



Age-Dependent Bromelain Enzyme Activity in Pineapple Rhizomes (“Smooth Cayenne” cultivar) Predicted by Fourier-Transform Near-Infrared Spectroscopy

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Abstract

Bromelain is a proteolytic enzyme complex derived from pineapple with extensive medicinal properties. The purpose of this study was to use near-infrared (NIR) spectroscopy to predict bromelain enzyme activity in “Smooth Cayenne” pineapple rhizomes. Samples from 18- and 24-month-old rhizomes were analyzed. A total of 574 NIR spectra (derived from 106 rhizomes, each divided into three anatomical sections and scanned twice) were used to develop the predictive models, with 459 spectra assigned to the calibration set and 115 spectra to the prediction set. NIR spectra were measured with an Fourier-transform NIR spectrometer in diffuse reflection mode at a wavenumber of 11000–4000 cm⁻¹ (909–2500 nm); then, bromelain enzyme activity was measured. A prediction model was established by partial least squares regression and validated by the test set validation method. A calibration equation was used to predict the activity of bromelain enzyme in a prediction set. The results showed bromelain enzyme activity of 0.0328 ± 0.0193 and 0.0293 ± 0.0182 CDU/mL in 18- and 24-month-old pineapple rhizomes, respectively. The calibration equation to predict bromelain enzyme activity based on spectral scanning of the middle of 18-month-old pineapple rhizomes had a correlation coefficient of 0.85, a root mean square error of prediction of 0.0097 CDU/mL, a bias of 0.0036 CDU/mL, and a ratio of prediction to deviation of 1.7. Although the model can be used for screening and predicting the approximate bromelain enzyme activity in 18-month-old pineapple rhizomes, it needs to be improved to provide more accurate predictions.

Keywords: Near-Infrared Spectroscopy, Bromelain enzyme, Pineapple, Rhizome

1. Introduction

Pineapple (*Ananas comosus*) is one of the most important commercial fruit crops in Thailand: It ranks as one of the top 10 exports of the country, and Thai pineapples have a significant global market share [1]. Today, about 25 million tons of pineapple are produced throughout the world, making it the third most consumed fruit after bananas and citrus fruits [2]. Thailand was a relatively early entrant to the processed food export trade [3]. Canned pineapple is a processed product of the Thai processed food sector and ranks among the top processed products in the world. Pineapples contain bromelain [4], a commercially important enzyme in food, pharmaceuticals, detergents, and textiles due to its strong proteolytic activity [5]. Moreover, bromelain can be used as an effective health supplement to prevent cancer [6], diabetes, and various cardiovascular diseases [7]. In addition, bromelain improves exploratory activity, memory, anxiety, and depression in a rat model of aluminum chloride-induced Alzheimer’s disease [8].

Bromelain is a collective term for a group of proteolytic enzymes belonging to the Bromeliaceae family and is primarily associated with thiol endopeptidases found in pineapple tissues [9,10]. It occurs in both the fruit and stem of the pineapple plant, although the term “bromelain” generally refers to stem bromelain because of its higher enzyme activity [2]. A previous study reported that mature pineapple stems contain the greatest concentration of bromelain, with a content of 53%, 36%, and 11% in 3-, 2-, and 1-year-old stems, respectively, whereas soft tissues contain comparatively lower amounts [11]. In addition to proteolytic enzymes, bromelain comprises several non-protease components, including phosphatases, glucosidases, peroxidases, cellulases, and protease inhibitors [7]. The pineapple rhizome, an underground extension of the stem, has also been identified as a significant source of stem bromelain [12].

Bromelain has the molecular formula $C_{39}H_{66}N_2O_{29}$ and is characterized by its solubility in water while remaining insoluble in alcohol, chloroform, and ether [13]. The optimal enzyme activity occurs within a temperature range of 50–60 °C and remains stable up to approximately 70 °C before thermal inactivation occurs [14]. Previous work isolated two major bromelain components from pineapple and purified them to electrophoretic homogeneity. They contained oligosaccharide groups composed of D-glucosamine, D-mannose, D-xylose, and L-fucose in the molar ratio of 2:2:1:1. Furthermore, bromelain was extensively degraded to yield glycopeptides containing only Asx, Glx, and Ser residues. Analyses involving periodate oxidation, methylation, and glycosidase digestion demonstrated that the oligosaccharide chain possesses a highly branched structure, with all neutral sugars located at nonreducing terminal positions and both *N*-acetyl-D-glucosamine residues situated internally [15].

Conventional methods used to analyze bromelain are often costly, time-consuming, and potentially hazardous due to their reliance on sophisticated laboratory instrumentation and complex chemical extraction procedures. In this context, near-infrared (NIR) spectroscopy has emerged as an important alternative because it provides a rapid, non-destructive, and chemical-free analytical approach. This technique enables real-time evaluation of enzyme activity without compromising the integrity of the product, so it is particularly suitable for commercial screening and quality control applications within the pineapple industry.

NIR spectroscopy is a widely recognized analytical technique based on the interaction between matter and electromagnetic radiation within the wavelength range of 780–2500 nm. This method allows reliable, cost-effective, multicomponent, and non-destructive analysis, with results obtainable within seconds or through continuous online monitoring [16]. To improve analytical accuracy and data interpretation, spectral preprocessing techniques are employed to enhance the signal-to-noise ratio by minimizing physical artifacts, while partial least squares regression (PLSR) addresses spectral overlap through decomposition into significant latent variables [17].

NIR spectroscopy has been extensively applied in the analysis of agricultural commodities within the food industry. Previous studies have utilized NIR spectroscopy to determine the chemical composition of rice, including protein and lipid contents [18,19], as well as gamma-oryzanol, gamma-aminobutyric acid (GABA), and amino acid contents in germinated brown rice [20,21]. In addition, the technique has been employed for monitoring lactic acid alcoholic fermentation [22] and the online monitoring of starch enzymatic hydrolysis [23].

To date, however, there have been no reported studies concerning the application of NIR spectroscopy for the evaluation of bromelain enzyme content in food products. Therefore, the present study aimed to apply NIR spectroscopy to assess the bromelain enzyme activity in the rhizomes of “Smooth Cayenne” pineapple.

2. Materials and methods

2.1 Materials

Pineapple rhizomes of the “Smooth Cayenne” cultivar were collected from orchards belonging to a single group of farmers in Pluak Daeng District, Rayong Province. Samples were harvested at 18 and 24 months after planting (Figure 1). A total of 106 rhizomes were obtained, comprising 53 rhizomes per age group. Each rhizome was divided into three anatomical sections—head, middle, and end (Figure 2)—yielding 318 individual samples. All samples were subjected to NIR spectroscopy, with measurements performed in duplicate to develop a comprehensive spectral database. Prior to analysis, harvested rhizomes were stored at 25 ± 2 °C until NIR spectroscopy data collection.

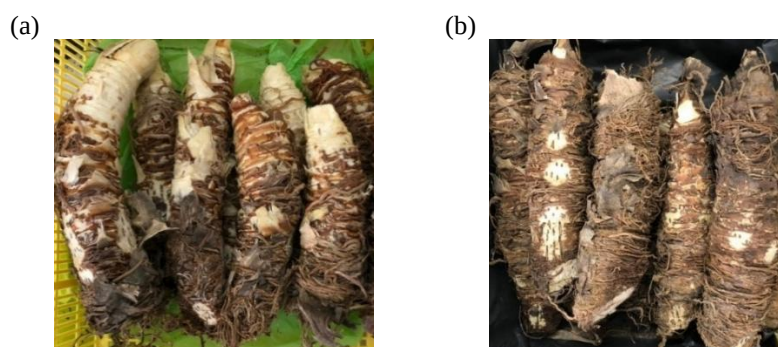


Figure 1 Images of (a) 18- and (b) 24-month-old pineapple rhizomes.

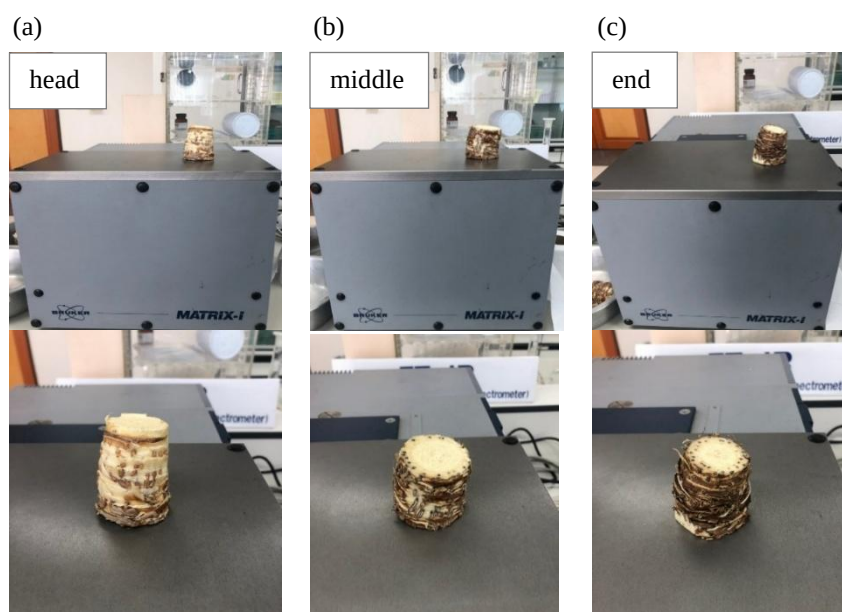


Figure 2 Images of near-infrared spectral acquisition of the (a) head, (b) middle, and (c) end sections of rhizomes.

2.2 NIR spectra of sample acquisition

Each anatomical section of the rhizome (3 cm long) was immediately subjected to spectral acquisition on the exposed cross-sectional surface using a Fourier transform near-infrared (FT-NIR) spectrometer (Matrix FT-NIR spectrometer, Bruker, Germany). Spectra were collected over the range of $11000\text{--}4000\text{ cm}^{-1}$ (corresponding to $900\text{--}2500\text{ nm}$) in diffuse reflectance mode at a spectral resolution of 32 cm^{-1} with 64 co-added scans per measurement. Each of the 318 samples was scanned twice, yielding a total of 636 spectra for subsequent analysis. Following spectral acquisition, the peel (skin) was removed, and approximately 30 g of each sample was used for bromelain extraction and enzyme activity determination. The resulting reference values were then correlated with NIR spectral data to develop a calibration model. Data processing and model development were performed using OPUS 7.8 software (Bruker Optics, Germany).

2.3 Bromelain extraction

All chemicals and reagents used were of analytical grade unless otherwise specified. Trichloroacetic acid (TCA, >99.5% purity) was purchased from Merck (Germany), and L-cysteine (>99.0% purity) was obtained from Himedia (India). L-Tyrosine (Biochemika, >99.0% purity) and ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) (>97.0% purity) were supplied by Fluka (Switzerland). Casein hydrolysate for microbiology was obtained from Sigma-Aldrich (Germany). Disodium hydrogen orthophosphate dodecahydrate (>99.0% purity) and sodium dihydrogen orthophosphate (>99.0% purity) were procured from Univar (Australia).

Pineapple rhizomes were chopped and blended with deionized ice in a 1:1 ratio using a food processor (HR2118, Philips, China) for 2 min. The resulting homogenate was filtered through a white cloth to separate the

solid residues. Bromelain was subsequently extracted from the filtrate by centrifugation (Z36HK, Hermle, Germany) at 10,000 rpm (65,395 g) for 20 min at 4 °C.

2.4 Bromelain enzyme activity

The enzyme activity of the extracted bromelain was determined according to the method of Shinya et al. [24]. Briefly, 1 mL of the crude enzyme extract was mixed with 1 mL of 0.05 M phosphate buffer (pH 8.0), 2 mL of 1 mM EDTA, and 1 mL of 5 mM L-cysteine. The reaction mixture was thoroughly homogenized to initiate enzyme activity. For the assay, 1 mL of diluted enzyme solution was added to a mixture containing 2 mL of 2% (w/v) casein and 2 mL of 0.05 M phosphate buffer (pH 8.0), and incubated in a water bath (WNB Series, Memmert, Germany) at 37 ± 0.1 °C for 20 min. A blank sample was prepared by substituting the enzyme solution with deionized water. The reaction was terminated by the addition of 5 mL of 3% (w/v) TCA. The mixture was then incubated at 37 ± 0.1 °C for 30 min, followed by centrifugation (Z36HK, Hermle, Germany) at 4,000 rpm (65,395 g) for 15 min at 4 °C.

Enzyme activity was calculated using Equation (1) based on the measured reaction products.

$$\text{Bromelain enzyme activity (CDU/mL)} = \frac{\text{Tyrosine } (\mu\text{g}) \times \text{Equal amount of enzyme solution dilution}}{\text{Tyrosine (MW)} \times \text{Curing time} \times \text{Enzyme content (mL)}} \quad (1)$$

In this equation, the dilution factor refers to the ratio used to dilute the crude enzyme extract prior to the reaction. The incubation (curing) time is defined as the period during which the enzyme reacts with the casein substrate. The enzyme volume (mL) denotes the specific volume of diluted enzyme solution added to the reaction mixture.

A tyrosine standard solution was prepared and diluted to concentrations of 0, 20, 40, 60, 80, and 100 µg/mL. The absorbance of each standard was measured at 280 nm using an ultraviolet–visible (UV–Vis) spectrophotometer (Cary 50 Scan, Varian, USA). The resulting values were used to construct a calibration curve relating tyrosine concentration to absorbance. UV absorption at 280 nm increased by 1 µg of tyrosine in 1 min. Based on the calibration curve, the concentration of tyrosine released during enzymatic hydrolysis was determined by substituting the measured absorbance into the regression equation. This concentration (µg/mL) was then used to calculate bromelain enzyme activity, expressed in casein digestion units (CDU/mL), where 1 CDU is defined as the amount of enzyme that catalyzes casein hydrolysis under assay conditions at pH 8.0 and 37 °C.

2.5 Using NIR spectroscopy to predict bromelain enzyme activity of pineapple rhizomes

Prior to developing the calibration model relating NIR spectral data to bromelain enzyme activity, PLSR was performed using OPUS 7.8 software. First, outliers in both the spectral data and reference enzyme activity were detected. A total of 62 spectra were identified as outliers based on Mahalanobis distance and subsequently removed, leaving 574 valid spectra to develop the model.

The remaining samples were divided into calibration and prediction sets. To ensure a uniform distribution of bromelain enzyme activity across the dataset, samples were first sorted in ascending order according to enzyme activity. Systematic sampling was then applied, allocating 80% of the samples to the calibration set and 20% to the prediction set. Spectral preprocessing techniques, including first derivative, second derivative, multiplicative scatter correction (MSC), and standard normal variate (SNV), were applied to both calibration and prediction spectra to reduce scattering effects and baseline variation. Model performance was evaluated using standard statistical parameters, including the correlation coefficient (R), the root mean square error of estimation (RMSEE), root mean square error of prediction (RMSEP), the ratio of performance to deviation (RPD), and prediction bias. The RMSEE and RMSEP were compared to assess model robustness; substantial differences between these values indicate potential overfitting and reduced generalizability of the model to independent samples.

3. Results and discussion

3.1 Bromelain enzyme activity in pineapple rhizomes

There was higher bromelain enzyme activity in 18-month-old pineapple rhizomes (0.0298 ± 0.017 CDU/mL) compared with 24-month-old pineapple rhizomes (0.0277 ± 0.017 CDU/mL), with the difference being statistically significant (Table 1). This variation may be attributed to endogenous regulatory mechanisms within pineapple tissues, including enzyme inhibition by endogenous protein inhibitors. For example, bromelain inhibitors such as “bromein” have been isolated from pineapple stems [25], and phytocystatins have also been reported to inhibit bromelain enzyme activity [26]. The accumulation of such inhibitory proteins and secondary metabolites over the plant’s developmental cycle may contribute to reduced enzyme activity in older rhizomes. In addition, environmental and physiological stress factors may influence bromelain expression and enzyme activity. Stress conditions such as salinity have been reported to negatively affect bromelain enzyme activity in pineapples

[27]. More generally, a prolonged growth duration may increase exposure to cumulative abiotic stresses, including nutrient limitation and water deficit, which can alter plant metabolism and reduce enzyme synthesis or catalytic efficiency. Consequently, older plants (24 months) may exhibit lower bromelain enzyme activity compared with younger, more physiologically active plants (18 months) [28].

Table 1 Bromelain enzyme activity in 18- and 24-month-old pineapple rhizomes.

Age (months)	Range (CDU/mL)	Mean $\pm$ standard deviation (CDU/mL)
18	0.0014–0.0718	0.0298 $\pm$ 0.017 ^a
24	0.0061–0.0870	0.0277 $\pm$ 0.017 ^b

Values with a different letter in the same column are significantly different according to the t-test ($P < 0.05$).

The ends of the 18-month-old pineapple rhizomes exhibited the highest bromelain enzyme activity (0.0328 $\pm$ 0.0193 CDU/mL) (Table 2). However, statistical analysis indicated no significant differences in enzyme activity among the anatomical sections within either age group. These results suggest that rhizome age, rather than internal spatial location, is the primary factor influencing bromelain enzyme activity. The finding is consistent with the study by Heinicke and Cortner [11], who demonstrated that bromelain concentration varies with plant age and is highest in the stem tissues.

Table 2 Bromelain enzyme activity in 18- and 24-month-old pineapple rhizome head, middle, and end sections.

Section	Bromelain enzyme activity (CDU/mL)	
	18 months old	24 months old
Head	0.0281 $\pm$ 0.0165	0.0283 $\pm$ 0.0178
Middle	0.0283 $\pm$ 0.0148	0.0254 $\pm$ 0.0151
End	0.0328 $\pm$ 0.0193	0.0293 $\pm$ 0.0182
F-test	ns	ns

The data are presented as the mean $\pm$ the standard deviation. The data were analyzed with the F-test; ns, not significant ($P > 0.05$).

3.2 NIR spectra of pure bromelain

Figure 3(a) shows the average NIR absorption spectrum of purified bromelain powder. The spectrum shows a general decrease in absorbance with increasing wavenumber (decreasing wavelength). There are prominent absorption bands at 4,382, 4,629, 4,875, 5,786, and 6,711 cm^{-1} (corresponding to 2,282, 2,161, 2,051, 1,728, and 1,490 nm, respectively). These bands are primarily associated with protein-related vibrational modes, particularly the amide I and amide III regions. The amide I band arises mainly from C=O stretching vibrations of the peptide backbone, while the amide III band results from a combination of C–N stretching and N–H bending vibrations [29,30]. Because bromelain is a protein-based enzyme containing peptide bonds, these spectral features are characteristic of its secondary structure. Structural alterations such as denaturation may lead to shifts in the amide I and amide III bands, reflecting changes in α -helix and β -sheet conformations, which are important indicators of enzyme stability and functionality [31]. In addition, there are absorption features related to the first overtone of O–H stretching vibrations of cellulose [32]. As a major structural component of plant cell walls, cellulose contributes distinct NIR signals due to overtone and combination bands arising from C–H, O–H, and C–O bonds. These include stretching and bending vibrations associated with the polysaccharide backbone [32].

Figure 3(b) shows the FT-NIR spectrum of pineapple rhizomes. There are characteristic absorption peaks at 5,156 cm^{-1} (1,939 nm), attributed to O–H stretching and deformation of water; at 6,911 cm^{-1} (1,447 nm), associated with the first overtone of O–H stretching in water and starch; and at 8,440 cm^{-1} (1,185 nm), corresponding to the second overtone of C–H stretching in CH_3 groups [33]. These features are not present in the spectrum of purified bromelain powder.

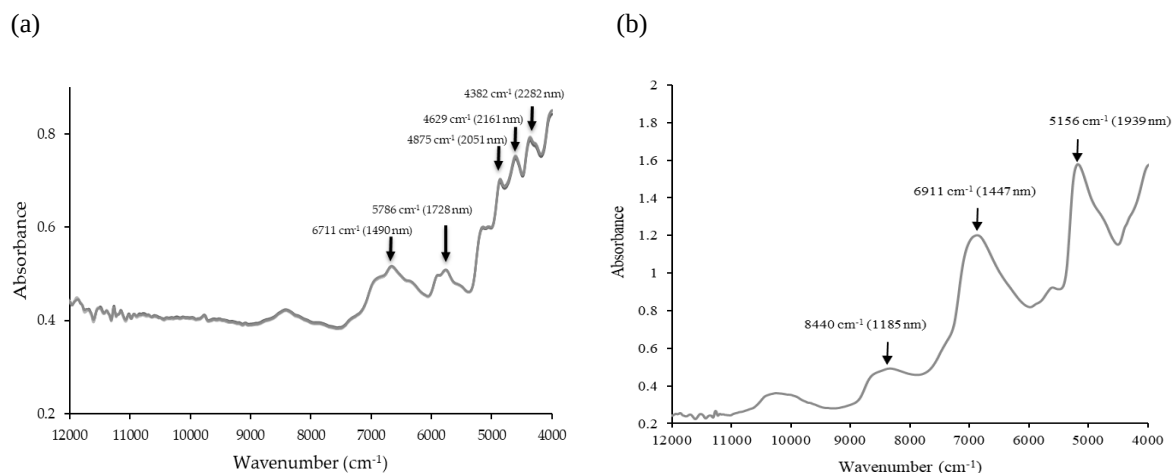


Figure 3 The NIR absorption spectrum of (a) purified bromelain powder from pineapple stem (≥ 3 units/mg protein) purchased from Sigma-Aldrich (USA) and (b) the FT-NIR spectrum of pineapple rhizomes.

3.3 Estimation of bromelain enzyme activity in pineapple rhizomes based on NIR spectroscopy

Table 3 presents the bromelain enzyme activity in total pineapple rhizome samples in the calibration and prediction sets, and standard deviation were 0.0014–0.0870 CDU/ml, 0.0014–0.0870 CDU/ml, and 0.0107–0.0249 CDU/ml, respectively.

Table 3 Bromelain enzyme activity (CDU/mL) of pineapple rhizomes for the PLSR calibration and prediction sets.

Parameter			Calibration set				Prediction set				
Age (months)	Section	No.	Max	Min	Mean	SD	No.	Max	Min	Mean	SD
18		232	0.0718	0.0014	0.0268	0.0130	57	0.0688	0.0014	0.0316	0.0182
24		227	0.0870	0.0061	0.0259	0.0156	57	0.0870	0.0061	0.0348	0.0209
	Head	83	0.0688	0.0025	0.0260	0.0130	20	0.0598	0.0025	0.0295	0.0183
18	Middle	82	0.0590	0.0014	0.0268	0.0118	19	0.0590	0.0014	0.0291	0.0157
	End	73	0.0854	0.0023	0.0308	0.0173	21	0.0854	0.0023	0.0407	0.0249
	Head	82	0.0870	0.0071	0.0266	0.0166	19	0.0870	0.0071	0.0353	0.0216
24	Middle	81	0.0623	0.0061	0.0236	0.0124	19	0.0576	0.0061	0.0278	0.0160
	End	75	0.0780	0.0067	0.0271	0.0156	19	0.0780	0.0067	0.0331	0.0209
Head		161	0.0870	0.0025	0.0267	0.0157	40	0.0870	0.0025	0.0343	0.0213
Middle		159	0.0483	0.0014	0.0240	0.0107	37	0.0476	0.0014	0.0251	0.0133
End		148	0.0854	0.0023	0.0299	0.0182	40	0.0780	0.0023	0.0360	0.0209
All		459	0.0698	0.0014	0.0239	0.0107	115	0.0598	0.0014	0.0301	0.0167

3.4 Analysis of the absorption spectra of pineapple rhizomes for bromelain content

Figure 4 illustrates the average first-derivative NIR spectra of 18- and 24-month-old pineapple rhizomes. The labeled wavenumbers indicate prominent peaks and troughs (inflection points) of the derivative spectra, which correspond to regions of maximum rate of change in absorbance (rather than the absolute absorption maxima). These derivative features, observed at 4675, 5045, 5292, 6696, 7159, and 8748 cm⁻¹, enhance spectral resolution by minimizing baseline shifts and resolving overlapping absorption bands. They are associated with chemical vibrations related to functional groups such as HC=CH, protein-related bonds (CONHR, CONH₂), cellulose, CH₂, and aromatic functional groups. This improved spectral separation facilitates more reliable interpretation of compositional differences between samples.

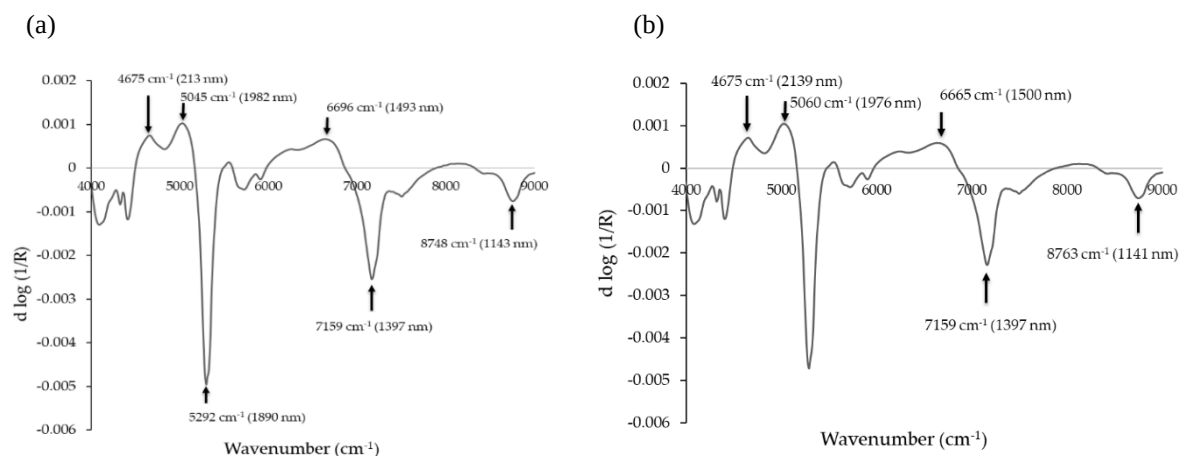


Figure 4 Average NIR absorption spectra of (a) 18- and (b) 24-month-old pineapple rhizomes.

3.5 Prediction of bromelain enzyme activity in pineapple rhizomes by PLSR

Table 4 presents the results of PLSR analysis conducted using OPUS software for predicting bromelain enzyme activity in pineapple rhizomes. The model developed using spectra from all anatomical regions of 18-month-old rhizomes demonstrated the best overall performance, with a calibration correlation coefficient (R_{cal}) of 0.52, a prediction correlation coefficient (R_{pre}) of 0.65, and an RPD of 1.3.

The model based on the middle section of the rhizome showed improved calibration performance ($R_{cal} = 0.65$), with an RMSEE of 0.0094 CDU/mL. The corresponding validation results showed an R_{pre} of 0.85, an RMSEP of 0.0097 CDU/mL, and a mean bias of 0.0036 CDU/mL (Table 4). Notably, this model exhibited an inverse relationship between calibration and prediction performance ($R_{pre} > R_{cal}$), which is statistically atypical. This phenomenon is primarily attributed to the limited size of the prediction set ($n = 19$), which may not adequately represent the full variability of the population. Therefore, the elevated R_{pre} should be interpreted with caution, as it may overestimate the true predictive capability when applied to larger and more diverse datasets.

These results may be explained by the relationship between bromelain enzyme activity and spectral variation in 18-month-old rhizomes. In contrast, 24-month-old rhizomes may exhibit biochemical and structural changes that are less consistently reflected in spectral features, potentially due to cumulative environmental stressors such as nutrient depletion or water scarcity over a longer growth period, as previously reported [28]. The middle section of the rhizome also exhibited lower variability in bromelain enzyme activity (a lower SD) compared with the other sections, there were no significant differences in enzyme activity among rhizome sections at either age (Table 2).

Overall, the 18-month-old rhizome dataset produced a lower SD and RMSEP compared with the 24-month-old dataset. Specifically, the SD was 0.0182 and 0.0209 CDU/mL, respectively, while the RMSEP was 0.0149 and 0.0203 CDU/mL, respectively (Table 4). These values resulted in an RPD of 1.3 and 1.1, respectively, indicating that differences in model performance were primarily driven by the RMSEP, despite the narrower variability in the 18-month dataset. Consequently, the model based on 18-month-old rhizomes demonstrated superior predictive performance for bromelain enzyme activity. In contrast, inclusion of 24-month-old samples reduced overall model performance, likely due to increased physiological variability associated with extended growth conditions.

A key limitation of the reference assay is its reliance on single measurements ($n = 1$), which represents a major source of uncertainty. As a result, both spectroscopic and analytical errors are incorporated into the RMSEP of the best-performing model (0.0097 CDU/mL). Nevertheless, the relatively high R_{pre} (0.85) and RPD (1.7) indicate that the reference measurements still provide a usable baseline for PLSR modeling. According to Williams [34], this level of performance supports the applicability of the model for screening and approximate prediction purposes. Future studies should incorporate replicate reference measurements to better quantify analytical uncertainty.

All PLSR models exhibited a consistent positive bias ranging from 0.0021 to 0.0090 CDU/mL, indicating consistent underestimation of bromelain enzyme activity. This bias may result from nonlinear relationships between NIR spectral features and enzyme functionality, as well as matrix effects arising from the complex fibrous structure of rhizome tissue. In addition, a systematic offset in the chemical reference method may have contributed to this consistent underestimation.

Further analysis of rhizome sections showed that the middle portion of 18-month-old samples exhibited lower variability compared with other parts. The SD for the head, middle, and end sections was 0.0183, 0.0157, and

0.0249 CDU/mL, respectively, while the corresponding RMSEP was 0.0140, 0.0097, and 0.0204 CDU/mL (Table 5). These resulted in an RPD of 1.3, 1.7, and 1.2, respectively. Despite narrower variability, the middle section model showed the lowest RMSEP, indicating that reduced prediction error rather than variance primarily drove model performance. Accordingly, the model based on the middle portion of 18-month-old rhizomes provided the best predictive performance for bromelain enzyme activity.

Figures 5 and 6 compare bromelain enzyme activity in 18- and 24-month-old pineapple rhizome samples as predicted by NIR spectroscopy and as measured by the reference laboratory method, respectively. Bromelain enzyme activity is a biochemical parameter that reflects the catalytic efficiency of the enzyme. Enzyme activity quantifies the rate at which a biochemical reaction is catalyzed and therefore represents enzyme “functionality” rather than absolute “quantity” of the substance itself, although activity units are expressed in concentration-equivalent terms to represent catalytic capacity.

According to Williams [34], R values between 0.81 and 0.90 indicate suitability for rough screening applications, whereas an RPD of 1.7 is generally considered low. This apparent discrepancy arises because RPD is strongly influenced by the low variance (SD = 0.0157) of the dataset, despite the relatively strong correlation (R = 0.85). Therefore, the RPD criterion was interpreted in the context of dataset variability, and the model was considered appropriate only for preliminary, non-critical screening rather than precise quantitative prediction.

Table 4 The PLSR results using the OPUS software for the prediction of bromelain enzyme activity in pineapple rhizomes

Parameter		No. of factors	Wavelength (cm ⁻¹)	Preprocessing	Calibration		Prediction			
Age	Part				R _c	RMSEE (CDU/mL)	R _{pre}	RMSEP (CDU/mL)	RPD	Bias (CDU/mL)
18		8	9411.2–5446.2 4613.1–4242.8	Straight line subtraction	0.52	0.0113	0.65	0.0149	1.3	0.0043
24		6	9411.2–7498.1 6109.6–5446.2	No spectral data preprocessing	0.33	0.0149	0.50	0.0203	1.1	0.0082
18	Head	6	8454.7–7498.1 4859.9–4242.8	Vector normalization (SNV)	0.49	0.0118	0.78	0.0140	1.3	0.0021
	Middle	8	7513.6–4242.8	Straight line subtraction	0.65	0.0094	0.85	0.0097	1.7	0.0036
	End	9	7513.6–4597.6	Straight line subtraction	0.65	0.0140	0.58	0.0204	1.2	0.0044
24	Head	1	9411.2–7498.1 6109.6–4597.6	First derivative + straight line subtraction	0.16	0.0165	0.50	0.0217	1.1	0.0090
	Middle	6	9411.2–7498.1 6109.6–5446.2 4613.1–4242.8	First derivative + MSC	0.58	0.0105	0.80	0.0111	1.4	0.0028
	End	7	9411.2–7498.1 6109.6–5446.2	Min-max normalization	0.46	0.0145	0.79	0.0153	1.4	0.0035
18,24	Head	4	9411.2–7498.1 5461.6–4242.8	Second derivative	0.34	0.0150	0.33	0.0210	1.1	0.0062
	Middle	10	9411.2–4242.8 9411.2–7498.1	Straight line subtraction	0.65	0.0084	0.58	0.0109	1.2	0.0022
	End	5	6109.6–5446.2 4427.9–4242.8	Constant offset elimination	0.35	0.0173	0.51	0.0183	1.1	0.0030
18,24		7	8454.7–7498.1 4427.9–4242.8	Constant offset elimination	0.41	0.0099	0.47	0.0158	1.1	0.0039

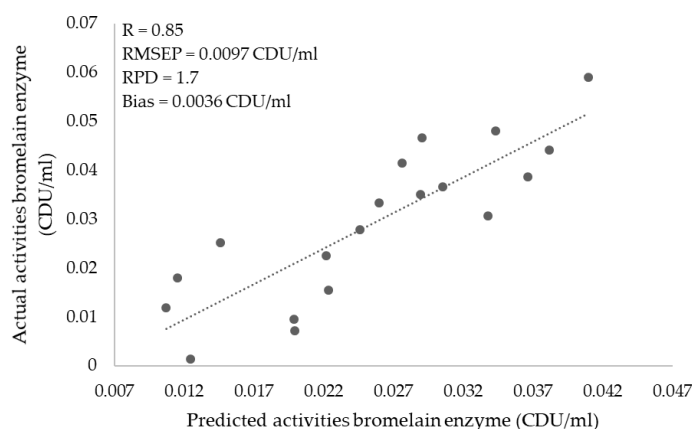


Figure 5 Comparison of bromelain enzyme activity of 18-month-old pineapple rhizomes samples predicted by NIR spectroscopy and measured in the reference laboratory.

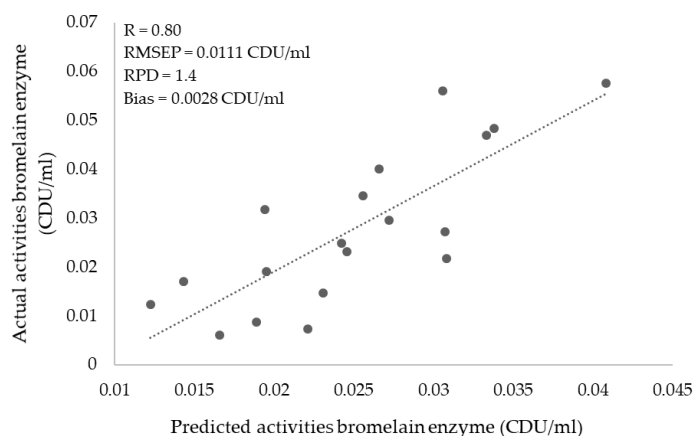


Figure 6 Comparison of bromelain enzyme activity of 24-month-old pineapple rhizomes samples predicted by NIR spectroscopy and measured in the reference laboratory.

Figure 7 presents the regression coefficient plot for bromelain enzyme activity in 18-month-old pineapple rhizome samples. There are distinct positive regression coefficients at the absorption bands of 4335, 4767, 4922, 5122, 6773, and 7051 cm^{-1} (2307, 2098, 2032, 1952, 1477, and 1418 nm, respectively). However, the characteristic bromelain-related absorption bands identified in Figure 3 (4382, 4629, 4875, 5786, and 6711 cm^{-1} , corresponding to 2282, 2161, 2051, 1728, and 1490 nm, respectively) are not prominently represented among the major regression coefficient peaks, suggesting limited correspondence between the spectral features associated with bromelain and those driving the predictive model. This mismatch is consistent with the relatively weak performance of the model.

The absorption band at 4335 cm^{-1} (2307 nm) and 7051 cm^{-1} (1418 nm) is associated with combination vibrations of C–H stretching and C–H deformation in CH_2 groups [33]. The band at 4922 cm^{-1} (2032 nm) corresponds to combination vibrations involving C=O stretching and the second overtone of CONH_2 groups [33]. In addition, the absorption at 6773 cm^{-1} (1477 nm) is attributed to the combination vibration of C–H stretching and the first overtone of CONHR groups [33].

Overall, spectral regions with higher regression coefficients exert a greater influence on the prediction of bromelain enzyme activity. In particular, the CONHR -associated band exhibited the strongest regression coefficient, indicating that vibrations related to C–H stretching first overtone play a dominant role in model prediction, despite their indirect relationship to bromelain-specific spectral features.

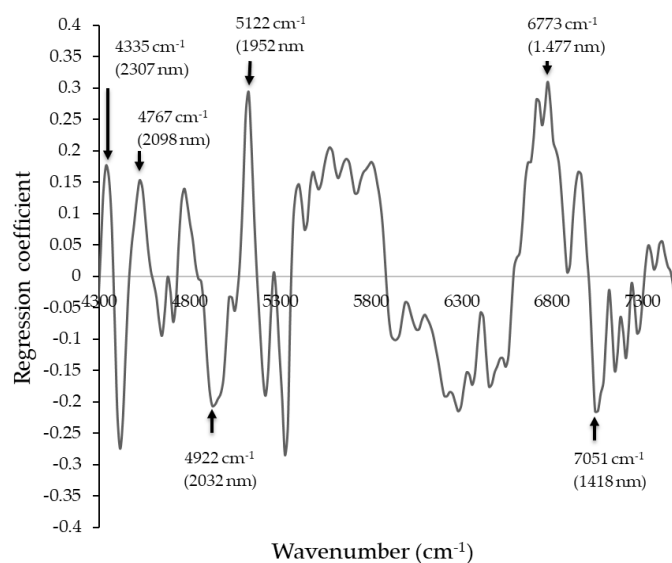


Figure 7 Regression coefficient plot of bromelain enzyme activity in 18-month-old pineapple rhizomes samples predicted by NIR spectroscopy.

4. Conclusions

The results indicate that the model developed from the middle section of 18-month-old pineapple rhizomes can be used for preliminary screening and estimation of bromelain enzyme activity. However, the absence of clear correspondence between regression coefficients and the spectral features of purified bromelain suggests that the model does not capture a distinct enzymatic “fingerprint,” which may contribute to its limited predictive performance. To improve model accuracy and robustness, future work should consider increasing both the number and diversity of samples to better represent biological variability. In addition, wavelength selection techniques may be applied to identify the most informative spectral regions and reduce redundant information. More advanced modeling approaches, such as Support Vector Regression (SVR) or Artificial Neural Networks (ANN), may also enhance predictive capability by capturing nonlinear relationships between spectral data and enzyme activity. Furthermore, the incorporation of advanced variable selection algorithms is recommended to improve model interpretability and to better identify subtle correlations between spectral features and enzymatic properties.

5. Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the author(s) used Gemini to assist with generating references in the appropriate paper format and QuillBot for grammar checking and paraphrasing text. After using these tools, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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7. Author Contributions

Keawsorn, K.: Conceptualization, validation, formal analysis, investigation, writing—original draft preparation, writing—review, editing, visualization, supervision, and funding acquisition; Meksophon, j.: validation, writing—original draft preparation; Chatchana, T.: validation, writing—original draft preparation; Bupta, C.: formal analysis, funding acquisition; Poonsri, W.: formal analysis, funding acquisition; Ratanacoon, M.: Conceptualization, validation, writing—original draft preparation, writing—review and editing, supervision; All authors have read and agreed to the published version of the manuscript.

8. Conflicts of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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