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Kinetic modelling of combined ultrasonication and Soxhlet based extraction of lipids from passion fruit seed using ethanol as solvent

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ABSTRACT: The aim of this study was to understand the extraction phenomena of oil from passion fruit seeds (PFS) using Soxhlet, ultrasonic-assisted extraction (UAE), and combined UAE-Soxhlet (UAES) methods and to investigate the physicochemical properties of the oil. The effect of solid-liquid ratio, temperature, and time of the UAE treatment on PFS oil (PFSO) yield was investigated and optimized. Phenomenological model parameters, viz. washing constant (0,807–1,139×10^{-1} min^{-1}) was faster than the diffusion constant parameter (5.0–5.4×10^{-3} min^{-1}) throughout the extraction process, and the washing constant was the highest for UAES. The composition of phenolics and fatty acids in the optimized oil extract was investigated by HPLC and GC, respectively. The comparison of the quality parameters of the extracted oils revealed that the combination of ultrasound and Soxhlet extraction is a viable alternative extraction procedure for obtaining better quality. The phenomenological model with combined extraction could be recommended for modelling extraction kinetics for better quality products and could be used for industrial purposes.

KEYWORDS: *Passion fruit seed oil; Phenomenological model; Quality parameters; Soxhlet extraction; Ultrasonication.*

RESUMEN*: Modelo cinético de ultrasonido combinado con extracción en Soxhlet de lípidos de semillas de maracuyá utilizando etanol como disolvente.* El objetivo de este estudio fue comprender los fenómenos de extracción del aceite de semilla de maracuyá (PFS) utilizando Soxhlet, extracción asistida por ultrasonidos (UAE) y métodos combinados UAE-Soxhlet (UAES) e investigar las propiedades fisicoquímicas. Se investigó y optimizó el efecto de la relación sólido-líquido, la temperatura y el tiempo del tratamiento con EAU sobre el rendimiento de aceite PFS (PFSO). Los parámetros del modelo fenomenológico, como la constante de lavado (0,807–1,139×10^{-1} min^{-1}) fue más rápido que el de la constante de difusión (5.0–5.4×10^{-3} min^{-1}) para todo el proceso de extracción y la constante de lavado fue mayor para la UAES. La composición de fenoles y ácidos grasos en el extracto de aceite optimizado se determinó mediante HPLC y GC, respectivamente. La comparación de los parámetros de calidad de los aceites extraídos mostró que la combinación de ultrasonido y extracción Soxhlet es un procedimiento de extracción alternativo viable que ofrece una mejor calidad. El modelo fenomenológico con extracción combinada podría recomendarse para modelar la cinética de extracción y obtener un producto de mejor calidad y podría usarse con fines industriales.

PALABRAS CLAVE: *Aceite de semillas de maracuyá; Extracción Soxhlet; Modelo fenomenológico; Parámetros de calidad; Ultrasonido.*

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1. INTRODUCTION

Passion fruit (*Passiflora edulis*) is an alluring, nutrient-rich exotic fruit that is valued for its distinctive aroma and flavor and is typically consumed fresh for its pleasant taste (Pereira *et al.*, 2017). It is also widely used in traditional medicine throughout the world and is being exploited by the food, pharmaceutical, and cosmetics industries (Corrêa *et al.*, 2016; dos Reis *et al.*, 2018). A significant issue with passion fruit in the manufacturing sector/industries is the waste generated from the discarded peels and seeds, which accounts for more than half of the fruit weight, and is gaining the interest of researchers as the seed and peel contain large quantities of bioactive compounds and has the potential to be used as a valuable material in food industries (dos Reis *et al.*, 2018).

Passion fruit seeds make up between 8.24 and 16.18% of the fruit weight (dos *Reis et al.*, 2018). The oil content in passion fruit seeds (PFS) ranges from 22–30% and is rich in polyphenols, phytosterols, and essential fatty acids (Ramaiya *et al.*, 2019; Ribeiro *et al.*, 2020; Purohit *et al.*, 2021). Antimicrobial properties are displayed by PFS oil (PFSO) (Jusuf *et al.*, 2020; Kawakami *et al.*, 2022). According to Ramiya *et al.* (2019), the PFSO has significant antioxidant activity at 34 mg/mL (EC_{50} DPPH) and roughly 570 mg GAE/kg oil of total phenolic compounds.

Leaching, or solid-liquid extraction, has been widely utilized in food processing and other sectors to extract the target compounds from plant food matrices by employing a proper solvent. This extraction/recovery method is mostly based on the diffusion of extractable compounds through the plant food matrix and subsequent dissolution in the solvent used (Milić *et al.*, 2013). Because seeds typically have low oil percentages, efforts must be made to increase yield while lowering overall process costs. The extraction process is mostly influenced by operating variables such as the solvent used, the extraction method, the operating temperature, the solid-to-solvent ratio, and the duration of extraction. By optimizing these factors, the extraction yield may be increased (Milić *et al.*, 2013; Orphanides *et al.*, 2014). On industrial scale, ethanol may have limitations in terms of oil solubility, leading to lower yields compared to hexane and require high temperatures to separate the oil from the solvent, but ethanol is the most widely used solvent because it is less harmful and has no impurities (Teng *et al.*, 2016).

High-energy extraction methods, which are based on the principles of green chemistry and bio-refinery techniques, have recently attracted attention and have become a hot topic in the field of oil extraction due to their quick extraction rates, high extraction yields, minimal negative effects on the extracted compounds, improved extraction of thermosensitive nutritional components, and lower processing temperatures (Goula *et al.*, 2017; Chutia and Mahanta, 2021).

Over the past few years, a number of novel techniques for obtaining seed oils have been investigated, including ultrasonic extraction, microwave extraction, and supercritical fluid extraction (Li *et al.*, 2016), which are often explored for their potential advantages in terms of efficiency, selectivity, and reduced environmental impact. However, their implementation on a larger scale may face challenges related to cost, equipment, and process optimization. Edible vegetable oils are traditionally extracted via mechanical pressing and/or liquid solvent-based extraction techniques, notwithstanding their limitations. The main drawbacks of mechanical pressing include low extraction rates, air and light exposure, and shelf-life loss. Solvent-based conventional extractions typically involve a lengthy process which consumes a lot of energy, resulting in high solvent usage with high toxicity and environmental risks, and also the loss of volatile compounds (Pereira *et al.*, 2017).

Supercritical extraction, subcritical fluid extraction, and ultrasonic-assisted extraction (UAE) are some of the most promising alternative processes (Pereira *et al.*, 2017). UAE is a quick and efficient extraction method that uses ultrasound to speed up the movement of solvents, accelerating extraction while also increasing mass transfer speed. UAE has been used to extract oil from raspberries (Teng *et al.*, 2016), sweet passion fruit seeds (Ribeiro *et al.*, 2020), Spanish flax seeds (García-Hernández *et al.*, 2017), and tara seeds (Li *et al.*, 2016). However, it has some drawbacks including the inability of the solvent to be refreshed during the process, which reduces its efficiency, the need for filtration and solvent evaporation in the subsequent phase, and the often low selectivity of extraction (Pereira *et al.*, 2017). So, combined UAE-Soxhlet extraction

(UAESO) is a potential method to increase the yield and quality of oils. With recoveries and chemical compositions comparable to those obtained by conventional Soxhlet extraction (SE) and no discernible differences in terms of quality, UAES extraction can be regarded as a novel technique which provides a quick method for the extraction of lipid contents (Luque-García and Luque De Castro, 2004; Djenni *et al.*, 2013). According to Luque-García and Luque De Castro, (2004), the official ISO method of sono-Soxhlet has been able to attain efficiencies comparable to or better than those offered by both conventional SE and UAE, saving both time and sample manipulation. The authors concluded that after applying ultrasound, the composition of the fat extracts remained the same and the precision was comparable to that obtained using the ISO reference technique. Investigation of Sono-Soxhlet procedures for obtaining oil from the seeds of passion fruit has not been reported.

Modelling can be used to forecast the outcome of an extraction process. Different types of mathematical modelling have been used in the field of food engineering, but the so-called phenomenological or thermodynamic models, which are based on irreversible thermodynamics, have garnered particular attention (Orphanides *et al.*, 2014). The most well-liked models, known as physical extraction kinetic models, are based on the phenomenon of mass transfer from solid matrices up to surfaces and from surfaces into the bulk extracting fluid. Despite being quite complex, they can be made simpler by applying the film theory and unsteady diffusion through matrices (Milić *et al.*, 2013).

It has already been discussed in a number of publications about how the phenomenological model might be used to extract the targeted compounds from plant materials such as resinoids from aerial parts using UAE (Milić *et al.*, 2013), essential oils from Congo-Brazzaville using hydro-distillation (Silou *et al.*, 2021), and juniper essential oils using microwave-hydrodistillation (Marković *et al.*, 2019). The use of phenomenological or thermodynamic models to comprehend the extraction processes of the sono-Soxhlet procedure has been the subject of limited research so far. In this context, this study is justified considering the scarce information and knowledge about the UAES-based oil extraction kinetics from passion fruit seeds and its characteristics.

The present work dealt with Soxhlet, ultrasound, and combined sono-Soxhlet extraction of oil from PFS. The effects of solid-liquid ratio, temperature, and time of UAE treatment on the yield of PFSO were investigated and also optimized based on extraction yield for industrial purposes. The phenomenological kinetics model was used to understand the extraction phenomena of oil using the sono-Soxhlet technique, and physicochemical characterization of optimized sono-Soxhlet extracted oil was made.

2. MATERIALS AND METHODS

2.1. Sample preparation

Yellow passion fruits (*Passiflora edulis spp*) were obtained from Bishnupur district, Manipur, India. Uniform-sized yellow-colored ripe fruits were cut into halves and parts were separated. Fresh seeds were dried in an oven at 50 °C to constant weight.

The standards used for HPLC were purchased from Sigma. All other chemicals were of analytical grade.

2.2. UAE extraction

The dried passion fruit seed was coarsely ground into powder to pass through a 40-mesh sieve. The powder was added to ethanol (extraction solvent) and placed in the ultrasonic bath (Riviera Glass Pvt. Ltd., India, Power 230 volts, 50 Hz). Solid-solvent ratio, extraction temperature, and extraction time were selected as extraction conditions. After the extraction, the ethanolic oil extract was recovered using a rotary evaporator followed by vacuum drying, until a constant weight (complete evaporation of ethanol) was obtained. This ethanolic extract was termed as UAEO.

The passion fruit seed oil was weighed and the extracted oil yield was calculated. A single factor analysis was performed to determine the solid-solvent ratio, extraction temperature, and extraction time. For the optimization of solid-solvent ratio, 5, 10, 15, 20, 25 and 30 g/100 ml solid-solvent ratios were selected and all extractions were performed for 30 min at 60 °C and the ratio at which maximum yield of oil occurred was selected as the optimized solid-solvent ratio. Similarly, for the selection of temperature, UAE was performed from 30 to 80 °C at each 10 °C interval using optimized solid-solvent ratio for 30 min. The extraction time periods used were 5, 10, 15, 20, 25, 30, 40, 50, 60 and 90 min at the optimized solid-solvent ratio and temperature conditions. For all experiments, oil yield was measured in triplicate.

2.3. Soxhlet method

Soxhlet extraction was performed using ethanol, as ethanol is the most widely used solvent because it is less harmful and has no impurities (Teng *et al.*, 2016). The optimized solid-solvent ratio was chosen for each extraction process. The seed powder was extracted for 8 h, and yield was calculated at 10, 20, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min. The extracted oil was named SEO.

2.4. Dual UAE-Soxhlet extraction (UAES or Sono-Soxhlet extraction)

Optimized ultrasound extraction followed by Soxhlet extraction up to 4 h in total was performed and its kinetics studied in terms of ethanolic extract yield. and tThe ethanolic extract was coded as UAESO. All experiments were conducted in triplicate.

2.5. Proximate analysis of passion fruit seed

Moisture content, total lipids, crude fiber, crude protein, and ash content were measured using standard AOCS methods (Firestone, 2009). All experiments were performed in triplicate.

2.6. Oil Extraction Yield

The extraction yield of ethanolic extracted oil was measured using Equation 1 in triplicate.

$$\text{Oil extraction yield} = \frac{Y_i - Y_f}{Y_i} \quad (1)$$

Where, Y_i and Y_f are the initial and extracted dried residual weights of passion fruit seed powder (g), respectively.

2.7. Physicochemical properties of seed ethanolic extract oil

The refractive index was measured at 25 °C using a hand refractometer. The acid value was determined by titration with 0.1 mol/L NaOH using phenolphthalein as an indicator (Pereira *et al.*, 2017). Iodine value (Firestone, 2009), peroxide value (Firestone, 2009), and conjugated diene (Farhoosh *et al.*, 2012) were also determined. All experiments were carried out in triplicate.

2.8. Total carotenoids content (TCC) and total phenolic content (TPC)

TCC was determined as per the method described by da Silva and Jorge (2016) and expressed as μg of β-carotene/g of oil. The TPC of PFSO was determined by the Folin-Ciocalteu reagent method (Purohit *et al.*, 2021) and reported in mg GAE/g of oil. All experiments were conducted in triplicate.

2.9. Antioxidant activity

2.9.1. 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of the oils was determined according to Chutia *et al.* (2020). All experiments were performed in triplicate.

2.9.2. 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS):

The ABTS activity was investigated according to Pereira *et al.* (2017) and the results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

2.10. HPLC analysis of phenolic acids

The phenolic acids were determined (Espin *et al.*, 2016) using a HPLC instrument with a C18 column. Phenolic acids were identified against standards and quantified.

2.11. Calculated oxidizability value (COX)

The COX value was calculated according to the formula in Equation 2, in triplicate.

COX value = [C18:1(%)+{10.3×C18:2(%)}+{21.6×C18:3(%)}]÷100 (2)

2.12. Fatty acid profile

The fatty acid profile of the PFSO samples was determined by preparation of methyl esters as described by Pereira *et al.* (2017) and separated by gas chromatography (GCMS-MS Model: GCMS-TQ8040, Shimadzu) and identified by comparison with retention times of the standard FAMEs. Each fatty acid was quantified as a percentage of the total methyl ester peak areas.

2.13. Phenomenological kinetics model

The mathematical model was based on the following assumptions (Milić *et al.*, 2013; Marković *et al.*, 2019): pseudo-single component of extracted oil; perfectly mixed solution; plant matrices were isotropic in nature; negligible resistance to the mass transfer; a fraction of the oils was located at the external surfaces, and rest was uniformly distributed in the matrices; extraction process occurred via two simultaneous mechanisms: (i) rapid penetration of solvent and dissolution of the oil located on or near surfaces of solid matrices known as washing, and (ii) mass transfer of oil soluble compounds from the plant matrices into the extractable solvent by diffusion and osmotic processes, well known as slow extraction; the fractions of oil extracted *via* washing, unhindered diffusion and hindered diffusion (*f1*, *f2* and *f3*, respectively) assumed to be constant:

$$-\frac{dw_p}{dt} = k \times w_p \tag{3}$$

Where, w_p was the average content of ethanolic extract in the plant particles (g/100g) at time t, and process rate constant (k)

For, t = 0, $w_p = w_\infty$, so the integration of Equation 3 gave the following expression for washing, unhindered diffusion and hindered diffusion, respectively:

$$\frac{w_{p,1}}{w_\infty} = exp(-k_1 t),\ \frac{w_{p,2}}{w_\infty} = exp(-k_2 t),\ \frac{w_{p,3}}{w_\infty} = exp(-k_3 t) \tag{4}$$

Based on the assumption,

$$\frac{w_p}{w_\infty} = f1 \times exp(-k_1 t) + f2 \times exp(-k_2 t) + f3 \times exp(-k_3 t) \tag{5}$$

Where, $f1 = \frac{w_{p,1}}{w_\infty}$, $f2 = \frac{w_{p,2}}{w_\infty}$ and $f3 = \frac{w_{p,3}}{w_\infty}$, and, $f1 + f2 + f3 = 1$.

Hence, the amount of ethanolic extract extracted until time t, $w = w_\infty - w_p$ follows

$$\frac{w}{w_\infty} = 1 - f1 \times exp(-k_1 t) - f2 \times exp(-k_2 t) - f3 \times exp(-k_3 t) \tag{6}$$

Or

$$w = w_\infty \begin{pmatrix} 1 - f1 \times exp(-k_1 t) - f2 \times exp(-k_2 t) \\ - f3 \times exp(-k_3 t) \end{pmatrix} \tag{7}$$

In general, for ideal conditions, it is assumed that hindered diffusion is negligible i.e. $f3 = 0$, the model can be represented by Equation 8:

$$w = w_\infty \left(1 - f1 \times exp(-k_1 t) - (1 - f1) \times exp(-k_2 t)\right) \tag{8}$$

if washing was faster than diffusion, ($k_1 >> k_2$), then Equation 9 follows:

$$w = w_\infty \left[1 - (1 - f1)\exp(-k_2 t)\right] \tag{9}$$

if $f = 0$, which implies that washing does not occur, means extraction yield increases exponentially due to diffusion:

$$w = w_\infty \left[1 - \exp(-k_2 t)\right] \tag{10}$$

Here Equation 8, Equation 9 and Equation 10 represent the Phenomenological model, model based on instantaneous washing followed by diffusion, and pseudo first order model, respectively.

2.14. Statistical analysis

The model parameters were estimated using MATLAB 7.14 (Release 2012a) and non-linear least squares regression using Microsoft Excel Solver (Microsoft office, USA). The performances of the developed model were statistically measured by coefficient of determination (R^2), mean squared error (MSE), and mean relative percent deviation (MRPD). The one-way analysis of variance (ANOVA) was performed to compare the means along with the Duncan's multiple range test to evaluate the significant difference using IBM SPSS software at $p < 0.05$.

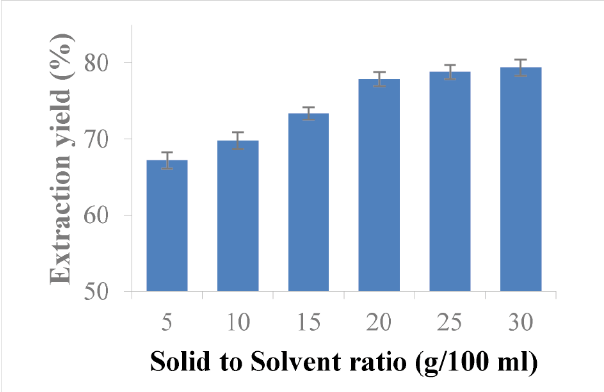
3. RESULTS AND DISCUSSION

3.1. Proximate analysis of passion fruit seed

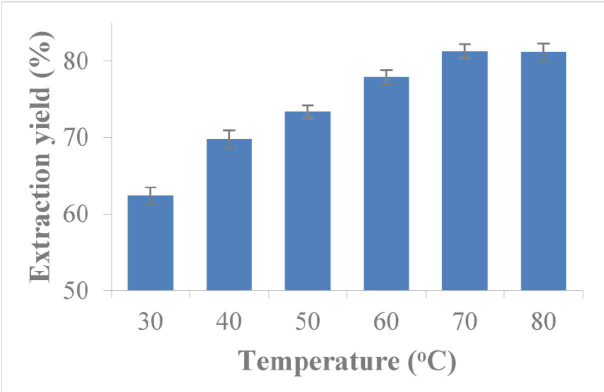
The PFS sample contained 8.64±0.64% moisture, 11.58±0.87% crude protein, 24.4 ±2.78% crude fat, 1.64±0.18% crude ash, and 51.59±3.81% crude fiber. The moisture content was 8.64%, whereas reported moisture content in yellow passion fruit seeds varied from 7.38 - 12.38% (Purohit *et al.*, 2021; Reis *et al.,* 2020; Malacrida and Jorge, 2012). The ethanolic extract content was 24.4%, in comparison, Purohit *et al.* (2021) found 24.6 and 28% oil in PFS cultivated in Manipur and Assam, respectively, and Malacrida and Jorge (2012) and Reis et al., (2020) obtained 30.39 and 23% oil, respectively, in Brazilian yellow PFS. PFS contained crude fiber (51.59 g/100g), crude protein (11.58 g/100g), and ash content (1.64 g/100 g), which agreed with previous reports (Malacrida and Jorge, 2012; Pereira *et al.*, 2017; Purohit et al., 2021). Malacrida and Jorge, (2012) found 7.38% moisture, 30.39% lipids, 12.23% protein, 1.75% ash and 48.73% fiber in Brazilian yellow passion fruit. The species of passion fruit used, and the diverse geo-climatic conditions may be the cause of variations in proximate composition (Reis *et al.,* 2020).

3.2. Effect of the solid-liquid ratio

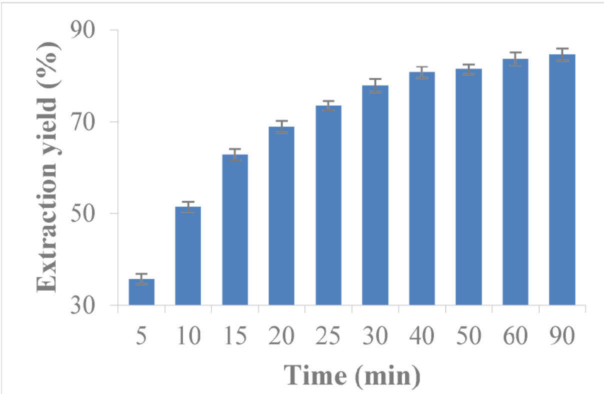
The best yield is achieved when the solution reaches saturation concentration. In our study, the solid-solvent ratio varied between 5 and 30 g/100 ml. The yield of ethanolic extract increased rapidly up to a solid-liquid ratio of 20 g/100 ml, after which a slight increase in yield was observed. At 20 g/100 ml solid-liquid ratio, 75.6% of total ethanolic extract was extracted by UAE; any further increase in the ratio increased the oil yield only marginally (Figure 1a). Therefore, a solid-liquid ratio of 20 g/100 ml was selected as the most suitable ratio for further tests. The most suitable liquid-solid ratio for UAE extraction of oil from tara seed using petroleum ether was 14:01 (Li et al., 2016).



(a) Solid-solvent ratio



(b) Temperature



(c) Time

Figure 1. Effect of extraction parameters on extraction yield of passion fruit seed oil (a) solid-solvent ratio, (b) temperature, and (c) time.

The results are expressed as mean ± standard deviation (n = 3).

3.3. Effect of extraction temperature

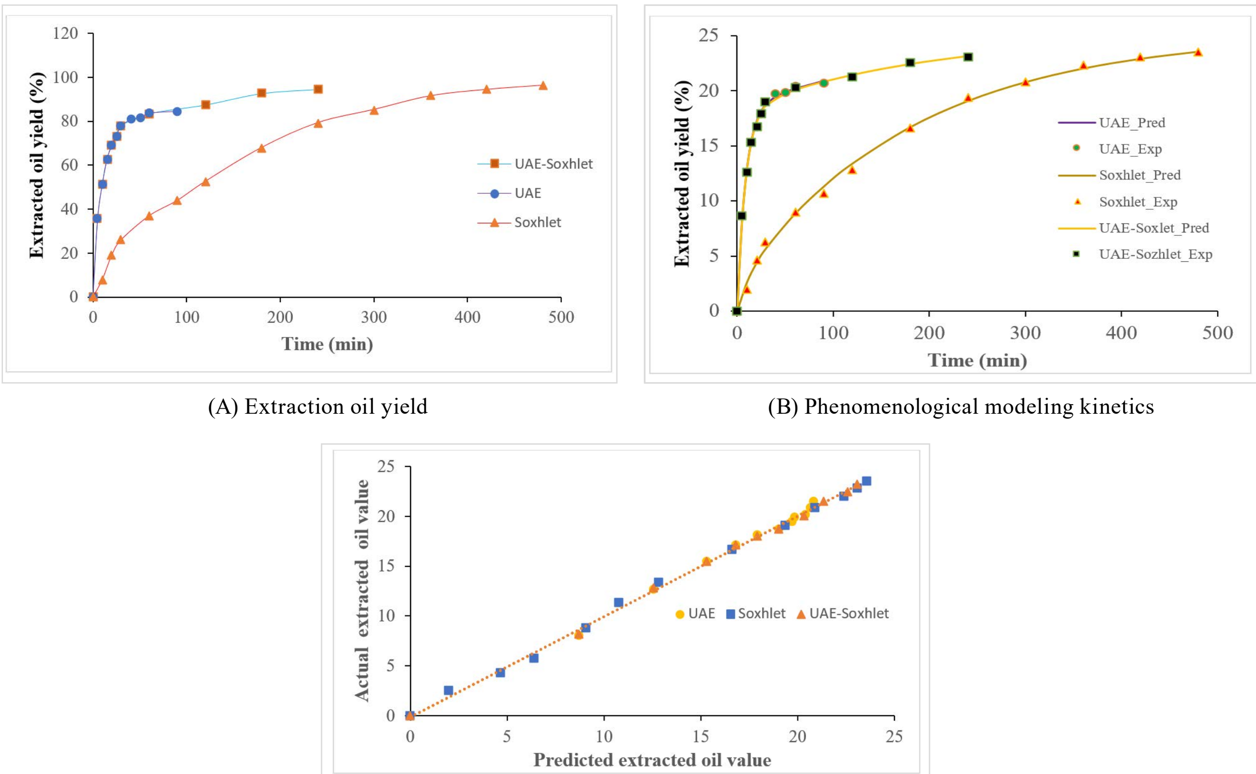
UAE temperature affects the interaction between the solid and liquid. When the ultrasonic temperature was increased from 30 to 70 °C, the yield of ethanolic extract increased from 62.4 to 81.3% (Figure 1b), whereas extraction at 80 °C caused a slight decrease (0.1%) in yield. 70 °C was selected as the most suitable temperature for subsequent experiments. Solvent and ethanolic extract viscosity decrease as temperature rises, improving its fluidity and facilitating its flow through solid matrices as well as raising the diffusion coefficients of the compounds that can be extracted from lipophiles. But at temperatures > 70 °C, the dissolution of cell impurities and decomposition of some constituents may increase and too high temperatures would enhance the speed of bubble collapse in the solvent, which may hamper the strength of the bubbles and thus decrease the yield (Goula *et al.,* 2017; Chutia and Mahanta, 2021).

3.4. Effect of extraction time on ultrasonic extraction of seed oil

Extraction times varying between 5 and 90 min were used to investigate the effect of ethanolic extract yield (Figure 1c), and the extraction yield was observed to increase with time. This might be because greater time allows the ultrasonic wave to penetrate cell walls and release the cell contents. The extraction yield was rapid up to around 30 min and thereafter the rate slowed down, which was also reported by Raj and Dash (2020). To extract approximately 75% of total oil, it required approximately 30 min and to extract the next 12% ethanolic extract, it required another 60 min. So, 30 min was chosen as the most suitable UAE time.

3.5. Soxhlet extraction

It is evident that when treatment time was extended at a steady temperature, oil extraction yield rose until it reached saturation (Figure 2a). Higher yield is a result of the solvent's viscosity being reduced, with the extracted oil being more fluid and soluble, and the solvent's diffusivity and mass transfer being improved. Similarly, higher treatment time means prolonged contact or exposure to the solvent (Goula et al., 2017; Chutia and Mahanta, 2021). To extract approximately 50%



(A) Extraction oil yield

(B) Phenomenological modeling kinetics

(C) Precited and Actual value of yield oil

Figure 2. UAE, Soxhlet and UAE-Soxhlet extraction of oil (a) Extraction oil yield, (b) Phenomenological modeling kinetics, (c) Precited and Actual value of oil yield

total extractable oil, it required approximately 120 min, to extract the next 30% oil approximately another 2 h was required. For a further 20% extract, it required another 4 h.

3.6. UAE-Soxhlet extraction

In this extraction process, optimized UAE extraction was performed for the first 30 min followed by Soxhlet extraction for 4 h (Figure 2a). The results revealed that combined UAE-Soxhlet extraction was able to extract approximately 95% of oil within a short period of time (4 h), and in comparison, the Soxhlet extraction process would require approximately 8 h. Using UAE only, it was very difficult to extract more than 85% of the total oil. The overall improvement may be due to the synergistic effect of ultrasound (due to breakdown of the cell and better penetration of the solvent) and Soxhlet extraction processes (due to high temperature and better solvent movement) (Reis *et al.*, 2020).

3.7. Kinetic modelling of all extraction processes

Figure 2b illustrates the variation in oil yields from PFS using different extraction techniques with ethanol as solvent. For all extraction processes, oil yield increased with extraction time. Using UAES process, overall extraction rate was higher compared to SE and UAE alone. The positive effect of UAES process on the extraction yield may be because UAE disrupted the cell wall and enhanced the accessibility of oil, and the later use of higher temperature in the Soxhlet method decreased the viscosity of the solvent and enhanced the overall fluidity (Djenni *et al.*, 2013) and solubility of the oil and gave synergetic effects. As a result, it enhanced the diffusivity and mass transfer (Hlaváč *et al.*, 2019). This study suggested that combined ultrasound and Soxhlet with ethanol as solvent can be used as a novel technology for higher extraction efficiency within a short time. UAE followed by Soxhlet extraction can be easily scaled up for industrial use (Deenu *et al.*, 2013).

The ethanolic extract extraction mechanism from PFS consisted of two stages. In the initial stage, rapid extraction of the extractable materials located at the surfaces occurred, and then the extractable materials from intact cells slowly diffused to the surface of the particles (Marković *et al.*, 2019; Silou *et al.*, 2021). This observation was very important for subsequent modelling of the extraction kinetics.

3.8. Phenomenological model

The kinetics of oil by UAE (UAEO), Soxhlet (SEO), and UAES (UAESO) was described by the phenomenological models represented in Table 1. The values for the model parameters of Equation 8 and the statistical criteria used for assessing the goodness of fit (R^2, MRPD, and MSE) are presented in Table 1. The experimental and predicted values for oil yield agreed very well with each other (Figure 2c) as the high coefficient of determination (R^2 > 0.997), low MSE (< 0.1118), and the acceptable MRPD (< ±1.79–1.55%) implied the good fitting that explained the extraction phenomena using the phenomenological model. This can be visually

Table 1. Parameters of phenomenological model for extraction of ethanolic extract from passion fruit seeds

Extraction	w_∞	$f1$	$k_1(min)^{-1}$	$k_2(min)^{-1}$	R^2	MRPD (%)	MSE
UAE	22.3 ± 1.6^a	0.7320 ± 0.0040^b	0.1121 ± 0.0090^b	0.0050 ± 0.0008^a	0.997	± 1.79	0.1118
Soxhlet	25.2 ± 1.0^b	0.0996 ± 0.0080^a	0.0807 ± 0.0030^a	0.0054 ± 0.0005^a	0.998	± 5.03	0.1386
UAE-Soxhlet	24.7 ± 1.4^{ab}	0.7259 ± 0.0070^b	0.1139 ± 0.0070^b	0.0051 ± 0.0007^a	0.998	± 1.55	0.0690

The results are expressed as mean ± standard deviation (n = 3). Values with different superscript letters (a,b) within the column represent significant differences (p < 0.05, one-way ANOVA with Duncan's multiple range test).
UAE: Ultrasonic-assisted extraction, UAE-Soxhlet: Combined ultrasonic-assisted extraction and Soxhlet extraction
w∞: The average content of ethanolic extract oil in the plant particles (g/100 g) at time t=0;*f*1: The fractions of oil extracted via washing;
k1: Process rate constant due to washing; *k1*: Process rate constant due to diffusion; *R2:* Coefficient of determination
MSE: mean squared error; MRPD: mean relative percent deviation.

observed in Figure 2b. The ethanolic extract extraction yield at saturation point increased with extraction techniques, which may be due to the increase in the extraction temperature as Soxhlet extraction temperature was higher than UAE.

The fraction of washable extractable substances decreased with the increase in the extraction temperature, for both Soxhlet and UAE extraction techniques. The "$f1$" values were higher for the UAE (0.7320) followed by combined UAES (0.7259) and SE (0.0096) (Table 1), which demonstrated the influence of ultrasound on the washing extraction process (Milić *et al.*, 2013). The k_1 and k_2 values for all extraction processes ranged from 0.0807 to 0.1139 min^{-1} and 0.0050 to 0.0054 min^{-1}, respectively, and for all extraction processes $k_1 >> k_2$, which emphasized the predominance of the washing process. The k_1 value was highest for UAES extraction followed by UAE and SE extraction processes, in that order, which indicated the synergistic effect of UAE and SE on the washing process as well as on the overall extraction process. Milić *et al.* (2013) observed a similar trend for UAE and conventional extraction processes. Marković *et al.* (2019) found similar values for phenomenological model parameters where k_1 was 0.1753 min^{-1}, k_2 was 0.0173 min^{-1} and $f1$ was 0.619 during the extraction of essential oil from juniper.

Two simpler models were developed, namely the pseudo-first order model (Equation 9), where the washing process was neglected, and the model based on instantaneous washing followed by diffusion, where the diffusion process was neglected (Equation 10). These models were evaluated for the purpose of comparison with the phenomenological model (Equation 8). The kinetics parameters of these two simpler models are presented in Table 2. The k_2 value for the pseudo first order model ranged between 0.0083 and 0.0754 min^{-1}, whereas the same parameter value for the model based on instantaneous washing followed by diffusion ranged from 0.0081-0.0714 min^{-1} (Table 2). For each extraction process, the k_2 value for the pseudo first order model was found higher than the model based on instantaneous washing followed by diffusion process. The pseudo-first order model and instantaneous washing followed by diffusion model had a small R^2 value and relatively high MSE and MRPD (%) values compared to the phenomenological model, regardless of the adopted extraction techniques. Therefore, only the pseudo first order model and instantaneous washing followed by diffusion models were less efficient compared to dual extraction (phenomenological model) for describing the extraction kinetics of oil from PFS. Thus, the phenomenological model better explained the variation in oil yield than the simpler models and could be recommended for modelling extraction kinetics.

3.9. Physicochemical properties of passion fruit seed oil

The physicochemical properties of the optimized ethanolic extract (UAESO) and conventional extraction (SEO) were calculated and are reported in Table 3. The acid values for the optimized UAESO and SEO were 2.4 and 2.8 mg KOH/ g oil, respec-

Table 2. Parameters of pseudo-first order model and model based on instantaneous washing followed by diffusion for extraction of ethanolic extract oil from passion fruit seeds

Extraction	Pseudo first order				Model based on instantaneous washing followed by diffusion				
	$k_2(min)^{-1}$	R^2	MRPD (%)	MSE	$f1$	$k_2(min)^{-1}$	R^2	MRPD (%)	MSE
UAE	0.0727 ± 0.0007^b	0.995	± 6.90	1.5887	0.0582 ± 0.0005^c	0.0676 ± 0.0006^b	0.978	± 5.81	1.4167
Soxhlet	0.0083 ± 0.0004^a	0.987	± 7.97	0.9881	0.0245 ± 0.0003^a	0.0081 ± 0.0007^a	0.988	± 7.44	0.9257
UAE-Soxhlet	$0.0754^c \pm 0.0007^c$	0.984	± 4.62	0.9357	0.0451 ± 0.0004^b	0.0714 ± 0.0007^c	0.983	± 4.62	0.8257

The results are expressed as mean ± standard deviation (n = 3). Values with different superscript letters (a,b,c) within the column represent significant differences ($p < 0.05$, one-way ANOVA with Duncan's multiple range test).
UAE: Ultrasonic-assisted extraction, UAE-Soxhlet: Combined ultrasonic-assisted extraction and Soxhlet extraction.
$f1$: The fractions of oil extracted via instantaneous washing followed by diffusion; $k2$: Process rate constant due to diffusion; $R2$:: Coefficient of determination.
MSE: mean squared error; MRPD: mean relative percent deviation.

tively (Table 3), which were within the permissible limit of 4.0 mg KOH/ g for crude oil as set by Codex Alimentarius (2015). Similarly, the peroxide value for the extracted oils was within the permissible range.

Between the two extraction methods, the UAESO demonstrated better oil quality. The low acid value and peroxide value for PFSO suggested the good quality and edibility of the oil. The scent and flavor of the oil are produced by free fatty acids. The percentage of unsaturated substance in oil is determined by the iodine value for the oil (Purohit *et al.,* 2021). For combined extraction, the iodine value for UAESO was determined to be 130.2 g I_2/100 g oil and for SEO 139.4 g I_2/100 g oil (Table 3).

Table 3. Physicochemical properties of passion fruit ethanolic extract seed oil

Parameters	UAESO	SEO
Acid value (mg KOH/ g oil)	2.42±0.03^a	2.81±0.04^b
Iodine value (g I_2/100 g)	130.24±0.6^{2}a	139.40±0.70^b
Peroxide value (meq O_2/100 g)	4.81±0.05^a	5.24±0.06^b
Refractive Index (at 25 °C)	1.47±0.02^a	1.54±0.04^b
Density (g/L) (at 25 °C)	918.23±0.80^a	902.12±0.78^b
Induction time (h)	2.42±0.04^a	2.14±0.03^b
Total phenolic content (mg GAE/g of oil)	28.97±0.09^a	26.87±0.09^b
Total carotenoids (µg ß-carotene/g)	5.80±0.01^a	5.12±0.02^b
DPPH scavenging activity (%)	71.28±0.07^a	67.84±0.08^b
ABTS (µM TEAC/g oil)	172.02±1.10^a	139.20±1.20^b

The results are expressed as mean ± standard deviation (n = 3).
Values with different superscript letters (a,b) within the row represent significant differences ($p < 0.05$, one-way ANOVA with Duncan's multiple range test).
UAESO: Ethanolic extract of ultrasound-assisted followed by Soxhlet extraction; SEO Soxhlet extracted oil.
DPPH scavenging activity: 2,2-Diphenyl-1-picrylhydrazylradical scavenging activity;
ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TEAC: Trolox equivalent antioxidant capacity; KOH: Potassium Hydroxide; GAE: Gallic acid equivalent.

Purohit *et al.* (2021) discovered the value between 126.9 and 131.4 g I_2/100 g oil for yellow passion fruit seed oil. The PFS possessed high values for iodine (124.67±0.67-131.00±0.58 g I_2/100 g) and peroxides (1.43±0.12-3.23±0.12 meq O_2/kg), which were similar to other edible seed oils as reported by Ramaiya *et al.* (2019).

The refractive index of PFSO of UAESO and SEO was 1.469 and 1.54, respectively (Table 3), which was within the range of refractive index for most vegetable oils. This value indicated the presence of long-chain unsaturated fatty acids and also intermolecular interaction in PFSO (Purohit *et al.*, 2021). The density of UAESO was found to be higher than SEO. The lower antioxidant activity observed in this study may be attributed to the high temperature used for extraction in the Soxhlet apparatus (Ramaiya *et al.*, 2019).

The total carotenoid contents of UAESO and SEO were 5.8 and 5.1 µg β-carotene/g of oil, respectively, and β -carotene was the major carotenoid in the seed oils. Similarly, 6.70 µg β-carotene/g sample of oil was reported by da Silva and Jorge (2016). Total phenolic contents, total carotenoid contents and overall antioxidant activity of the oil extracted by the UAES technique was higher as compared to the Soxhlet process. Prolonged heating in the Soxhlet process (SEO) may degrade the total phenolic content and total carotenoids, which affected the overall antioxidant activity of the oil (Goula *et al.*, 2017; Chutia and Mahanta, 2021).

3.10. HPLC analysis of total phenolic acids

The total phenolic content in PFSO was 33.57 mg GAE/g of oil, whereas 36.02-39.11 mg GAE/g of total phenolic content in yellow passion fruit seed oil from the northeast region was reported by Purohit *et al.* (2021).

Four major phenolic compounds were detected by the HPLC method, namely gallic acid (20%), salicylic acid (16.4%), piceatannol (54.6%) and kaempferol (8%). da Silva and Jorge (2016) found three main phenolic acids: caffeic acid, coumaric acid and salicylic acid, with salicylic acid being the major phenolic acid. de Santana *et al.* (2017) identified stilbene piceatannol as the major compound in yellow passion fruit seed extract, accounting for 50% of the total phenolic content.

3.11. Fatty acids composition

The fatty acid composition and its relative percentage in yellow PFSO are presented in Table 4.

The fatty acid methyl ester (FAME) composition indicated the presence of unsaturated fatty acids, which was also observed from the high iodine value. The presence of long chain unsaturated fatty acids in the PFS indicated by the refractive index values was confirmed by the presence of high amounts of linoleic and oleic acids. The major fatty acids found in UAESO were linoleic acid (67.3%), oleic acid (16.1%), palmitic acid (13.4%), stearic acid (2.5%), and linolenic acid (0.6%). The total content of saturated fatty acids (SFA) was 15.9%, while the content of unsaturated ones (UFA) was 84%. Palmitic acid was predominant among the SFAs. The results agreed with the report of Purohit *et al.* (2021) and Pereira *et al*., (2017).

Table 4. Fatty acid composition of combined UAE-Soxhlet extracted passion fruit seed oil

Parameters	Amount*
Palmitic acid (C16:0, %)	13.4
Stearic acid (C18:0, %)	2.5
Oleic acid (C18:1, %)	16.1
Linoleic acid (C18:2, %)	67.3
Linolenic acid (C18:3, %)	0.6
Saturated fatty acid (SFA, %)	15.9
Unsaturated fatty acid (UFA, %)	84.0
Monounsaturated fatty acid (MUFA, %)	16.1
Polyunsaturated fatty acid (PUFA, %)	67.9
COX value	7.2

* The values refer to a single determination using GC-MS instrument. The fatty acid content was calculated using GC-MS software.
COX: Calculated oxidizability value; UAE-Soxhlet: Combined ultrasonic-assisted extraction and Soxhlet extraction.

The COX value for UAESO was 7.2, which was calculated by considering the percentages of oleic, linoleic, and linolenic acids present in the PFSO. Higher unsaturated fats lead to an increase in the COX value in oils. UAESO was, therefore, stable and the process can be employed for the prevention of oxidative deterioration. Purohit *et al.* (2021) reported almost similar COX values (7.4) in PFSO. In comparison, the COX values for fresh palm oil, peanut oil, and camellia oil are 1.615, 4.631, and 1.772, respectively (Xu *et al*., 2015).

4. CONCLUSION

A new and efficient approach based on the dual extraction of passion fruit seed oil in ethanol using ultrasound and Soxhlet methods is proposed. Using optimized conditions of UAE extraction of PFSO, along with combined extraction of ultrasound and Soxhlet based techniques was used for the efficient extraction of oil from seeds. Study results indicated that combined ultrasound and Soxhlet procedures were more effective in extracting the oil from PFSO than Soxhlet (in terms of time consumption) and UAE (in terms of extraction yield). The developed phenomenological model for explaining the kinetics of the extraction process demonstrated that the combined techniques only strongly influenced the washing process. The generalization ability of this kinetics model should not be ignored in the future modelling of the extraction kinetics and the phenomenological model better explained the variation in oil yield than the simpler models and could be recommended for modelling extraction kinetics. The overall quality and oil yield was found better compared to Soxhlet extraction. Future work may explore the use of hexane as it may help to reduce the cost of industrial processing of ethanolic extract.

AUTHOR'S CONTRIBUTION

Hemanta Chutia: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Validation; Visualization; Roles/Writing - original draft;

Charu Lata Mahanta: Conceptualization; Resources; Supervision; Validation; Visualization; Writing - review & editing.

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Not applicable

DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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