed (Vlassa et al., 2022), increased protein; decreased ash and fiber in Goroho banana stem. (Najoan, Wolayan, & Sompie, 2020) increased protein, fat, fiber in *Pistia* leaf (Mandal & Ghosh, 2009), increased protein and fiber; decreased ash, fat and carbohydrate in watermelon epicarp (Ojokoh & Orekoya, 2016), increased protein and ash in tamarind seed (Olagunju, Ezekiel, Ogunshe, Oyeyinka, & Ijabadeniyi, 2018), and increased carbohydrate and fiber were also observed in fermented banana peels (Ozabor, Ojokoh, Wahab, & Aramide, 2020). However, in the present study, a notable reduction in the fiber content of the fermented sample was observed.

Accordingly, fermentation biologically enriches food substrates with protein, essential amino acids, essential fatty acids, and vitamins, and reduces anti-nutrients and complex molecules (Olasupo, Okorie, & Oguntoyinbo, 2016; Steinkraus, 1995). The significant levels of nutritional content (crude protein, crude fiber, crude fat, ash) of molasses fermented PFPW obtained in the study indicate its potential as a feed additive. Higher protein values of the fermented samples in the current study could be attributed to the capacity of autochthonous microbiota to secrete extracellular enzymes into the fermented substrates during its metabolic activities. It suggests its utility as a supplemental source of protein in animal feed formulations. While according to Ojokoh and Orekoya (2016), higher ash content in fermented samples can be attributed to partial consumption of minerals in the substrate by fermenting microorganisms during their metabolism. Ash in animal nutrition is very essential as it indicates the presence of essential minerals necessary for various physiological functions. The minimal crude fat can contribute to the energy content when used in animal feed formulations. Moreover, several studies have pointed out that pineapple processing waste, particularly the peel, can be a source of dietary fibers, bringing beneficial contributions to digestive health and reduction of blood glucose.

The crown and stem, on the other hand, are rich in cellulose and hemicellulose that can be used as potential animal feedstock and raw material for biofuel production (Pardo, Cassellis, Escobedo, & García, 2014). Although increased dietary fiber can impede nutrient absorption, the fermentation of pineapple waste reduces fiber content, resulting in shorter fibrous particles. As a result, incorporating fermented pineapple waste into animal feed can enhance feed passage rates and improve digestibility compared to conventional feed ingredients.

3.2. Phytochemical composition and antioxidant activity

Results of the phytochemical composition analyses in Table 2 showed the positive effect of fermentation on PFPW, indicating an increase in its total phenolic content (TPC) by 105–395% and total flavonoid content (TFC) by 306–744%. Furthermore, the free radical scavenging activity was also enhanced, as reflected by the results of the DPPH assay, with an increase of 67–192%. These findings conform with the results of several conducted studies, which observed a significant increase in TPC, TFC, and antioxidant activity due to the application of fermentation processes on agricultural by-products/wastes such as tamarind seed and mixed tamarind peel and seed (Santos et al., 2020); pistachio green hull (Ordoñez-Cano et al., 2025); mango seed (Torres-León et al., 2019); pineapple waste (Rivera et al., 2023) and extracts from date by-products of three different varieties (Khosravi, Razavi, Castangia, & Manca, 2024).

Table 2. Phytochemical composition and antioxidant activity of fermented pineapple fruit processing waste (PFPW).

Molasses (%)	Fermentation period (Days)	DPPH (% scavenging activity)	TPC (mg GAE/g)	TFC (mg CE/g)
0	0	28.95 ± 2.31 g	1.07 ± 0.15 g	0.16 ± 0.00^{f}
0	15	25.07 ± 1.75 g	$2.13\pm0.02^{\rm f}$	0.61 ± 0.02^{e}
0	30	$33.60 \pm 1.63^{\mathrm{fg}}$	3.18 ± 0.27^{c}	1.01 ± 0.06^{bc}
0	60	39.17 ± 0.00^{f}	3.15 ± 0.02^{c}	1.26 ± 0.02^{a}
10	15	48.45 ± 2.12^{e}	2.99 ± 0.14^{cf}	$0.76 \pm 0.03^{\mathrm{de}}$
10	30	71.78 ± 1.61^{cd}	$2.26 \pm 0.20^{\mathrm{def}}$	$0.86 \pm 0.05^{\rm cd}$
10	60	73.77 ± 1.65^{bc}	4.62 ± 0.13^{a}	1.35 ± 0.06^{a}
20	15	$69.71 \pm 0.99^{\text{cd}}$	$2.19\pm0.10^{\rm ef}$	0.65 ± 0.01^{de}
20	30	84.39 ± 0.63^{a}	$2.70 \pm 0.15^{\text{cdef}}$	0.70 ± 0.03^{de}
20	60	75.18 ± 0.61 bc	4.77 ± 0.20^{a}	1.04 ± 0.05 bc
30	15	$81.00\pm\ 1.16^{ab}$	3.33 ± 0.20^{bc}	1.10 ± 0.07^{bc}
30	30	$80.35 \pm 1.27^{\mathrm{ab}}$	5.30 ± 0.13^{a}	1.32 ± 0.03^{a}
30	60	65.71 ± 0.71^{d}	$4.14\pm 0.25^{\rm b}$	1.30 ± 0.09^{a}

Note: Values are mean ± SE of triplicate determinations. Means having a common letter superscript in the same column are not significantly different at p>0.05

Molasses fermented PFPW of the current study contains considerable amounts of antioxidants: total phenolic content (TPC) with the highest value of 5.30± 0.13 (mg GAE/g) and total flavonoid content (TFC) with the highest value of 1.32± 0.03 (mg CE/g). The highest DPPH value of 84.39± 0.63 is recorded in the current study. Accordingly, fruit wastes are a source of bioactive compounds such as TPC and TFC, which can be beneficial for health considering their higher antioxidant capacity present in fruit waste than that of the edible part generally consumed by humans, as mentioned by Sengar, Sunil, Rawson, and Venkatachalapath (2022); Ali, Bashmil, Cottrell, Suleria, and Dunshea (2021); Vijaykumar, Murchana, and Kumar (2019) and Bazinet, Labbé, and Tremblay (2007). In the biological system, these polyphenols act as hydrogen atom donors, metal chelators, reducing agents, and free radical scavengers. Moreover, fermentation can further amplify antioxidant activity by increasing the production of phytochemicals, antioxidant polysaccharides, and antioxidant peptides through microbial hydrolysis or biotransformation (Zhao et al., 2021). It also promotes the breakdown of plant cell walls, facilitating the release or formation of additional antioxidant compounds.

3.3. Anti-nutritional contents

The anti-nutritional contents of pineapple fruit processing waste (PFPW) at varying days of fermentation and molasses concentration are shown in Table 3. Fermentation decreased the values of the anti-nutritional contents of samples in the current study. Tannin content is reduced by 45–56%, while phytate content is likewise reduced by 10–40% when compared to unfermented samples. Similar observations on the effect of fermentation were noted in the following studies: banana peels, cashew apple residue, Irish potato peels, and tamarind (*Tamarindus indica L.*) seed in the production of daddawa-type condiment (Akinnibosun & Oyetayo, 2018; Kareem, Ojokoh, & Baba, 2017; Olagunju

et al., 2018; Ozabor et al., 2020). In the current study, PFPW fermented with 20% molasses for 60 days had the lowest tannin value, which is 11.38 ± 0.07 mg TAE/g, while PFPW fermented with 30% molasses for 15, 30, and 60 days had the lowest phytate content of 0.06 ± 0.00 .

Tannins and phytates are categorized as anti-nutritional factors found in plant-based raw materials that can affect how nutrients are absorbed and digested (Mandal & Ghosh, 2009). In the current study, a lower value of phytate content in the fermented samples of PFPW was obtained; however, the values of tannin were slightly higher when compared with previously conducted studies. These anti-nutritional contents may have both positive and negative effects on organisms, as the presence of these plant compounds in feed formulations at low levels acts as antioxidants, antimicrobials, and immune-boosting agents. Conversely, if present in high concentrations, they have negative implications, as they hinder growth of organisms due to poor nutrient absorption and digestion. As reported in the study of Hossain and Jauncey (1989), fed diets containing tannins of 0.57 and 1.14% resulted in poor growth in common carp (*Cyprinus carpio*). This growth-inhibitory effect may be attributed to the inhibition of proteases and other digestive enzymes, or it might be due to the formation of indigestible complexes with dietary protein, resulting in diminished digestibility parameters (Becker & Makkar, 1999; Krogdahl, 1989). The reduction in anti-nutrients observed in the fermented samples in this study may be attributed to the increased activity of anti-nutrient-degrading enzymes. Consequently, lower levels of anti-nutrients can enhance feed utilization and nutrient digestibility.

Table 3. Anti-nutritional contents of fermented pineapple fruit processing waste (PFPW).

Molasses (%)	Fermentation Period (Days)	Tannin (mg TAE/g)	Phytate (%)
0	0	26.54 ± 0.04 g	$0.10 \pm 0.00g$
0	15	14.37 ± 0.04^{e}	0.09 ± 0.00^{de}
0	30	$17.97 \pm 0.02^{\rm f}$	0.10 ± 0.00^{fg}
0	60	13.99 ± 0.05^{d}	0.09 ± 0.00^{ef}
10	15	14.67 ± 0.02^{e}	$0.08 \pm 0.00^{\text{cde}}$
10	30	14.43± 0.05e	0.07 ± 0.00^{ab}
10	60	$14.27 \pm 0.15^{\text{de}}$	0.06 ± 0.00^{a}
20	15	$12.89 \pm 0.06^{\circ}$	0.07 ± 0.00^{ab}
20	30	11.51 ± 0.04^{a}	0.07 ± 0.00^{ab}
20	60	11.38 ± 0.07^{a}	0.09 ± 0.00^{de}
30	15	12.82 ± 0.09^{c}	0.06 ± 0.00^{a}
30	30	$12.05\pm0.11^{\rm b}$	0.06± 0.00a
30	60	11.68 ± 0.05^{a}	0.06± 0.00a

Note: Values are mean ± SE of triplicate determinations. Means having a common letter superscript in the same column are not significantly different at p>0.05.

4. CONCLUSION

The significant levels of nutritional content (crude protein, crude fiber, crude fat, ash), enhanced antioxidant properties (TPC and TFC), and reduced values of anti-nutritional factors (ANF) can be attributed to the positive effects of the fermentation process of pineapple fruit processing waste (PFPW) using molasses. The obtained results further indicate that fermented PFPW can be a potential feed additive.

Funding: This work was conducted with support from the Science Education Institute (SEI) - Science and Technology Regional Alliance of Universities for National Development (STRAND), and the Inland Aquatic Resources Research Division (IARRD) - Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (PCAARRD), funded by the Department of Science and Technology (DOST). Institutional Review Board Statement: Not applicable.

Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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