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Research article

Application of low methoxyl pectin powder derived from palmyra palm pulp wash water to enhance toddy palm cake quality

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Abstract

Importance of the work: One key aspect of the circular economy involves harnessing the potential of waste to create added value. Utilizing products from wastewater collected during the palmyra palm pulp (PPP) washing process might have value and avoid causing environmental pollution.

Objectives: To recover and characterize pectin from water collected from the PPP washing process and to demonstrate a practical application for the recovered low methoxyl pectin (LMP).

Materials & Methods: The pectin recovery involved washing the fresh PPP with distilled water, separating the pulp, precipitating the pectin in 95% ethanol and subsequently drying and grinding. Then, the pectin powder was characterized and incorporated into the production process of toddy palm cake (TPC) to demonstrate a practical application.

Results: The yield of the recovered pectin was in the range 10–15% weight per weight. Characterization of the pectin powder from PPP confirmed it to be LMP, with a degree of esterification of 32.28 ± 3.63% (mean ± SD). The pectin solution formed a gel with calcium ions, with a minimum concentration of 30 mg/g of pectin. In the absence of pectin, the introduction of calcium had detrimental effects on the TPC properties, resulting in increased hardness and reduced surface porosity. The addition of pectin significantly improved the quality of the TPC reducing the hardness of the TPC sample and increased its springiness, specific volume and surface porosity, surpassing the properties of the controlled sample.

Main finding: Utilizing the LMP in the palmyra palm pulp wash water presented an opportunity to extract pectin in a sustainable and cost-effective manner. The enhancement in the TPC texture highlighted the positive impact of incorporating pectin, leading to a more enjoyable eating experience.

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Introduction

The utilization of natural ingredients in the food industry has gained much attention in recent years due to consumer demand for healthier and more sustainable products (Basak and Annapure, 2022). Pectin, a complex polysaccharide found in various plant tissues, is one such natural ingredient that exhibits unique functional properties and has been widely used in the food industry as a gelling agent, thickener, stabilizer and emulsifier due to its ability to form gel networks, enhance texture and improve product stability (Zeeb et al., 2021). Additionally, pectin offers health benefits, such as cholesterol-lowering effects, prebiotic properties and potential anti-cancer activity (Devi et al., 2023).

The palmyra palm (*Borassus flabellifer* L.) is a versatile tropical plant that offers various valuable products. For example, palmyra palm fruits are rich in pulp, the main component needed for various applications (Basava et al., 2023). During the extraction process, a substantial amount of water is required to separate the pulp from the fruits, resulting in wash water as a byproduct. Notably, this wash water contains low methoxyl pectin (LMP), which has gained popularity in the low-calorie food industry due to its ability to form gels without sugar (Grant et al., 1973), enabling the production of reduced-sugar or sugar-free products that cater to the growing consumer demand for healthier alternatives (Wan et al., 2021; Yang et al., 2022). Prior to the current study, it was discovered that the water collected from the palmyra palm pulp washing process contained natural LMP in the approximate range 5–15% by weight. Because the availability of natural sources for LMP production is limited, this finding suggested that the value of these byproducts from the pulp separation process could be increased. Notably, this practice aligns with the principles of 'the circular economy', which prioritize the adding value to waste of natural resources.

Traditionally, toddy palm cake (TPC), a popular bakery product in Thailand, is made from palmyra palm pulp. Since the palmyra palm pulp loses some pectin during the pulp separation process, the addition of pectin into the palmyra palm pulp could potentially enhance the quality and functionality of TPC. Therefore, this study investigated the potential application of palmyra palm pectin, extracted from the wash water, in TPC preparation. The physicochemical properties and rheological behavior were evaluated of the pectin derived from palmyra palm, along with its effects on TPC properties, to gain valuable insights into the potential use of palmyra palm pectin as a functional ingredient in the bakery industry.

Material and Methods

Materials

Mature and ripe fruits of the palmyra palm (*Borassus flabellifer* L.) were obtained from a palmyra palm plantation in Suphan Buri province, Thailand. The fruits were collected at a stage of maturity in the range 100–110 d. Each fruit weighed (approximately 1.2–1.6 kg). All the chemical reagents used were of analytical grade.

Preparation of palmyra palm pulp wash water

To prepare the palmyra palm pulp wash water (PW), the fruit skin was manually separated from the pulp using a knife. All the collected pulp samples from the palm fruits were combined to ensure consistency. The pulp was stirred in distilled water using a mixer for 5 min at 25–30°C. For one batch, the total amounts of pulp and water utilized were 2 kg and 4 L, respectively. The total soluble solid content (mean ± SD) in the filtered wash water was 19.88±0.70 g/L. After washing, a commercial cold-press juicer (X1; Goodnature, USA) with a 35-mesh filter bag was used to separate the pulp from the water. The resultant PW was promptly collected and utilized within 1 hr to preserve its freshness and ensure its suitability for subsequent use.

Preparation of pectin powders by precipitation

Crude pectin powder (CPP) and purified pectin powder (PPP) were prepared using a traditional method for pectin extraction, as described in Belkheiri et al. (2021), with the exception of acid extraction because the pectin molecules were already dissolved in the wash-water, rendering the sample ready for the precipitation process. Ethanol (95%) was added to the PW at a PW-to-ethanol volume ratio of 1:3. The mixture was left undisturbed for 10–15 min or until a light-yellow pectin lump formed on the surface. Then, the precipitated pectin was separated from the mixture using a 100-mesh sieve. The separated pectin was dried in a hot-air oven (UF110; Memmert, Germany) at 60°C for approximately 18 hr. The dried pectin product was milled using a commercial blender (HR2120; Philips; Thailand). Then, the milled pectin was filtered using a 60-mesh sieve to obtain the CPP, which was promptly stored in an aluminum foil bag containing a silica gel bag to absorb any moisture. To prepare the PPP,

the PW sample underwent centrifugation at 10,000 revolutions per minute (12,475×*g*) for 15 min, after which the supernatant was mixed with 95% ethanol at a supernatant-to-ethanol volume ratio of 1:3. Similar to the procedure used for CPP, the precipitated pectin was obtained by drying the sample at 60°C, followed by milling and sieving using a 60-mesh sieve.

Characterization of the crude and purified pectin powder

Moisture content, total solids and bulk density

The moisture content (MC) and total solids were determined by accurately weighing an amount (1–2 g) of the sample (for both CPP and PPP) and placing in an aluminum can. The sample can was placed in a hot-air oven (UF110; Memmert, Germany) at 105°C. The MC and total solids were calculated based on difference between the mass of the sample before and after drying.

The bulk density (in grams per cubic meter) was determined by measuring the mass (m_{CPP}, in grams) of 5 mL of powder and using Equation 1:

$$\text{Bulk density} = m_{CPP} / 5 \quad (1)$$

Water activity and color measurement

The water activity in the sample was examined at 25°C using a water activity meter (Series 3TE; AquaLab; USA). The color of the sample was measured based on the $L^*a^*b^*$ system using a spectrophotometer (MiniScan EZ 4500; HunterLab, USA). The instrument had an aperture size of 12 mm and was adjusted using the CIELAB color scale, a D65 illuminant and an observer at 10°. The sample weighed about 1 g.

Degree of esterification, equivalent weight and methoxyl content

The degree of esterification (DE) was determined using a modified method of Jin et al. (2022). A 1.5 g sample (W_p) of CPP or PPP pectin powder was mixed with 5 mL ethanol, 1 g sodium chloride, 95 mL deionized distilled water and six drops of 0.4% phenol red indicator. The sample was titrated with 0.05 N NaOH to the color change endpoint (V1). Then, 10 mL of 0.05 N NaOH was added, followed by 10 mL of 0.05 N HCl. A second titration with 0.05 N NaOH yielded V2. The DE (as a percentage), equivalent weight (W_e; in g/mol) and methoxyl (MeO) content (as a percentage) were calculated using Equations 2–4, respectively:

$$DE = V_2 / (V_1 + V_2) \times 100\% \quad (2)$$

$$W_e = W_p \times 1{,}000 / (0.05 \times V_1) \quad (3)$$

$$MeO = V_2 \times 0.05 \times 3.1 / W_p \times 100 \quad (4)$$

Galacturonic acid

The galacturonan content was determined using a colorimetric method, as described by del Amo-Mateos et al. (2023), with galacturonic acid (GalA) as the standard. A 0.1 g pectin powder sample was mixed with 100 mL of 0.05 M NaOH to form P_1. Then, 10 mL of P_1 was diluted to 100 mL with deionized water, creating P_2. In another container, 2 mL of P_2 was mixed with 1 mL of 0.1% weight per volume (w/v) carbazole solution. After 25 min, 12 mL of concentrated sulfuric acid was added. Absorbance at 525 nm was measured using a spectrophotometer (UV-1800; Shimadzu; Japan). The GalA concentration (GalAC) in micrograms per milliliter was determined from a standard curve. The GalA content (as a percentage weight per weight, %w/w) in the pectin powder sample was calculated using Equation 5:

$$GalA = GalA_C \times 15 \times 100\% / (W_p \times 10^6) \quad (5)$$

where W_p represents the weight of the pectin powder sample in grams, 15 is the total volume in milliliters of the GalA solution being measured and 10^6 is the unit conversion factor from grams to micrograms.

Protein content

The protein contents in the CPP and PPP samples were determined following Nwachukwu and Aluko (2019) by dissolving 0.1 g of pectin powder in 10 mL of deionized water, then hydrolyzing 0.1 mL of this solution with 2N NaOH (1:1 ratio) at 100°C for 10 min. Afterward, 1 mL of Lowry agent was added and incubated at 25°C for 10 min, followed by adding 0.1 mL of Folin-Ciocalteu reagent and further incubation for 30 min. Absorbance at 550 nm was measured using a spectrophotometer (UV-1800; Shimadzu; Japan). The protein content was determined using a bovine serum albumin (BSA) standard curve.

Crude fiber content

The fiber content of each sample was determined following the procedure described by Nielsen (2010). After drying at 110°C for 1 hr and grinding into a powder, approximately 1 g of CPP or PPP (W) was digested in 1.25% sulfuric acid for 30 min. Next, this was washed with water and then boiled in 1.25% NaOH for 30 min, reaching a neutral pH. After washing,

the sample was rinsed with 95% ethanol, dried at 101±1°C for 4–6 hr and the weight was recorded (A). Following ignition at 600°C for 1.5 hr, the sample was reweighed (B). The crude fiber (CF) content (as a percentage on a weight-per-weight basis) was calculated using Equation 6:

$$CF = (A - B) \times 100 / W \quad (6)$$

Fourier-transform infrared spectroscopy

The dry powder samples (CPP of PPP) were blended and their spectra were recorded using a spectrophotometer (Thermoscientific; USA). The range of wavelengths scanned was 400–4000 cm^{-1}, with 32 scans conducted. For reference, the Fourier-transform infrared spectroscopy (FTIR) spectra of the palmyra palm pulp were also obtained.

Rheological properties

The rheological properties of the pectin solutions were evaluated using a controlled-stress rheometer (RheoStress 1; Haake; Germany) equipped with a cone-and-plate geometry (P35-MPC60). The cone-plate gap distance was maintained at a constant value of 0.3 mm. The pectin solutions (5%w/w), each containing varying concentrations of Ca^{2+} (0 mg/g, 10 mg/g, 20 mg/g or 30 mg/g of pectin) were transferred onto the pre-equilibrated geometry plate at 25°C**.** Amplitude sweep tests were conducted by varying the shear strain in the range 0.5–1,000% while maintaining a constant frequency of 1 Hz at 25°C. The time dependency sweep test was performed at a constant frequency of 1 Hz and a constant strain of 0.5% at 25°C.

Using pectin powder in toddy palm cake recipe

Toddy palm cake (TPC) served as the bakery food model for this study. To assess the impact on the properties of the TPC, varying quantities of crude pectin powder (CPP, DE32%—as mentioned in Table 1) and commercial low methoxyl pectin (COM, DE16%) were incorporated into the batter at different calcium levels.

Toddy palm cake preparation

The TPC was prepared using the following ingredients: 100 g of all-purpose wheat flour (Kite; United Flour Mill PCL; Thailand), 100 g of coconut milk (with approximately 18% coconut oil and 82% water), 80 g of sugar, 50 g of palmyra palm pulp, 5 g of yeast, 3 g of baking powder and 1 g of salt. Different quantities of pectin (CPP or COM) and $Ca(OH)_2$ were added according to the test conditions specified in Table 1. The ingredients were thoroughly mixed in the blender for approximately 10 min using a circular motion. Then, the batter was aged in a sealed container for at least 1 hr before being poured into 20 mL stainless steel molds and steamed for 25 min. After steaming, the samples were cooled to room temperature for approximately 30 min before further analysis. The water activity and color of the TPC samples were determined using the methods described in subsection 2.4.2.

Textural properties

The hardness and springiness index of the TPC samples were measured using a universal texture analyzer (LR 5K; Lloyd Instruments; UK) equipped with a 35 mm diameter cylinder probe. The shape of the TPC sample was controlled by cutting using a hollow stainless cylindrical mold. The cylindrical mold had an inner diameter of 13 mm and a height of 20 mm. Thus, each TPC sample was a cylindrical shape with dimensions of 13 mm in diameter and 20±1 mm in height. The texture profile analysis was conducted with the following parameters: 2 mm/s compression speed, 0.005 N trigger force set and 10 mm deformation level, which was 50% of the original height.

Table 1 Summary of toddy palm cake formulas

Sample	Pectin (g)	*Pectin (%weight per volume of water)	$Ca(OH)_2$ (mg)	Ca^{2+} (mg/g of pectin)
Control	0	0	0	N/A
0-1	0	0	136	N/A
0-2	0	0	272	N/A
(COM or CPP) 3-0	2.46	3	0	0
(COM or CPP) 3-1	2.46	3	136	30
(COM or CPP) 3-2	2.46	3	272	60
(COM or CPP) 6-0	4.92	6	0	0
(COM or CPP) 6-1	4.92	6	136	15
(COM or CPP) 6-2	4.92	6	272	30

CPP = crude pectin powder and COM = commercial pectin powder; N/A = not applicable.

* Calculated from ratio of pectin mass and total volume of water (82 mL).

Specific volume, expansion ratio and surface porosity

The volume of the TPC samples was determined using a rapeseed displacement method (Zanoletti et al., 2017). The specific volume was calculated by dividing the volume by the weight of the sample; then, the expansion ratio of the TPC samples was calculated by comparing the volumes of the TPC and the batter used to prepare it. To estimate the surface porosity of the TPC samples, thin slices of the samples were obtained with a thickness of less than 1 mm. Images of the sample surfaces were captured using a light microscope (CHS; Olympus; Japan) and the surface porosity was analyzed using the ImageJ software (National Institutes of Health; USA). The surface porosity was determined from the ratio of the void area to the total area.

Statistical analysis

A full factorial design was used in this study. All experiments involved three independent replicates. Analysis of variance was conducted to assess the significance of the factors in influencing differences between the means. Duncan's new multiple range test was used to compare among means at a confidence level of 0.95 (α = 0.05). The results from the experiments were recorded as the mean ± SD.

Results and Discussion

Yields and physicochemical properties of pectin powder

Fig. 1 illustrates the appearance of the CPP and PPP samples, showing a yellowish-brown powder, which could be attributed to the presence of β-carotenoid residues in the fruit pulp. Table 2 provides the yield and various properties of the CPP and PPP. The yield of PPP was significant lower (by approximately 5%w/w) than the CPP yield, primarily due to the removal of impurities during centrifugation. These values were comparable to those reported for pectin extracted from various sources (Belkheiri et al., 2021; Tran et al., 2023). Consequently, the GA content of the PPP was higher than that of the CPP by approximately 11%w/w. Both CPP and PPP had high %GA values (51% and 61%, respectively). Both the CPP and PPP samples were classified as low methoxyl pectin since their DE values were below 50% (Einhorn-Stoll et al., 2021). No significant difference was observed between the DE values of the CPP and PPP samples. The DE values and GA content of the CPP and PPP were comparable to the low methoxyl pectin extracted from various kinds of fruits and vegetables, such as apple, blue burry, pitahaya and tangerine (Ripoll and Hincapié-Llanos, 2023). The W_e value of the CPP was comparable to that of the PPP samples. Notably, the average W_e value of the obtained pectin samples was relatively high compared to values reported in the literature, which may be attributed to variations in pectin sources and the extraction methods used. For example, pectin extracted from apple peel waste had a W_e value of approximately 650 g/mol (Kumar and Chauhan, 2010), while pectin extracted from *Nephrolepis biserrata* leaves had a W_e value of 1,250 g/mol (Pagarra et al., 2018). The bulk density was likely related to the water activity and the moisture content. The values for the bulk density, water activity and moisture content of the CPP were higher than those of the PPP. Because centrifugation removed more impurities from the PPP sample, the moisture content in

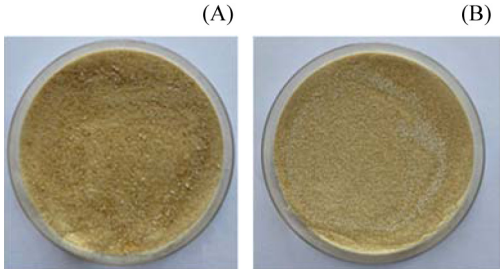

Fig. 1 Appearance of samples obtained from precipitation and passing through 60-mesh sieve: (A) crude pectin powder; (B) purified pectin powder

Table 2 Yield and physicochemical properties of the crude pectin powder (CPP) and purified pectin powder (PPP)

Sample	CPP	PPP
Yield (%w/w-db)*	14.57±0.62[a]	9.65±1.67[b]
Bulk density (g/mL)	0.723±0.008[a]	0.682±0.013[b]
L^*	74.80±0.48[a]	71.13±1.08[b]
a^*	4.44±0.28[a]	3.90±0.12[b]
b^*	21.24±1.17[a]	18.93±0.46[b]
a_w	0.367±0.013[a]	0.345±0.007[b]
Moisture content (%w/w)	17.57±0.18[a]	11.83±0.83[b]
Protein (%w/w)	6.72±1.08[a]	5.72±1.16[a]
Crude fiber (%w/w)	not detected	not detected
Degree of esterification (%)	32.28±3.63[a]	33.82±1.83[a]
Equivalent weight (g/mol)	6938±399[a]	7063±590[a]
Methoxyl content (%)	21.01±3.16[a]	22.22±2.19[a]
Galacturonic acid (%w/w)	50.89±4.28[a]	61.43±4.01[b]

Mean ± SD (n = 3) in each row superscripted with different lowercase letters are significantly ($p < 0.05$) different.

CPP = crude pectin powder; COM = commercial pectin powder; %w/w = % weight per weight; -db = on a dry basis; * yield based on dry weight of palmyra palm pulp.

the PPP sample should have been effectively reduced during the precipitation process with ethanol, compared to the CPP sample. Consequently, the PPP sample contained less water, resulting in a lower bulk density than for the CPP sample. Overall, the properties of the CPP were similar to those of the PPP. Due to the simplicity of production, only the CPP samples were primarily utilized in the rheological experiments and used in toddy palm cake preparation.

Fourier-transform infrared spectroscopy spectra

Fig. 2 depicts the FTIR spectra of the pectin powder and dried palmyra palm pulp samples. The CPP spectrum had similarities to that of the PPP. The peak observed at 1740 cm^{-1} was attributed to the presence of the carbonyl group in the esterified methyl group ($COOCH_3$) and the carboxylic acid group (COOH) (Lira-Ortiz et al., 2014). Additionally, the peak observed at 1600 cm^{-1} corresponded to the stretching vibration of the carboxylate anion (COO-), according to Jong et al. (2023). The absorbance bands at 1740 cm^{-1} and 1600 cm^{-1} in the FTIR spectra corresponded to the esterified and free carboxyl groups, respectively (Lira-Ortiz et al., 2014). The peaks observed at 1417 cm^{-1}, 1225 cm^{-1} and 1010 cm^{-1} indicated the bending of CH_2, $–CH_3CO$ stretching and C–O–H deformation, respectively (Jong et al., 2023). Notably, the positions of all absorbance peaks in the dried palmyra palm pulp spectrum closely resembled those observed in the spectrum of the pectin powder samples. Indeed, the higher ratio between the absorbance heights at 1600 cm^{-1} and 1740 cm^{-1} in the dried palmyra palm pulp spectrum compared to the pectin powder samples suggested a higher proportion of free carboxyl groups relative to esterified methyl groups. This observation suggested that native pectin molecules in the palmyra palm pulp might have undergone esterification during the washing process, potentially catalyzed by enzymes.

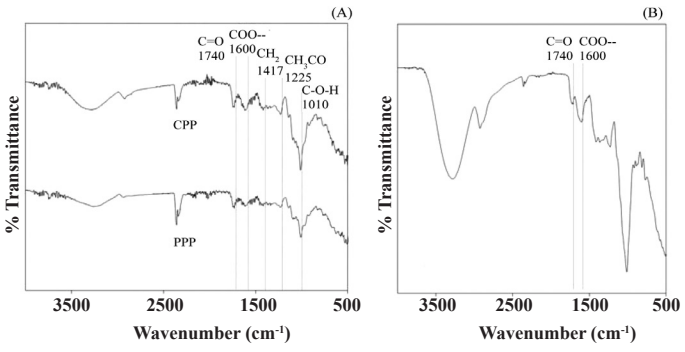

Fig. 2 Fourier-transform infrared spectra of: (A) purified pectin powder (PPP) and crude pectin powder (CPP); (B) dried palmyra palm pulp

Rheological properties

The amplitude sweep test was conducted on 5% pectin solutions with varying concentrations of Ca^{2+}. The upper limit of the linear viscoelastic region, defined as the point where the storage modulus (G') decreases to 95% of its initial value, varied depending on the concentration of Ca^{2+}. When the Ca^{2+} concentrations were 0 mg/g of pectin, 10 mg/g of pectin, 20 mg/g of pectin and 30 mg/g of pectin, the corresponding strain values at which this limit was reached were 1,460%, 1,152%, 12.2% and 7.5%, respectively. To ensure that the subsequent rheological experiments were conducted within the linear viscoelastic region, a strain of 1% was used unless stated otherwise.

Fig. 3 depicts the rheological behavior of a 5% w/v pectin solution during oscillatory time sweeps. In the absence of calcium ions, the solution exhibited more liquid-like characteristics, as evidenced by the loss modulus (G'') value being approximately 4.7 times higher than the G' value. Both G' and G'' remained relatively constant over time, indicating the absence of gel formation. However, when Ca^{2+} was added at 30 mg/g of pectin, the solution rapidly transitioned to a more solid-like state, with gel formation occurring within approximately 1.2 min. The G' value steadily increased over time, indicating ongoing gel formation. After about 2.6 min, the rate of G' increase accelerated noticeably, reaching approximately 10.6 Pa/min. These results demonstrated gel formation when the pectin concentration was at least 5% w/v and the Ca^{2+} concentration was 30 mg/g of pectin or higher.

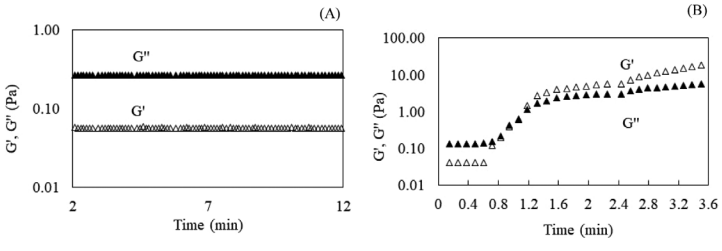

Fig. 3 Plots between storage modulus (G') and loss modulus (G'') of 5 %weight per volume pectin solution for concentrations of Ca^{2+} at: (A) 0 mg/g of pectin; (B) 30 mg/g of pectin

Effects of pectin and calcium on toddy palm cake properties

Color

TPC is a popular Thai dessert known for its distinct flavor. Its natural amber-yellow color is attributed to the presence of palmyra palm pulp. Fig. 4A visually represents the control sample, which did not contain pectin or calcium (Ca^{2+}). The color evaluation was based on the average values for L^*, $a^*$and b^*, of 48.75, 9.97 and 39.34, respectively for the natural sample. Changes in color were observed upon adding calcium to the pectin-free samples. The L^* value decreased significantly while the a^* value increased (Table 3). The higher calcium content intensified the brown color in the TPC samples. These shifts indicated that the samples underwent a browning reaction during the steaming process. This observation aligned with the findings of Kocadağlı and Gökmen (2016), who studied the impact of different salts on browning development in cookies during baking. They reported that adding $CaCl_2$ led to pronounced glucose degradation after heating at 180°C for 15 min. The degradation reaction of glucose predominantly formed furfurals as dark brown, insoluble components that increased the browning intensity. Adding the CPP or COM pectin powder to the TPC samples provided similar results and significantly improved the color stability during the steaming process compared to samples without pectin as visually seen in Fig. 4. The L^*, a^* and b^* values of the pectin-containing samples fell within narrow ranges that did not differ much from the control sample. The L^* values were in the range 48–52, the a^* values were in the range 8.7–10.6 and the b^* values were in the range 38–42, indicating minimal color changes in the TPC samples containing pectin. The presence of pectin hindered the browning reaction, resulting in similar color values to the control sample. This effect could be attributed to the interaction between calcium ions and the carboxyl groups in low methoxyl pectin that inhibited or slowed down the color change. As a result, the color of the pectin-containing samples did not differ from the control sample (p-values of 0.21, 0.74 and 0.32 for L^*, a^* and b^*, respectively).

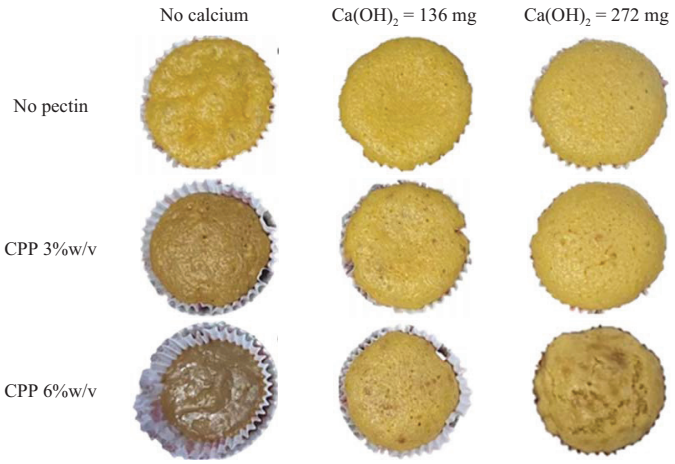

Fig. 4 Appearance of toddy palm cake samples containing different amounts of crude pectin powder (CPP) and calcium, where w/v = weight per volume

Table 3 Effects of calcium on color of toddy palm cake containing no pectin

Sample	L*	a*	b*
Control	48.75±1.71[a]	9.97±0.85[b]	39.34±2.71[a]
0-1	43.83±1.91[b]	10.21±0.47[b]	35.75±7.62[a]
0-2	32.87±4.04[c]	12.11±3.19[a]	35.06±6.51[a]

Mean ± SD (n = 3) within each column superscripted with different lowercase letters are significantly (p < 0.05) different.

Surface porosity and specific volume

Fig. 5 provides images captured from the TPC slices. In the control sample, most hole diameters were in the range 100–500 µm, with an average hole area of 64%.

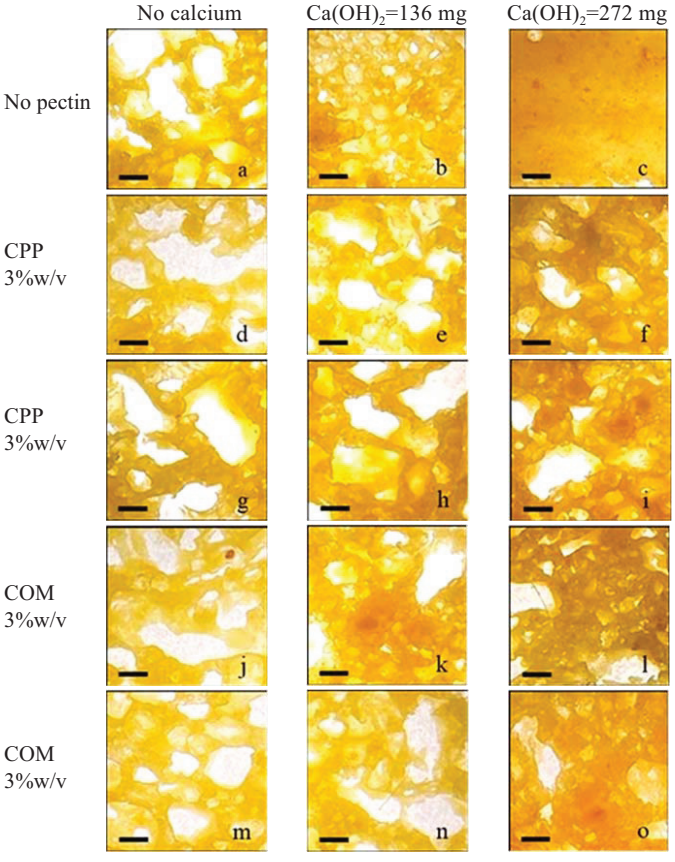

Fig. 5 Light microscope images of samples containing different amounts of pectin and Ca^{2+}, where CPP = crude pectin powder, COM = commercial pectin powder and length of scale bar = 500 µm

However, significant changes were observed in samples with no pectin and increasing levels of Ca^{2+} ions (see Table 4). The sample containing the highest calcium level (sample 0–2) had a remarkably stiff texture, with a substantially reduced average hole diameter of 10 μm and a surface porosity of only 16%. These observations corresponded to the visual appearance (Figs. 4A, 4D and 4G). Guo et al. (2023) conducted a study to examine the impact of salt ions on the texture, structure and microstructure of a composite protein gel made from mung bean protein and wheat gluten. Their findings revealed that introducing salt ions enhanced the electrostatic interactions, hydrophobic interactions and disulfide bonds within the protein networks. In turn, this promoted the formation of larger aggregates between the proteins. In the context of the current research, it is plausible that the increased presence of calcium ions might have further strengthened the network formation within the gluten component. Consequently, this could have impeded gas expansion during steaming, resulting in a minimal specific volume observed in sample 0–2 (0.88 ± 0.05 mL/g).

Adding pectin increased the surface porosity and specific volume in the samples without calcium (Figs. 5A, 5D, 5G, 5J, 5M and 6). This observation was consistent with the study conducted by Cui et al. (2023) that investigated the influence of the degree of esterification of the pectin on the wheat gluten network. These authors reported that LMP competed with gluten for water binding, resulting in partial dehydration and disruption of the continuity of the gluten network. In the current study, both types of pectin were LMP. Therefore, adding pectin could disorder and weaken the gluten network, facilitating gas expansion during the steaming process. This resulted in a higher specific volume and an increase in the size of the TPC samples by approximately 10–20%. Furthermore, reducing the DE of the pectin slightly enhanced the surface porosity and specific volume. For example, the specific volume of sample COM 6-0 was 2.56 ± 0.05 mL/g compared to 2.33 ± 0.13 mL/g for sample CPP 6-0. As pectin is generally recognized as safe (GRAS) (U.S. Food and Drug Administration, 1983) and was extracted using a mild solvent, such as ethanol, without further chemical modification, it can be considered a safe ingredient for use in TPC.

Significant variations in specific volumes were observed in the samples containing both pectin and calcium. For example, samples CPP 6-1 and COM 6-1 had notably higher specific volumes than the control sample, whereas samples CPP 6-2 and COM 6-2 had lower specific volumes. At high calcium levels, it was possible that some excess calcium ions, which were not bonded with the carboxyl groups of the pectin molecules, strengthened the gluten network and may have led to a slight decrease in gas expansion during the steaming process.

Hardness and springiness

The hardness of the samples without pectin increased significantly when calcium was added, with values rising from

Table 4 Effects of pectin and calcium on toddy palm cake properties

Sample	No pectin	Pectin3% (CPP)	Pectin6% (CPP)	Pectin3% (COM)	Pectin6% (COM)
Surface porosity (%)					
Ca-0	63.63±1.05^{aA}	65.55±5.71^{a-bA}	66.25±6.34$^{a-b,A}$	68.90±5.76$^{a-b,A}$	68.79±4.58^{bA}
Ca-136	55.14±15.97^{aB}	60.07±0.75^{bA}	61.71±4.75$^{b-c,A}$	57.67±2.86^{bB}	66.08±0.27^{cA}
Ca-272	16.43±1.00^{aC}	60.54±4.26^{bA}	61.00±7.67^{bA}	59.00±8.76$^{b,A-B}$	57.85±1.37^{bB}
Specific volume (mL/g)					
Ca-0	2.08±0.07^{aA}	2.21±0.17^{a-bA}	2.21±0.13^{a-bA}	2.42±0.20$^{b-c,A}$	2.56±0.05^{cA}
Ca-136	1.35±0.10^{aB}	2.17±0.10^{bA}	2.33±0.14^{b-cA}	2.08±0.10^{bB}	2.37±0.01^{cB}
Ca-272	0.88±0.05^{aC}	1.99±0.07^{bA}	1.84±0.10^{bB}	1.97±0.10^{bB}	1.90±0.07^{bC}
Hardness (N)					
Ca-0	2.59±0.05^{aA}	0.97±0.02^{bA}	0.94±0.04^{bA}	0.99±0.02^{bA}	0.69±0.05^{cA}
Ca-136	3.69±0.06^{aB}	1.20±0.13^{bB}	0.69±0.05^{cB}	0.64±0.12^{cC}	0.49±0.01^{dB}
Ca-272	41.07±2.52^{aC}	1.07±0.07^{cB}	0.54±0.31^{dB}	0.80±0.03^{dB}	2.12±0.04^{bC}
Springiness index (%)					
Ca-0	0.83±0.03^{aA}	0.91±0.03^{bA}	0.93±0.03^{bA}	0.86±0.01$^{a-b,A}$	0.91±0.03^{bA}
Ca-136	0.82±0.04^{aA}	0.84±0.02^{aB}	0.87±0.02^{aA}	0.88±0.02^{aA}	0.93±0.02^{bA}
Ca-272	0.46±0.03^{aB}	0.85±0.01^{bB}	0.90±0.09^{bA}	0.89±0.01^{bA}	0.89±0.01^{bA}
Water activity					
Ca-0	0.945±0.002^{aA}	0.943±0.001^{aA}	0.932±0.003^{bA}	0.943±0.001^{aA}	0.932±0.003^{bA}
Ca-136	0.950±0.010^{aAB}	0.936±0.010^{bcAB}	0.919±0.005^{cB}	0.936±0.002^{bB}	0.934±0.005^{cA}
Ca-272	0.958±0.002^{bB}	0.921±0.006^{bB}	0.923±0.008^{bB}	0.925±0.006^{bC}	0.924±0.008^{bA}

Mean ± SD in each column superscripted with different lowercase superscriptions are significantly ($p < 0.05$) different, and different capital letters denote significant ($p < 0.05$) differences among means within each row; CPP = crude pectin powder; COM = commercial pectin powder

2.59 N to 3.69 N and 41.07 N when 136 mg and 272 mg of $Ca(OH)_2$, respectively, were introduced. Conversely, adding pectin positively affected the texture of the TPC by increasing its softness, regardless of the presence of calcium. There was a noticeable difference in hardness between samples CPP 6-2 and COM 6-2. Since the calcium amount was the same in both samples, the variation could be attributed to the different properties of the pectin used. It was observed that the batter containing COM had a higher viscosity compared to that containing CPP. Therefore, it was plausible that the higher viscosity of the batter contributed to a harder TPC sample. Nevertheless, the hardness of the COM 6-2 sample remained lower than that of the control sample.

The springiness index is a crucial characteristic of bakery products, as it affects the overall acceptability (Fayaz, et al. 2021.). According to Fig. 6, adding pectin led to an approximate 10% increase in the springiness of all TPC samples, elevating the springiness values from 0.82 to 0.9. Conversely, when calcium was added alone without pectin at a dosage of 272 mg, the springiness of the TPC samples decreased significantly to only 0.45. These results suggested that the incorporation of natural dietary fiber, such as pectin, could potentially mitigate the adverse effects of adding calcium alone on the textural properties of TPC. Therefore, it could be possible to develop calcium-rich bakery foods by supplementing them with natural dietary fiber to counteract the negative impact of calcium addition on the final texture of the product.

Water activity

The water activity values for all samples fell within a narrow range (0.92–0.96). When calcium was added alone, there was a slight increase in the water activity of the TPC samples. The water activity reached a minimum value of approximately 0.92 in the samples CPP 3-2, CPP 6-1, CPP 6-2, COM 3-2 and COM 6-2. In the pectin-calcium system, gel formation occurred when the concentrations of pectin and calcium ions were sufficiently high. This gel structure bonded a portion of the water, leading to a decrease in the water activity of the sample. Overall, there was a limited effect of pectin and calcium on the water activity of the TPC samples.

Conclusion

LMP in the PW presented an opportunity to extract valuable pectin in a sustainable and cost-effective manner. The rheological and physicochemical properties of the obtained pectin powder, such as DE and GA, play a crucial role in determining the functionality and stability of pectin in various food applications. Using palmyra palm pectin in the TPC formulation improved the cake texture and aligned with the goal of developing healthier bakery products, while promoting the sustainable utilization of byproducts to minimize waste and achieve a more environmental-friendly approach. Furthermore, the results of the study highlighted the potential of palmyra palm pectin as a functional ingredient in the food industry. The incorporation of natural dietary fiber, such as pectin, into other bakery products has the potential to create new products with enhanced nutritional value and the potential for positive health and economic impacts. The primary cost associated with pectin recovery from wash-water was approximately USD 0.20/g of pectin. This cost was comparable to the price of low methoxyl pectin in the Thai domestic market, which was approximately USD 0.29/g . However, notably, the key to cost-effectiveness lay in the highly efficient recycling of ethanol during the precipitation process. By understanding the physicochemical properties of pectin powder, food manufacturers can make informed decisions regarding its incorporation into different products and optimize its functionality for specific applications.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Basak, S., Annapure, U.S. 2022. Trends in “green” and novel methods of pectin modification – A review. Carbohydr. Polym. 278: 118967. doi.org/10.1016/j.carbpol.2021.118967

Basava, P.A.R., Vignesh, S., Elumalai, A., Anandharaj, A., Chidanand, D.V., Baskaran, N. 2023. Nutritional and pharmacological properties of palmyra palm. Food Hum. 1: 817–825. doi.org/10.1016/j.foohum.2023.07.030

Belkheiri, A., Forouhar, A., Ursu, A.V., et al. 2021. Extraction, characterization, and applications of pectins from plant by-products. Appl. Sci. 11: 6596. doi.org/10.3390/app11146596

Cui, Y., Chen, J., Zhang, S. 2023. The effect of degree of esterification of pectin on the interaction between pectin and wheat gluten protein. Food Hydrocoll. 136: 108272. doi.org/10.1016/j.foodhyd.2022.108272

del Amo-Mateos, E., Fernández-Delgado, M., Lucas, S., et al. 2023. Valorization of discarded red beetroot through the recovery of bioactive compounds and the production of pectin by surfactant-assisted microwave extraction. J. Clean. Prod. 389: 135995. doi.org/10.1016/j.jclepro.2023.135995

Devi, R., Sharma, E., Thakur, R., et al. 2023. Non-dairy prebiotics: Conceptual relevance with nutrigenomics and mechanistic understanding of the effects on human health. Food Res. Int. 170: 112980. doi.org/10.1016/j.foodres.2023.112980

Einhorn-Stoll, U., Archut, A., Eichhorn, M., Kastner, H. 2021. Pectin - plant protein systems and their application. Food Hydrocoll. 118: 106783. doi.org/10.1016/j.foodhyd.2021.106783

Fayaz, U., Hussain, Dar, A., Kumar, N., et al. 2021. Formulations and quality characterization of low salt flat bread: Effects on functionality, rheological and sensory properties. Appl. Food Res. 1: 100005. doi.org/10.1016/j.afres.2021.100005

Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C., Thom, D. 1973. Biological interactions between polysaccharides and divalent cations: The egg-box model. FEBS Lett. 32: 195–198. doi.org/10.1016/0014-5793(73)80770-7

Guo, R., Liu, L., Huang, Y., Lv, M., Zhu, Y., Wang, Z., Zhu, X., Sun, B. 2023. Effect of Na^{+} and Ca^{2+} on the texture, structure and microstructure of composite protein gel of mung bean protein and wheat gluten. Food Res. Int. 172: 113124. doi.org/10.1016/j.foodres.2023.113124

Jin, L., Zuo, F., Gao, Y., Sui, S., Zhang, D. 2022. Purification of pectin by ultrafiltration in combination with sodium citrate. J. Food Eng. 335: 111158. doi.org/10.1016/j.jfoodeng.2022.111158

Jong, S.H., Abdullah, N., Muhammad, N. 2023. Optimization of low-methoxyl pectin extraction from durian rinds and its physicochemical characterization. Carbohydr. Polym. Technol. Appl. 5: 100263. doi.org/10.1016/j.carpta.2022.100263

Kocadağlı, T., Gökmen, V. 2016. Effects of sodium chloride, potassium chloride and calcium chloride on the formation of α-dicarbonyl compounds and furfurals and the development of browning in cookies during baking. J. Agric. Food Chem. 64: 7838–7848. doi.org/10.1021/acs.jafc.6b03870

Kumar, A., Chauhan, G.S. 2010. Extraction and characterization of pectin from apple pomace and its evaluation as lipase (steapsin) inhibitor. Carbohydr. Polym. 82: 454–459. doi.org/10.1016/j.carbpol.2010.05.001

Lira-Ortiz, A.L., Reséndiz-Vega, F., Ríos-Leal, E., Contreras-Esquivel, J.C., Chavarría-Hernández, N., Vargas-Torres, A., Rodríguez-Hernández, A.I. 2014. Pectins from waste of prickly pear fruits (*Opuntia albicarpa* Scheinvar 'Reyna'): Chemical and rheological properties. Food Hydrocoll. 37: 93–99. doi.org/10.1016/j.foodhyd.2013.10.018

Nielsen, S.S. 2010. Food Analysis, 4th ed. Springer. New York, NY, USA.

Nwachukwu, I.D., Aluko, R.E. 2019. A systematic evaluation of various methods for quantifying food protein hydrolysate peptides. Food Chem. 270: 25–31. doi.org/10.1016/j.foodchem.2018.07.054

Pagarra, H., Rahman, R.A., Rachmawaty, Hartati, Azis, A.A. 2018. Characterization and monosaccharide content of pectin from *Nephrolepis biserrata* leaves. AIP Conf. Proc. 2030: 020178. doi.org/10.1063/1.5066819

Ripoll, C.S.S., Hincapié-Llanos, G.A. 2023. Evaluation of sources and methods of pectin extraction from fruit and vegetable wastes: A systematic literature review (SLR). Food Biosci. 51: 102278. doi.org/10.1016/j.fbio.2022.102278

Tran, N.T.K., Nguyen, V.B., Tran, T.V., Nguyen, T.T.T. 2023. Microwave-assisted extraction of pectin from jackfruit rags: Optimization, physicochemical properties and antibacterial activities. Food Chem. 418: 135807. doi.org/10.1016/j.foodchem.2023.135807

U.S. Food and Drug Administration. 1983. Food and drugs: Direct food substances affirmed as generally recognized as safe. www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1588, 29 November 2023.

Wan, L., Yang, Z., Cai, R., Pan, S., Liu, F., Pan, S. 2021. Calcium-induced-gel properties for low methoxyl pectin in the presence of different sugar alcohols. Food Hydrocoll. 112: 106252. doi.org/10.1016/j.foodhyd.2020.106252

Yang, J., Wan, L., Duan, X., Wang, H., Yang, Z., Liu, F., Xu, X., Pan, S. 2022. Potential low-calorie model that inhibits free fatty acid release and helps curcumin deliver *in vitro*: Ca^{2+}-induced emulsion gels from low methyl-esterified pectin with the presence of erythritol. Int. J. Biol. Macromol. 200: 449–457. doi.org/10.1016/j.ijbiomac.2022.01.069

Zanoletti, M., Marti, A., Marengo, M., Iametti, S., Pagani, M.A., Renzetti, S. 2017. Understanding the influence of buckwheat bran on wheat dough baking performance: Mechanistic insights from molecular and material science approaches. Food Res. Int. 102: 728–737. doi.org/10.1016/j.foodres.2017.09.052

Zeeb, B., Roth, M., Endreß, H. 2021. Commercial pectins. In: Phillips, G.O., Williams, P.A. (Eds.). Handbook of Hydrocolloids, 3rd ed. Woodhead Publishing. Sawston, UK, pp. 295–315.