

# Kinetic study of bioactive compound extraction from cacao shell waste by conventional and deep eutectic solvent

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## Abstract

Cacao shells contain bioactive compounds such as phenolic acids and flavonoids. This study investigated the potential of bioactive compound extraction in cacao shells using conventional and green solvents like deep eutectic solvent (DES) (choline chloride: lactic acid). Specifically, it investigated the extraction kinetic models and parameters, which are critical to scale up the extraction process. The extraction of cacao shell was conducted using various conventional solvents (ethanol, methanol, n-hexane, and water) and DES (100 % and 70%) in which the result showed that DES 100% had the highest total phenolic content of  $337.92 \pm 9.55$  mg GAE/g dry weight. Meanwhile, pseudo-second order and Peleg's model provided the best fit for the experimental data with higher  $R^2$  values. DES 70% showed a higher total flavonoid content of  $76.51 \pm 1.59$  mg RE/g dry weight. FT-IR and Raman spectroscopy confirmed the presence of bioactive compounds in DES-based extracts, which revealed characteristic vibrational bands associated with polyphenolic structures. These include bands corresponding to hydroxyl ( $-OH$ ), carbonyl ( $C=O$ ), and aromatic  $C=C$  stretching—functional groups commonly found in quercetin and other bioactive compounds.

**Keywords:** Cacao shell; green solvent; deep eutectic solvent; bioactive compounds; kinetic study

## 1. Introduction

Cacao fruit (*Theobroma cacao* L.) is one of the plantation commodities in Indonesia. Sulawesi Island is the most significant contributor of this fruit with about 61% of the total production in Indonesia in 2021 [1]. The high production of cacao fruit, however, has also led to an increase in cacao shell waste. Cacao fruit has a composition of 76-86% cacao shell and 13-24% cacao beans or pulp [2]. From the composition of the fruit, in 2022 cacao shell waste achieved 556,514 tons.

Cacao shells in cacao plantation areas can pose environmental problems. When the shells degrade, the spores of fungi, such as *Moniliophthora perniciosa* that grow on the shells, can give witches broom disease to cacao trees and affect cacao fruit production [3]. In addition to the fungus *M. perniciosa*, cacao shell waste is a source of the pathogen *Phytophthora* sp., which causes black cacao fruits and can reduce cacao shell production by 20-30% globally [4]. One utilization of cacao shell waste as animal feed has a number of limitations, such as alkaloid compounds in cacao shells that will reduce livestock growth if continuously consumed [5]. Besides alkaloid compounds, cacao shells contain macromolecular and other bioactive compounds.

Cacao shells consist of several macromolecular

components (proteins, carbohydrates, and lipids) and minerals (Ca, Fe, K, Mg, and Na) [6–8]. They also contain bioactive compounds such as phenolic acids (19.6-49.5 mg GAE/g), flavonoids (4-22.4 mg rutin equivalent (RE)/g), theobromine (11.158-17.598 mg/g) and caffeine (0.047-0.563 mg/g) [7,9]. Fresh cacao shells contain a variety of phenolic acids and flavonoids, predominantly catechin (36%), followed by quercetin and epicatechin (each accounting for 21%), along with gallic acid (11.3%), p-coumaric acid (6.5%), and protocatechuic acid (4.5%) [10]. The flavonoid and phenolic acids compounds have antioxidant, anti-inflammatory, and antibacterial abilities that can be used for biomedical applications [11].

Extracting bioactive compounds from biomass in common uses organic solvents that can dissolve the bioactive compounds. These solvents, nevertheless, are volatile and highly toxic to humans and environment. To avoid this, solvents such as deep eutectic solvents (DES) can be used in view of their environmentally friendly nature. DES is an eutectic mixture often created by simultaneously heating hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) compounds having a substantially lower final melting point for each component. [12]. In addition, DES is a green solvent derived from pure compounds that are available, so the purity of the solvent is known as non-flammable, non-toxic, and biodegradable [13–15].

Several studies showed that DES using choline chloride

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(HBA) and lactic acid (HBD) can extract bioactive compounds from natural materials. A study by Fanali et al. [16] showed that DES from a mixture of choline chloride and lactic acid in a molar ratio of 1:2 showed potential in extracting bioactive compounds in hazelnut shells of 16.96 g gallic acid equivalent (GAE) / 100 g sample. Lactic acid showed better results than xylitol, urea, and triethylene glycol that had 7.14, 13.98, and 12.92 g GAE/100 g samples, respectively. The extraction of bioactive compounds was also compared with 50% ethanol organic solvent and showed that the DES solvent of choline chloride and lactic acid mixture could extract bioactive compounds by 39% compared to ethanol 50%.

In evaluating the appropriate type of solvent to utilize, it is beneficial to study extraction kinetics. Applying the kinetic model and analyses of model parameters can provide an exemplary realization of the extraction mechanism, which is critical to scale up the extraction process [17]. This study aimed to determine the effect of solvent types, namely conventional solvent (ethanol, methanol, n-hexane, and water) and DES (choline chloride:lactic acid), on bioactive compounds in cacao shell extract. The analyses of total phenolic content (TPC) and total flavonoid content (TFC) were conducted to provide an overview of the amount of bioactive compounds in cacao shell extract samples. Meanwhile, a kinetic study of TPC was conducted based on three models (pseudo-first order, pseudo-second order, and Peleg's model). Cacao shell extract was also analyzed by functional group (FT-IR and Raman Spectroscopy) to identify the presence of bioactive compounds in cacao shell extract.

## 2. Materials and Methods

### 2.1. Raw materials

In this study, dried cacao shell waste, as a mix of 75% *trinitario* and 25% *forastero*, was collected from Pangandaran, West Java and milled using a grinder to a small size. The fine cacao shell was then sieved using a Tyler screen with a particle size of -40 +60 mesh.

Chemicals used were choline chloride ( $C_5H_{14}ClNO$ , Himedia,  $\geq 99.00\%$ ), lactic acid ( $C_3H_6O_3$ , Himedia,  $\geq 90.00\%$ ), Folin-Ciocalteu reagent (Merck), sodium carbonate p.a. ( $Na_2CO_3$ , Merck,  $\geq 99.90\%$ ), sodium nitrite p.a. ( $NaNO_2$ , Merck,  $\geq 99.90\%$ ), aluminum chloride p.a. ( $AlCl_3$ , Merck,  $\geq 98.00\%$ ), sodium hydroxide p.a. ( $NaOH$ , Merck,  $\geq 99.00\%$ ), gallic acid p.a. (Merck, 100.00%), rutin p.a. (Merck,  $\geq 96.00\%$ ), ethanol p.a. ( $C_2H_6O$ , Supelco,  $\geq 99.90\%$ ), methanol p.a. ( $CH_3O$ , Merck,  $\geq 99.90\%$ ), n-hexane p.a. ( $C_6H_{14}$ , Supelco,  $\geq 99.50\%$ ), and aquadest.

### 2.2. Preparation of deep eutectic solvent

A DES was prepared based on Xia et al. [18] with a minor modification. It was made using choline chloride ( $C_5H_{14}ClNO$ ) and lactic acid ( $C_3H_6O_3$ ). These components were combined in a molar ratio of 1:2 with a total mixture mass of 100 g. Subsequently, the mixture was heated at a temperature of 80°C with the stirring speed of 250 rpm for 2 hours until a homogeneous and colorless liquid was obtained.

### 2.3. Extraction of bioactive compound

Solid-liquid extraction was carried out using six different solvents (water, ethanol, methanol, n-hexane, DES in pure form (DES 100%), and DES as a 70% aqueous mixture v/v (DES 70%)) to extract the bioactive compounds in cacao shells. To this end, the extraction operating parameters included the temperature of 40°C and a solid-to-solvent volume ratio of 1:10 g/ml. The experiments were undertaken in a 250 ml laboratory three-neck flask with a continuous stirring at 250 rpm. For the kinetic study, sampling was carried out at 1 min, 5 min, 10 min, 20 min, 40 min, 60 min, 80 min, and 100 min. After extraction, the samples were centrifuged for 10 minutes at 3000 rpm to remove any supernatant to conduct further examination. For conventional solvents (water, ethanol, methanol, and n-hexane) extraction, the bioactive compound separation was conducted by means of a rotary vacuum evaporator (*Yamato RE301*). The extracts of cacao shell were then stored in a freezer at -25°C for further analysis.

### 2.4. Determination of total phenolic content and total flavonoid content

The TPC of extracts was determined by a visible spectrophotometer (*Optima SP-300 Spectrophotometer*) using the Folin-Ciocalteu method based on Singleton et al. [19] with modifications. An extract of 0.3 mL was mixed with 2.5 mL of diluted Folin-Ciocalteu reagent (1:10; v/v). Next, 2.0 mL of 7.5% sodium carbonate was added, and the mixture was allowed to react at room temperature for 2 hours. Gallic acid here was utilized as a reference, and absorbance was measured at 760 nm. The results were expressed in milligrams of gallic acid equivalent/ gram of dry weight (mg GAE/ g dry weight).

TFC analysis was determined using the method as described by Blasa et al. [20]. TFC of extracts was also determined by means of a visible spectrophotometer (*Optima SP-300 Spectrophotometer*). 1.0 mL of extracts was mixed with 0.3 mL sodium nitrite (5%) and 0.3 mL aluminum chloride (10%); the mixture was then reacted for 3 minutes at room temperature. The wavelength used to measure absorbance was 510 nm with rutin used as the standard. The results were expressed in milligrams of rutin equivalent/ gram of dry weight (mg RE/ g dry weight).

### 2.5. Kinetic studies

This study employed pseudo-first order, pseudo-second order, and Peleg's model for modelling the extraction of bioactive compounds from cacao shells using different solvents. The pseudo-first order equation of Lagergren [21,22] can be rewritten in its differential form as follows:

$$r_e = \frac{dc_t}{dt} = k(C_{eq} - C_t) \quad (1)$$

where  $r_e$  is the rate of extraction (mg GAE/g dry weight. min),  $C_t$  (mg GAE/g dry weight) is the extraction capacity (concentration of TPC) at a given extraction time ( $t$ ),  $C_{eq}$  (mg GAE/ g dry weight) is the concentration of TPC at saturation point, and  $k$  is the pseudo-first order extraction rate coefficient.

Eq. (1) was integrated with an application of the boundary conditions of  $C_t = 0$  at  $t = 0$ , and  $C_t = C_t$  at  $t = t$  as follows:

$$\ln \left[ \frac{C_{eq}}{C_{eq} - C_t} \right] = kt \quad (2)$$

The kinetic behavior of the extraction process was evaluated based on the pseudo-second-order model as introduced by Ho et al. [23]. The equation can be written as:

$$r_e = \frac{dC_t}{dt} = k'(C_{eq} - C_t)^2 \quad (3)$$

Eq. (3) was integrated using the boundary conditions of  $C_t = 0$  at  $t = 0$  and  $C_t = C_t$  at  $t = t$  as follows:

$$\frac{t}{C_t} = \frac{t}{C_{eq}} + \frac{1}{C_{eq}^2 k'} \quad (4)$$

The pseudo-second order coefficient was calculated from the intercept obtained by plotting  $t/C_t$  vs.  $t$ . The coefficients obtained were the pseudo-second order constant  $k'$  (g dry weight/mg GAE minutes) and the concentration of TPC at the saturation point ( $C_{eq}$ ).

Peleg's model, a widely recognized semi-empirical kinetic approach that was originally introduced by Peleg [24], has been extensively applied due to its strong ability to describe the extraction behavior of bioactive compounds from plant matrices. This model conceptualizes plant material as a uniformly mixed solid in solute distribution. The modified Peleg's equation, explaining the behavior of the extraction process, can be represented as follows:

$$C_t = \frac{t}{k_1 + k_2 t} \quad (5)$$

$C_t$  can be calculated by determining the Peleg constant ( $k_1$  and  $k_2$ ) from Eq. (5) by depicting the curve between  $1/C_t$  and  $1/t$ . The Peleg rate constant  $k_1$  relates to the beginning extraction rate ( $B_0$ ).

$$B_0 = \frac{1}{k_1} \quad (6)$$

The Peleg rate constant  $k_2$  relates to the maximum extraction equilibrium concentration of phenolic compounds  $C_{eq}$ . When  $t \rightarrow \infty$ , Eq. (7) gives the relations between equilibrium concentration and  $k_2$  constant.

$$C|_{t \rightarrow \infty} = C_{eq} = \frac{1}{k_2} \quad (7)$$

## 2.6. Characterization of bioactive compounds with FT-IR and raman spectroscopy

FT-IR Spectra were collected using *Thermo Scientific Nicolet iS10 FTIR Spectrometer* used to detect the functional groups of extract ranging from 600 to 4000  $\text{cm}^{-1}$  with 8  $\text{cm}^{-1}$  resolution. Raman spectra were also collected using a *LabRam HR Evolution* equipped with a 537 nm diode laser for excitation with a maximum output power of 100mW.

## 3. Results and Discussion

### 3.1. Kinetic Study of total phenolic content

Cacao shell extraction was performed at a controlled

temperature of 40°C with a fixed solid-to-solvent ratio of 1:10. The extraction temperature was deliberately maintained at 40°C to minimize the degradation of thermolabile bioactive compounds as the elevated temperatures are known to compromise the structural integrity and functionality of these compounds. Previous studies, such as that by Niamnuy et al. [25], reported the significant degradation of bioactive at the temperatures in the range of 50 to 70°C, resulting in a decline in antioxidant and antibacterial activities. This degradation was primarily attributed to the susceptibility of bioactive molecules—particularly those containing phenolic, thiol, and unsaturated functional groups—to oxidation and thermal breakdown. These groups readily interacted with oxygen, generating free radicals and reactive intermediates, or underwent bond cleavage under heat, ultimately reducing bioactivity or transforming into inactive derivatives. Therefore, the moderate extraction temperature employed in this study was deemed critical in preserving the target compounds' chemical stability and functional properties.

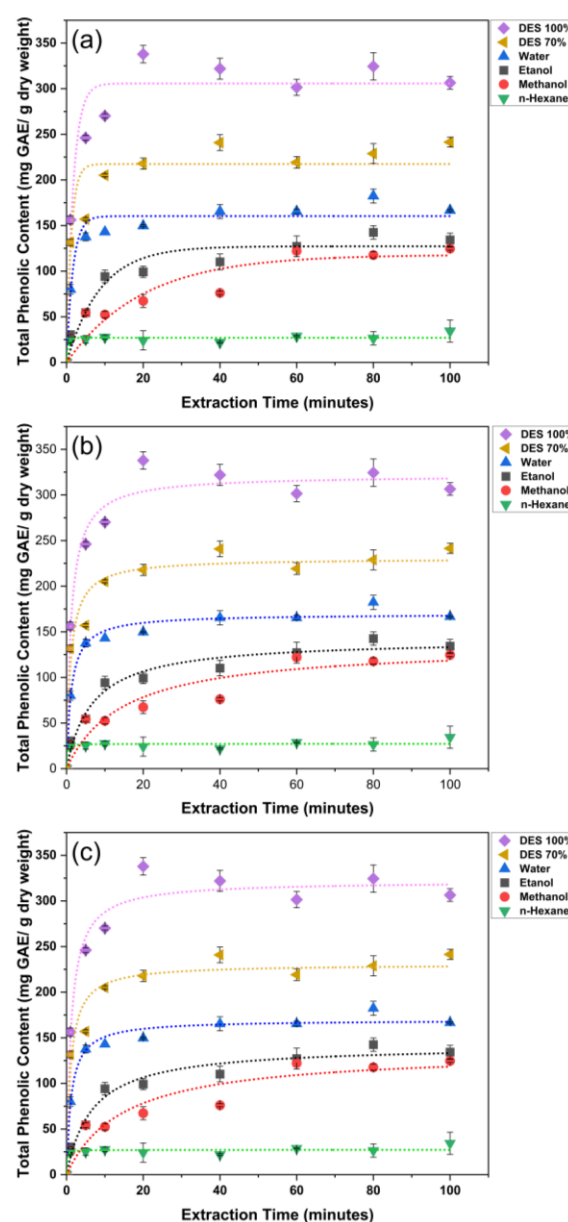


Fig. 1. Kinetic study of TPC using different solvents by (a) Pseudo-first order, (b) Pseudo-second order, and (c) Peleg's model (Note: n=2; GAE= Gallic Acid Equivalent)

Experimental data on the extraction of cacao shells, as presented in Fig. 1, showed that the green solvent DES, specifically choline chloride: lactic acid, yielded a significantly higher TPC compared to conventional solvents. The DES 100% extract achieved a TPC of  $337.92 \pm 9.55$  mg GAE/g dry weight, indicating its superior efficiency in extracting bioactive compounds. This enhanced performance was attributed to the unique molecular properties of DES that are able to maintain the biological and functional activity of extracted compounds and increase their stability by forming a strong hydrogen bonding network within the solvent matrix [26]. The interaction between HBA and HBD, such as choline chloride and lactic acid, creates a tunable medium tailored to target specific bioactive compounds such as phenolics. As shown from molecular dynamics simulations, DESs possess a heterogeneous nanoscale structure with polar and apolar domains, further enhancing the extracted molecules' solubility and mobility [27,28]. Additionally, direct interactions between DES components and target compounds reduce competition for hydrogen bonding, increasing extraction selectivity and efficiency [29]. The carbon chain length and number of functional groups in the HBD and HBA also play a pivotal role in modulating these interactions [30].

The addition of water to the DES system resulted in a 29% decrease in the TPC of the extract, indicating a significant reduction in extraction efficiency. This decline is primarily attributed to changes in the physicochemical properties of DES upon water incorporation, particularly its polarity and hydrogen bonding capacity [31,32]. Water disrupts the original hydrogen bond network between the HBD and HBA, weakening DES's

solvation power for phenolic compounds. Consequently, the altered solvent environment becomes less effective in interacting with and stabilizing phenolic structures, reducing solubility and extraction yield.

In comparison, conventional solvents such as water, ethanol, and methanol exhibited considerably lower extraction efficiency with the TPC values of  $182.35 \pm 7.71$ ,  $142.51 \pm 7.39$ , and  $124.61 \pm 1.76$  mg GAE/g dry weight, respectively. The non-polar solvent n-hexane yielded the lowest TPC value at  $34.44 \pm 12.09$  mg GAE/g dry weight, reflecting its limited capacity to extract polar phenolic compounds due to weak or absent hydrogen bonding interactions. Furthermore, previous studies utilizing ultrasonic-assisted extraction (UAE) with organic solvent ethanol reported the lower TPC values ( $263.69$  mg GAE/g sample) compared to this study using DES [33]. This underscores conventional solvents' limitations even when supported by advanced techniques such as sonication.

Typically, organic solvents are less selective and often fail to extract hydrophilic and hydrophobic compounds simultaneously, leading to inefficiencies in the extraction process [34]. The extraction mechanism with organic solvents primarily relies on solubility and partitioning with interactions occurred via van der Waals forces, dipole-dipole interactions, and hydrogen bonding; however, these are generally weaker and less specific than DES's extensive hydrogen bonding networks. These findings reinforce the superior extraction capability of DESs, particularly under optimized conditions, in isolating phenolic-rich fractions from plant-based materials such as cacao shells.

Table 1. Kinetic parameters of Pseudo-first order, Pseudo-second order, and Peleg's model

Model	Parameter	DES 100%	DES 70%	Ethanol	Methanol	Water	n-Hexane
Pseudo-first Order	$k$ ( $\text{min}^{-1}$ )	0.57	0.81	0.11	0.86	0.60	2.50
	$C_{eq}$ (mg GAE/ g dry weight)	305.73	217.40	127.27	117.98	160.34	26.95
	$R^2$	0.95	0.90	0.94	0.89	0.95	0.87
Pseudo-second Order	$k'$ (g dry weight/mg GAE. min)	$2.64 \times 10^{-3}$	$4.14 \times 10^{-3}$	$1.06 \times 10^{-3}$	$5.02 \times 10^{-4}$	$4.83 \times 10^{-3}$	0.31
	$C_{eq}$ (mg GAE/ g dry weight)	321.73	230.59	141.98	135.79	169.59	27.19
	$R^2$	0.98	0.96	0.97	0.89	0.98	0.87
Peleg's Model	$B_0$ (mg GAE/ g dry weight. min)	273.22	219.78	21.34	9.25	138.69	229.36
	$C_{eq}$ (mg GAE/ g dry weight)	321.54	230.41	142.05	135.87	169.49	27.19
	$R^2$	0.98	0.96	0.97	0.89	0.98	0.87

Fig. 1 also shows the kinetic model with three different proposed models. The proposed kinetic model was applied to describe the extraction kinetics of cacao shells. Nonlinear regression analysis was used to fit the proposed models to the experimental data, and  $R^2$  was used to assess the models. In statistics, the  $R^2$  number is frequently used to show how effectively the experimental data fits the theoretical model, particularly for model-fitting applications. A higher  $R^2$  value indicates a better fit between the model and experiment data.

Table 1 provides the kinetic parameters for each of the proposed models. The data showed that several solvents, including ethanol, water, DES 70%, and DES 100%, fit the pseudo-second order model better than the pseudo-first order one, as seen by higher  $R^2$  values. This model described an initial rapid dissolution of easily accessible compounds followed by a slower diffusion-controlled phase [35]. Interestingly, though DESs and conventional organic solvents (e.g. ethanol, water)

possess distinct physicochemical properties, they both demonstrated compatibility with this kinetic behavior, implying that despite their differences in viscosity, polarity, and hydrogen bond donor/acceptor characteristics, the dominant mechanism governing extraction remains similar under the conditions tested.

A comparable level of model accuracy was observed for both Peleg's and pseudo-second-order kinetics with similar error values and strong correlation coefficients. This is also illustrated in Fig. 1(b) and 1(c), showing nearly overlapping fitting curves for both models. The close agreement was primarily attributed to the structural similarity of their equations (Eq. (4) and Eq. (5)) [36]. Peleg's model, in particular, effectively characterizes the biphasic nature of the extraction process, capturing both the initial solubilization phase and the subsequent diffusion-driven phase. Such behavior aligns with the established theories of bioactive compound extraction,

where solutes initially located near the surface are quickly released, followed by a slower diffusion of compounds embedded deeper within the plant matrix [37–40].

Further analysis of the kinetic constants ( $k$  and  $k'$ ) revealed that dilution of DES with water enhanced the extraction rate, as evidenced by a 30% and 36% increase in the pseudo-first order and pseudo-second order rate constants, respectively, when comparing DES 100% to DES 70%. This improvement was related to the reduction in viscosity caused by the addition of water, facilitating molecular mobility and accelerating diffusion. However, despite the faster extraction kinetics, DES 100% maintained a higher equilibrium concentration  $C_{eq}$  of TPC than DES 70%, likely due to its superior solvent-solute interaction capacity.

In contrast to DES, conventional organic solvents exhibited comparatively lower saturation capacities during the extraction of phenolic compounds. Of these solvents, n-hexane showed the most rapid extraction kinetics with the highest rate constants observed in both the pseudo-first order ( $2.50 \text{ min}^{-1}$ ) and pseudo-second order ( $0.31 \text{ g dry weight}/(\text{mg GAE} \cdot \text{min})$ ) kinetic models. Notably, TPC extracted with n-hexane reached saturation within the first minute of the extraction process. This rapid equilibrium reflects a fast initial mass transfer; however, due to its non-polar nature, n-hexane possesses a limited solubilizing ability for polar bioactive compounds such as phenolics, resulting in lower extraction efficiency. Similarly to n-hexane, methanol exhibited comparable  $R^2$  values for all kinetic models, indicating analogous fitting quality. Both n-hexane and methanol preferentially extracted compounds based on polarity, targeting non-polar and polar constituents, respectively. Unlike other solvents, which did not exhibit rapid dissolution at the initial stage but demonstrated slower diffusion rates, methanol's extraction kinetics warranted further investigation focused on mass transfer properties to determine its diffusion coefficient accurately.

### 3.2. Effect of different solvents on total flavonoid content

Fig. 2 illustrates the effect of solvent type on the TFC of cacao shell extracts. The DES 70% yielded the highest TFC value of  $76.51 \pm 1.59 \text{ mg RE/g dry weight}$  after 40 minutes of extraction. However, a notable decline to  $56.32 \pm 0.84 \text{ mg RE/g dry weight}$  was observed at 100 minutes. A similar trend was evident in the water extraction where TFC decreased from  $60.87 \pm 0.79 \text{ mg RE/g dry weight}$  at 40 minutes to  $51.63 \pm 0.39 \text{ mg RE/g dry weight}$  at 100 minutes. This reduction is attributed to the thermal degradation of flavonoids during prolonged exposure to elevated temperatures [41–44].

In contrast, flavonoid extraction conducted at room temperature from *Merremia mammosa* demonstrated increased flavonoid content without any degradation over a 2-hour [45]. Comparable decreases in bioactive compounds with extended extraction time have been reported in the extraction of tannins from *Melastoma malabathricum* and *Mimosa pigra*, as well as in *Cajanus cajan L.* extraction [46,47]. Furthermore, regardless of extraction duration, the water content in solvents may promote the oxidation of polyphenolic compounds, contributing to their degradation [48].

At DES 100%, the highest TFC achieved was  $74.72 \pm 4.01 \text{ mg RE/g dry weight}$ , followed by a slight decline to  $70.72 \pm 2.14 \text{ mg RE/g dry weight}$  at prolonged extraction times. This relatively slower increase and subsequent decline in TFC when

using pure DES (DES 100%) was attributed to its high viscosity, which reduced the diffusivity of bioactive compounds into the solvent matrix. The addition of water to DES, specifically choline chloride:lactic acid, has been shown to significantly enhance the diffusion process by lowering solvent viscosity and improving mass transfer. Benitez-Correa et al. [31] reported a progressive increase in the diffusivity coefficient of cacao bean shell bioactive compounds with increasing water content in DES with the values of  $9.8 \times 10^{-12}$ ,  $20.2 \times 10^{-12}$ , and  $29 \times 10^{-12} \text{ m}^2/\text{s}$  for 30%, 40%, and 50% water addition, respectively.

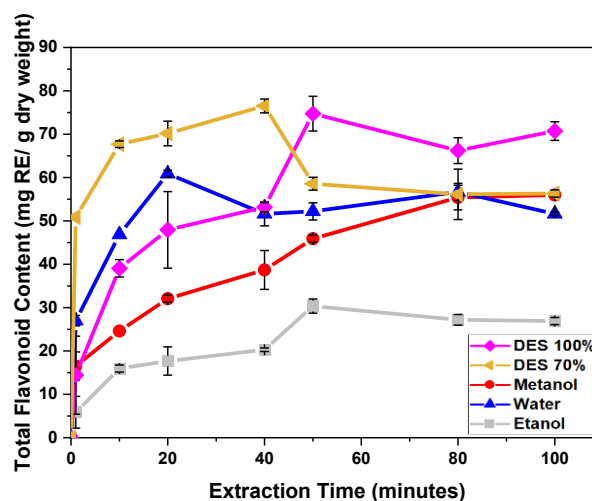


Fig. 2. Effect of various solvents (DES 100%, DES 70%, ethanol, water, and methanol) on TFC (Note:  $n=2$ ; RE= Rutin Equivalent)

In contrast, the conventional solvent methanol consistently increased TFC over time, ultimately reaching a stable value of  $55.38 \pm 2.34 \text{ mg RE/g dry weight}$  at 100 minutes. This value was comparable to the TFC obtained using DES 70% and water at the same extraction duration. A similar trend was observed for ethanol; however, a slight reduction in TFC was noted at 50 minutes with a maximum TFC of only  $26.90 \pm 0.81 \text{ mg RE/g dry weight}$ . Methanol extracted 48.57% more flavonoids than ethanol, likely due to its higher polarity, enhancing its capacity to solubilize polar bioactive compounds such as flavonoids [49].

### 3.3. Characterization of bioactive compound on cacao shell extract

The characterization of functional groups in each cacao shell extract was carried out using FT-IR analysis and Raman Spectroscopy. Fig. 3 shows the effect of solvent type on cacao shell extract. Conventional solvents such as methanol, water, and ethanol peaked at  $3361 \text{ cm}^{-1}$ , indicating the presence of hydroxyl groups (-OH) [50]. Hydroxyl groups indicated the presence of phenolic compounds in the extract. Hydroxyl peak intensity in methanol and water showed the highest peak among conventional solvents. These results were correlated with the high TPC values in both extracts.

The ethanol extract not only showed the lower transmittance intensity of hydroxyl peak but also showed peaks around  $2923 \text{ cm}^{-1}$  and  $1730 \text{ cm}^{-1}$ . These peaks indicated the presence of -CH saturated and carbonyl groups ( $\text{C}=\text{O}$ ) that also indicated the presence of oil or fat compounds in the extract. It is important



to note that a high ethanol concentration is capable of dissolving non-polar compounds in the form of oil or fat [51]. A similar peaks also occurred during the extraction using an n-hexane solvent. The n-hexane extract lacked the O–H stretch at 3361  $\text{cm}^{-1}$ , indicating the absence of hydroxyl groups, and instead displayed peaks associated with oil constituents (2923  $\text{cm}^{-1}$  and 1730  $\text{cm}^{-1}$ ). N-hexane solvent is a non-polar solvent that has a high efficiency in extracting oil [52].

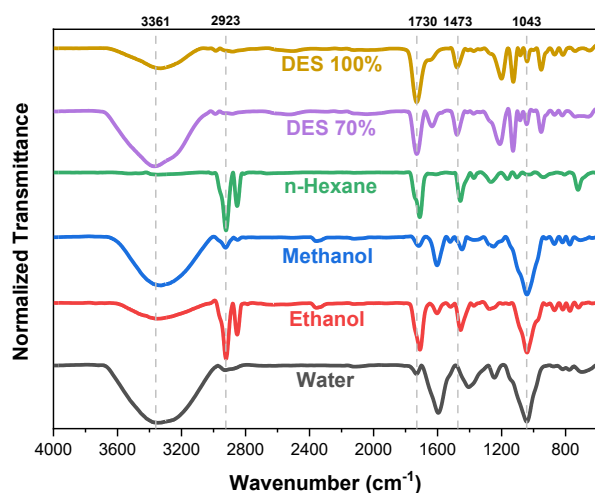


Fig. 3. FT-IR analysis of cacao shell extract at various solvents

The FT-IR spectra in Fig. 3 also showed some functional groups in DES 100% and DES 70% extracts. DES 100% and 70% showed almost the same functional groups as the conventional solvent and the addition of several peaks with a small intensity below the wavenumber of 1473  $\text{cm}^{-1}$ . The peak around 1473  $\text{cm}^{-1}$  was attributed to C–H bending vibrations of aliphatic chains, suggesting the presence of alkyl groups possibly derived from DES components or solubilized matrix compounds. In contrast to conventional solvents, DES extracts did not undergo a separation process; thus, the presence of solvents and water in the extract possibly interfered with the analysis of functional groups, including the detectable water content [53]. To overcome these problems, analysis using Raman spectroscopy can be performed.

As illustrated in Fig. 4, the Raman spectra of DES (choline chloride: lactic acid) and cacao shell extracts extracted with DES (DES 100% and DES 70%) showed that the presence of the extract induced new vibrational features and increased peak intensities. The relative weakness of the Raman water bands enables the acquisition of clear Raman signals even in aqueous environments [54], which is advantageous when analysing DES systems with water content. The cacao shell extracts displayed several new bands compared to the DES alone, indicating the successful extraction of additional compounds from the biomass matrix. In particular, enhanced signals at 709, 1446, and 1647  $\text{cm}^{-1}$  provided strong evidence for incorporating bioactive aromatic and carbonyl-containing compounds into the DES extract. These signals and FT-IR data confirmed the presence of polyphenolic structures such as flavonoids.

The presence of bioactive compounds in the DES-based extracts was confirmed through characteristic vibrational features observed in both FT-IR and Raman spectra. Raman analysis of the extracts revealed the peaks at 709, 1446, and 1647  $\text{cm}^{-1}$ , which corresponded to out-of-plane aromatic C–H

bending,  $\text{CH}_2$  bending, and C=C or C=O stretching, respectively, while FT-IR spectra showed key absorption bands at 3381  $\text{cm}^{-1}$  (O–H stretching), 1730  $\text{cm}^{-1}$  (C=O stretching), and 1043  $\text{cm}^{-1}$  (C–O stretching) [55]. All of these peaks were consistent with polyphenolic structures. These spectral features were in good agreement with the vibrational modes reported for quercetin [56] where hydroxyl groups at positions C-3, C-5, C-7, C-3', and C-4' exhibited O–H stretching vibrations in the 3400–3200  $\text{cm}^{-1}$  range and in-plane bending at 1504, 1350, and 1160  $\text{cm}^{-1}$  in FT-IR, while corresponding Raman bands appeared at 1498, 1367, and 1179  $\text{cm}^{-1}$  for in-plane bending. Moreover, the carbonyl group of quercetin produced stretching vibrations at around 1600  $\text{cm}^{-1}$  in both Raman and FT-IR spectra with additional out-of-plane bending modes below 900  $\text{cm}^{-1}$  attributed to C=O and C–O–H groups. These results align with previous findings confirming the presence of flavonoids like quercetin in cacao shell extracts [10].

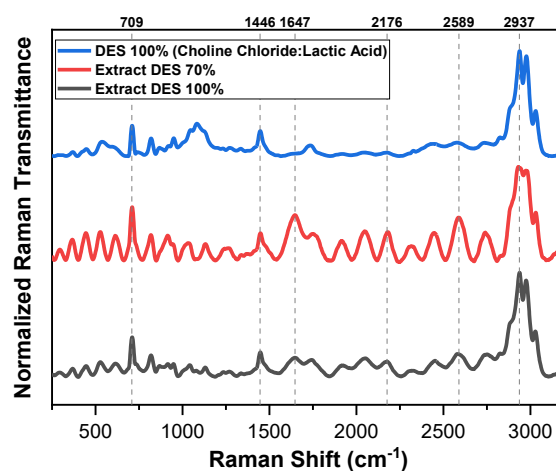


Fig. 4. Raman spectra of DES 100% (choline chloride: lactic acid) and extract cacao shell from DES

While these spectral characteristics closely resemble those of quercetin, the overlapping nature of vibrational bands in both Raman and FT-IR spectra suggests the potential presence of other structurally related polyphenolic or aromatic compounds like other flavonoids in the extract. This indicates that DES not only extracts quercetin but may also facilitate the recovery of a broader range of bioactive compounds inherent in cacao shells. These findings highlight the effectiveness of DES as a green solvent. Future studies should identify and quantify individual compounds present in DES extracts using complementary techniques such as LC-MS or NMR and optimize the DES composition and extraction parameters for targeted compound recovery and functional application in food, pharmaceutical, or cosmetic formulations.

#### 4. Conclusion

This study highlights the strong potential of DES, specifically a choline chloride and lactic acid mixture, for extracting bioactive compounds from cacao shells. DES 100% resulted in the highest TPC ( $337.92 \pm 9.55$  mg GAE/g dry weight), better than conventional solvent, while DES 70% was most effective for flavonoids, reaching  $76.51 \pm 1.59$  mg RE/g dry weight at 40 minutes. Kinetic analysis showed that the pseudo-

second-order and Peleg's models closely matched the experimental data, suggesting these models are suitable for describing the extraction process. Although DES and conventional solvents like ethanol and water differ significantly in their physical and chemical properties, they follow similar kinetic patterns, pointing to a shared underlying mechanism. Functional group analysis further confirmed the presence of phenolic compounds like quercetin. These findings support the use of DES as a green and selective extraction solvent. Further studies should explore identifying individual compounds using LC-MS or NMR and refine DES formulations for specific food, pharmaceutical, and cosmetic applications.

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