

EXTRACTION AND CHARACTERIZATION OF PECTIN FROM PINEAPPLE PEELS (ANANAS CONOSUS) AND COCOA HUSK

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Abstract

Pineapple peels and cocoa husks are typically discarded as agricultural waste from food processing in many areas of Chiang Rai. Here, we aim to extract and characterize the pectin from the pineapple waste and cocoa husk collected in the Chiang Rai area. Pectin was initially extracted using organic solvents (hydrochloric acid and citric acid), followed by ethanol precipitation. The pectin extracted was then evaluated for its dry matter yield, equivalent weight, and degree of esterification (%DE). The results showed that the pineapple residue contained 1.50-2.97% pectin, whereas the cocoa husk contained 6.20-15.86% pectin. The gram equivalent weight of cocoa husk was approximately 800-900, while pineapple waste was 1,300. The extracted pectin displayed low methoxy content, with a low level of DE (11-22%). Finally, the functional groups of pectin extracted were confirmed by Fourier transform infrared spectroscopy, showing the existence of the C=O and C-O stretching peaks at 1600-1700 and 1100-1200 cm^{-1} , respectively. This preliminary study provides useful information for future studies on the gelling properties of the pectin from pineapple and cocoa husk waste.

Keywords: Cocoa husk; Pectin; Pineapple waste; Proximate analysis; Waste management

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Introduction

Endemic plants of Chiang Rai, such as cocoa husks and pineapple peels, are typically discarded as waste, despite their significant potential for economic and environmental renewal. Various value-added studies have been conducted on these pineapple waste and cocoa husk wastes, including the extraction of pectin (Voragen *et al.*, 1995; Syamsiro, 2012; Dai *et al.*, 2018; Rodsamran and Sothornvit, 2019; Ramos *et al.*, 2021). Pectin is a carbohydrate polymer found in plants, and it serves as a substrate for the enzyme pectinase, which is present in plant cells, between fibers at the top of the first layer of the cell wall. Pectin binds cells together and is a major component of parenchyma tissue, along with arabinose, galactose, and galacturonic acid (Rathinavelu and Graziosi, 2005). With its high molecular weight and pectinic acid content, pectin can function as a gelling agent when mixed with food and water. It is also commonly used as a thickener and stabilizer in a variety of foods, including jams and jellies (Saha and Bhatthacharya, 2010).

The gelling properties of pectin are largely determined by the location of ester groups and the degree of methylation (DM). The hydrophobic interaction between the CH₃-groups was found to be inversely related to rupture strength and gel threshold in model systems (Oakenfull and Scott, 1984). However, factors such as pectin composition, DM, solubility, pH, temperature, and the presence of soluble solids can influence its activity. Commercial pectin extraction usually aims to obtain water-soluble pectin with a high molecular weight (Voragen *et al.*, 1995). Various methods, such as Nuclear Magnetic Resonance (NMR), have been used to investigate the structural properties of pectin in dietary carbohydrates (Cheng and Neiss, 2012; Sila *et al.*, 2009), but NMR's quantitative analytical capability in carbohydrate detection remains limited (Simmler *et al.*, 2014). Fourier transform infrared (FT-IR) spectroscopy has emerged as a powerful tool for studying the structure, physical properties, and interactions of carbohydrates, providing a new interpretive and experimental framework for the study of complex natural polymer systems (Kacurakova and Wilson, 2001; Chargot and Zdunek, 2013).

This research focuses on the extraction and characterization of pectin from pineapple and cocoa husk waste, which were subsequently evaluated for their physical and chemical characteristics, using equivalent weight, degree of

esterification, and functional-group methods. The findings in this work could be utilized for developing gelling agents, thickening agents, and emulsifying agents in the food industry.

Materials and Methods

Preparation waste samples

Samples of pineapple peel, pineapple eye bud, cocoa pod husk, and cocoa husk wastes were collected from difference area Chiang Rai Province, Thailand, which were utilized as raw materials for pectin extraction. Pectin was extracted from 100 g of dried cocoa pod husk, cocoa bean husk, and pineapple peel, and pineapple floret, which were dried in an oven at 50°C. The experimental procedure involved the preparation of a solution comprising 0.05 M hydrochloric acid (HCl) in water at a ratio of 1:12 (w/v), alongside a solution of 0.05 M citric acid, with the same ratio. Furthermore, solutions of 0.05 M HCl with 6% sodium hexametaphosphate (SHMP), and 0.05 M citric acid with 6% SHMP were prepared. These solutions were employed in treating the samples at a temperature of 95°C, for a duration of 90 minutes, as part of the extraction process. The selection of these specific solvent combinations was deliberate, and intended to assess their respective impacts on pectin yield within the context of the study. Following the extraction process, the pectin solution was filtered using a white filter cloth, and 95% acidified alcohol was employed to precipitate the filtered solution. The residue was subsequently dried using acetone through five successive iterations, or until a colorless filtrate was obtained. The resulting gel was allowed to precipitate at 25 °C for 24 hours, after which the pectin was dried at 65°C for another 24 hours. The dried pectin was then ground to pass through 60 mesh sieves for further analysis.

Proximate composition analysis

The waste residues obtained from pineapple peel, pineapple eye bud, cocoa pod husk, and cocoa husk were subjected to proximate analysis by AOAC approved methods for dry matter content (AOAC, 1998, 2005). Standard techniques were employed to assess the levels of crude protein, crude fat (ether extract), crude fiber, and ash content. All analyses were conducted, and the results were recorded and reported.

Equivalent weight

To determine the equivalent weight (Ranganna, 1995), 0.5 g of the sample was weighed and placed in a 250 mL conical flask. Subsequently, 5 mL of ethanol, 1.0 g of NaCl, and 100 mL of distilled water were added to the flask. Then, 6 drops of phenol red were introduced, and the solution was titrated against of 0.1 M (N) NaOH. The endpoint of the titration was indicated by the appearance of a purple color. This neutralized solution was stored for the subsequent determination of the methoxy content. The equivalent weight was calculated using the following formula:

$$\text{Equivalent weight} = (1000S)/(N \times V)$$

Where S represents the dry weight of the sample, N denotes the normality concentration of NaOH, and V represents the quantity of NaOH used in the titration.

The degree of esterification

To determine the degree of esterification (%DE) of the extracted pectin, 0.5 g of pectin powder was dissolved in 2 mL of ethanol and mixed with 100 mL of water. The mixture was stirred until a homogeneous solution was obtained. Subsequently, 10 mL of 0.5 M NaOH was added and stirred for 15 minutes. Afterward, 10 mL of 0.5 M HCl was added and stirred until the solution became colorless. A phenolphthalein indicator was then added, and the solution was titrated with 0.5 M NaOH (V2). The %DE was calculated using the following equation (Jittra *et al.*, 2004):

$$\%DE = (\text{NaOH}v1 / (\text{NaOH}v1 + 2)) \times 100$$

Where NaOHv1 represents the volume of 0.5 M NaOH used in the titration.

Functional group analysis

The functional groups of the extracted pectin were investigated using Fourier transform infrared spectroscopy (FT-IR), specifically employing the Nicolet 6700 FT-IR spectrophotometer (Bruker, Germany). Two milligrams of each sample were

mixed with 100 mg of KBr powder. The mixture was placed into the die set, and a hydraulic pressure of 8-10 tons was applied to form a pellet. The pellet was then mounted in the sample holder of the FTIR spectrometer, and the spectrum was recorded. The spectra were collected by averaging 64 scans at a resolution of 4 cm⁻¹ within the range of 400-4000 cm⁻¹.

Results and Discussion

Proximate analysis of pineapple and cocoa husk

To analyze the composition of pineapple and cocoa husk samples, proximate analysis were performed, and the results were collected in Table 1 for the four samples (pineapple peel, pineapple floret, cocoa pod husk, and cocoa bean husk). This data highlights the abundance of dry matter as the primary ingredient, followed by carbohydrates, crude fiber, crude protein, and ash, respectively. The dry matter composition ranged from 87 to 91%. Pineapple samples and cocoa pod husk accounted for 53-56% of dry matter, while cocoa bean husk represented less than half, at 29%. These findings indicate that the cocoa bean husk gave a lower sugar content compared to other materials (Chanprasartsuk *et al.*, 2020).

The crude fiber content ranged from 17 to 28%, with the pineapple peel and cocoa bean husk having the higher fiber content compared to the other samples. This fiber-rich residue can be utilized in the production of dietary fibers, absorbents, gelling agents, and thickeners.

Crude protein content in pineapple peel, pineapple floret, and cocoa pod husk ranged from 4 to 7%, with cocoa pod husk exhibiting double the protein level, at 15%. The ash content across all four samples ranged only 3 to 7%, with pineapple peel showing the lowest content, indicating a lower mineral content in the pineapple peel, compared to the waste samples reported in the literature (Chareekhot, 2016). Fat content in pineapple peel, pineapple floret, and cocoa pod husk were approximately 1-2%, while cocoa bean husk contained a significantly higher fat content, of 11%.

Table 1. Proximate composition (% weight) of pineapple waste and cocoa husk (mean ± standard deviation)

Composition	Pineapple peel	Pineapple floret	Cocoa pod husk	Cocoa bean husk
Dry matter	89.81±0.04	87.88±0.04	91.51±0.15	91.60±0.00
Ash	3.09±0.03	6.39±0.03	6.67±0.13	7.13±0.10
Crude Fiber	26.83±0.65	17.75±0.23	18.67±0.32	28.48±0.40
Fat	2.21±0.07	1.48±0.01	1.90±0.33	11.19±0.03
Crude Protein	4.60±0.05	6.60±0.06	7.50±0.13	15.80±0.07
Carbohydrates	53.10±0.62	55.60±0.38	56.80±0.14	29.0±0.27

Additionally, the findings presented in Table 1 indicate that the ash, protein, and lipid content of cacao pod husk align with the research conducted by Vega *et al.* (Vega *et al.*, 2018).

Characterization and properties of pineapple and cocoa husk samples

Previous studies (Attri and Maini, 1996; Handee *et al.*, 2016) investigated the impact of different organic solvent conditions on the performance of pectin extraction. Different organic solvents were tested for the optimal percent yield of the pectin extraction, using different combinations of sodium hexametaphosphate (SHMP), hydrochloric acid (HCl), and citric acid, as shown in Table 2. Results indicate that although HCl solution is less extractable than SHMP (data not shown), it exhibits superior extraction properties compared to citric acid (see Table 2). Besides, HCl is often used in the industry processes of pectin extraction. Most samples gave a dark brown color from the resulting pectin, except the cocoa bean husk, showing a light brown color. Among the samples and if HCl is used, cocoa bean husk had the highest pectin concentration (7.10%), followed by cocoa pod husk (6.20%), pineapple floret (2.97%), and pineapple peel (1.70%). These results are consistent with the data reported in the literature (Petkovic *et al.*, 2012). Furthermore, the data in the Table 2 also points out that both cocoa pod and bean husks can be utilized

as ingredients in various dishes, similar to the pectin extracted from durian peel reported by Suwannarat *et al.* (Suwannarat *et al.*, 2019). We also found that this pectin had a taste acceptance of jam and jelly, in comparison to commercially produced jam supplemented with additional pectin.

When adding SHMP to HCl, the pectin concentration was determined to be higher in the cocoa husks (i.e., 15.86% of the cocoa pod husk and 13.90% of the cocoa bean husk). Pineapple samples contained pectin at a very low concentration (1.5–2.67%). On the contrary, when citric acid was utilized, the amount of pectin was relatively lower than when using HCl and HCl + SHMP, and the pectin from pineapple samples could't be determined, probably due to its low content.

When the equivalent weights of pectin from various samples were analyzed (Table 3), it was observed that the equivalent weights of pectin produced from waste materials were 883.33 for cocoa pod husks, 946.58 for cocoa bean husks, and 13035.71 for pineapple peel. These results suggest that the resulting weights of the extracted pectin had varying quantities. This could be because of low pH at the time of extraction. Temperature and time of extraction are different variables. Due to the equivalent weight being a value that indicates the amount of free carboxyl groups in the structure that are not esterified, a low equivalence weight indicates that pectin has a small amount of free

Table 2. Percent yield and physical appearance of pectin, extracted using different extraction conditions of sodium hexametaphosphate (SHMP), hydrochloric acid (HCl), and citric acid (Citric)

Samples	% Yield				Physical appearance
	HCl	HCl SHMP	Citric	Citric + SHMP	
Cocoa pod husk	6.20±0.25	15.86±0.43	6.20±0.07	11.42±0.56	Dark brown
Cocoa bean husk	7.10±0.29	13.90±0.24	7.10±0.16	12.60±0.62	Light brown
Pineapple peel	1.70±0.02	1.50±0.06	ND	ND	Dark brown
Pineapple floret	2.97±0.04	2.67±0.08	ND	ND	Dark brown

ND = not detected

Table 3. Equivalent weight and degree of esterification of the extracted pectin from HCl + SHMP solution extraction

Pectin	Weight (g)	NaOH Volume, V (mL)	NoH Concentration, N (mol/L)	1000S/NV	Equivalent Weight, Eq.Wt.
Cocoa Pod Husk	0.11	1.20	0.10	916.67	883.33
	0.10	1.00	0.10	1000.00	
	0.11	1.50	0.10	733.33	
Cocoa Bean Husk	0.13	1.20	0.10	1083.33	946.58
	0.10	1.20	0.10	833.33	
	0.12	1.30	0.10	923.08	
Pineapple waste	0.90	0.80	0.10	11250.00	13035.71
	0.90	0.60	0.10	15000.00	
	0.90	0.70	0.10	12857.14	

carboxyl groups in its structure, that are not esterified (Tangwongchai *et al.*, 2006). The amount of methoxyl and carboxyl in the molecular structure was shown by the equivalent weight. The higher the equivalent weight, the more significant the methoxyl group is in the structure. Pectins with a higher equivalent weight possess a more complex structure and enhanced gel-forming capacity compared to those with a lower equivalence weight (Saraswaty *et al.*, 2017). As a result, pectin from cocoa husks and pineapple can be extracted under different conditions, such as varying pH levels, leading to differences in physicochemical properties. These differences in properties affect gelation factors, such as the addition of sugar and Ca^{2+} , resulting in various applications.

The degree of esterification (DE) of pectin ranges from 0 to 100 and is used as a grading criterion. A DE score of more than 50% typically indicates high methoxy pectin (HMP), which can gel at pH 4, or in the presence of more than 55% sucrose. Conversely, a DE score of 50% or less suggests low methoxy pectin (LMP), which can gel in the presence of calcium ions. LMP tends to gel quickly due to its lower degree of esterification. A gelatinous framework with cations, particularly calcium, can form under constant electrostatic conditions (Table 4). The pectin derived from the three wastes has an LMP characteristic, making it appropriate for providing texture to food, particularly low-fat cuisine (Ciriminna *et al.*, 2016).

Table 4. Equivalent weight and degree of esterification of the extracted pectin from HCl + SHMP solution extraction

Pectin	v1	v2	$(v2/(v1+v2)) \times 100$	Average
Cocoa Pod Husk	3.80	0.40	9.52	11.41±1.9
Pod	3.90	0.60	13.33	
Husk	3.90	0.50	11.36	
Cocoa Been Husk	2.30	0.90	28.13	22.01±6.72
Been	2.30	0.40	14.81	
Husk	2.00	0.60	23.08	

Interestingly, the pectin derived from cocoa husk using mineral an acid like HCl, at 95°C for 90 min, exhibited a degree of esterification (%DE) ranging from 11 to 22% (Table 4). This indicating a prevalence of low methoxy pectin where the glycosidic group of methyl ester of pectin tends to be hydrolyzed to produce galacturonic acid (Musita, 2021). This finding suggests the potential applicability of cocoa husk pectin in gelation processes. Commercial pectin derived from citrus fruit typically exhibits a higher degree of esterification (DE), often exceeding 50%, classifying it as high methoxyl pectin (HMP) (Tangwongchai *et al.*, 2006). Such pectins necessitate high sugar concentrations and acidic

conditions to achieve gelation. In contrast, pectins extracted from alternative plant sources, including sunflower heads (Iglesias and Lozano, 2004), display a wide range of DE values, to the variability observed in pectins from cocoa pod husks and cocoa bean husks. The low DE of pectin from cocoa pod and cocoa bean husks suggests that these pectins gel in the presence of calcium ions (Ca^{2+}), as opposed to requiring high sugar content and acidic conditions. Consequently, these pectins are particularly suitable for applications where low sugar content is essential, such as in low-calorie or diabetic-friendly food products.

Functional analysis of pectin extracts by FT-IR

The FT-IR technique is used to further describe the pectin structure, as shown in Figure 1. Important wave numbers at 1740, 1610, 1240, or 1370, 1361 cm^{-1} (Kacurakova and Wilson, 2001) correspond to the ester C=O stretching, COO-antisymmetric stretching, C-O stretching, and CH_2 bending, respectively. The structural characteristics of the extracted pectin were similarly detected with the C=O stretching peak at 1600-1700 cm^{-1} , the C-O stretching peak around 1100-1200 cm^{-1} , and close to those reported for commercial pectin (Musita, 2021, Mada *et al.*, 2022)

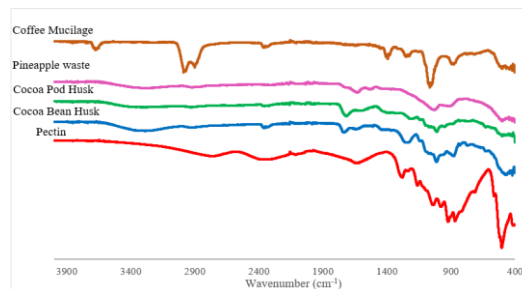


Figure 1. FT-IR graph of the pectin extracted

Conclusions

This study reports the extraction and characterization of pectin from pineapple (*Ananas comosus*) waste and cocoa husk. The investigation also aimed to elucidate key characteristics of the extracted pectin. The pectin yield obtained from the extraction process was found to be 15.86%w/w, which falls within the range observed for apple pomace (3.5-14.32%w/w). Furthermore, the equivalent weight of cocoa husk was estimated to be between 800 and 900, whereas that of pineapple peel was determined to be 1303. In light of the FT-IR spectrum, a common practice involves modestly scaling the frequency axis to align significant bands with those detected experimentally. By employing

this approach, the predicted structure of the extracted pectin was confirmed through FT-IR structural analysis. Based on our initial results, future investigations will focus on forecasting and examining gelling parameters such as pH, acylation, temperature, and molecular weight of the extracted pectin. By undertaking these endeavors, we aim to enhance our understanding of the pectin's properties and its prospective applications.

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