Kinetics Modelling of Bioactive Compounds Extraction from Rambutan (Nephelium lappaceum L.) By-Products and their Antioxidant Activities

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Abstract

Rambutan (Nephelium lappaceum) and its by-products have great potential in the agricultural, pharmaceutical, and food industries due to its antimicrobial, antioxidant, anti-diabetic, and anticancer properties. This study aimed to obtain rambutan extract rich in phenolic compounds with possible antioxidant properties by using extraction parameters such as temperature, solvent, and liquid to solid ratio. Once total phenolic compounds (TPC) from rambutan peel and seed were extracted, antioxidant assays such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH•), 2, 2’-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS•), and ferric reducing antioxidant power (FRAP) were investigated and optimization was performed by means of Second-order, Power, Peleg and Page’s law models. The results show that rambutan extract possesses scavenging effects on DPPH• radical and ABTS• radical cation, as well as FRAP capacity. The antioxidant profile and phenolic content of rambutan extract was noted to be altered by temperature and extraction time with the highest extraction efficiency achieved after 120 min at 45 °C. Furthermore, Peleg model with R² > 0.89 was found to be the best model to describe the generated extraction data. The outcome of this study showed that the solid-liquid extraction could be an efficient extraction technique to obtain rambutan extract rich in phenolic compounds with high antioxidant properties.

Keywords

Rambutan, Extraction kinetics, Phenolic compounds, Antioxidant activities

Abbreviations

ABTS: 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid); DPPH: 1,1-Diphenyl-2-Picrylhydrazyl; FRAP: ferric reducing antioxidant power; GAE: Gallic acid equivalents; TPC: Total phenolic content

Introduction

Rambutan (Nephelium lappaceum L.) belongs to the Sapindaceae family which is widely distributed in Southeast Asia and Africa. In Cameroon, rambutan is mainly consumed as a fresh fruit, which results in a large amount of waste from the seeds and peels. As reported by Solís-Fuentes et al. [1], rambutan peels represent 45.7-64.7% of fresh fruit weight. However, rambutan peels and seeds...
have been reported as a rich source of antioxidant components, with several health attributes like antibacterial, antidiarrheal and it is also used as a remedy for dysentery [2, 3]. Recent studies have highlighted the presence of some valuable bioactive compounds in rambutan peels, such as geraniin, ellagic acid, rutin, quercetin and corilagin. In addition, the ethanolic extract of rambutan peel would contain ellagic acid, corilagin and geraniin [4]. The phenolic profile of rambutan peels revealed constituents that could be responsible of its antiradical and antioxidant properties [2, 5]. Furthermore, rambutan seeds have been studied for their phenolic content from which it is possible to find ellagic acid, corilagin, and geraniin same phenolic compounds that have been studied for their important health benefits [2, 4, 6]. Therefore, Rambutan extract could be used in food industries as sources of nutraceutical compounds. According to Rohman et al. [2] and Yongliang et al. [4] rambutan peel extract has a high content of bioactive constituents with significant bioactivities and non-toxic activity to cells in vitro at less than 125 µg.mL⁻¹.

Extraction is an essential unit operation in food processing that enables the isolation and characterization of bioactive compounds from natural sources [4, 5, 7]. Several researchers have evaluated the influence of various extraction parameters [7, 8] on the extraction of phenolic compounds from plants. However, to date, there is still no standardized method for the simultaneous extraction of all bio-compounds. Therefore, there is a need for systematic and extensive research on the extraction of dietary phenolic compounds from plants [8]. Moreover, there is a lack of information regarding the simulation and modeling of solid-liquid extraction of phenolic compounds from rambutan. Hence the need for a mathematical modeling of the extraction process. Mathematical models are useful tools used in food engineering enabling the simulation, design, optimization, and control of processes that contribute to efficient use of time, energy and raw materials.

Low cost rambutan by-products such as seed and peel, present a great antioxidant potential and an interesting profile of bioactive compounds of different categories which can be altered by extraction. It seems necessary to develop a fast, economical and green extraction method that guarantees high extraction yields of total phenols. Therefore, the current study aims to investigate the extraction of phenolic compounds from rambutan peels and seeds extract, and modelling the impact of extraction conditions on the profile of antioxidants from rambutan peel and seed extract.

**Material and Methods**

**Material**

Rambutan (*Nephelium lappaceum*) fruits of similar maturity and weight were purchased in the local market of Madagascar, Douala, Cameroon. The peels and seeds were obtained by manually removing the thick layer of peel and pulp from the fruit. Thereafter, they were carefully washed under running tap water to remove any impurities adhering to the surface of the peel. Then, the peels and the seeds were dried in an oven (60°C. for 24 h), ground using a grinder (CGOLDENWALL Electric Grain Mill Grinder) and sieved (500 µm). The obtained samples were kept in a sealed plastic bag and stored in a desiccator pending extraction experiments.

**Methods**

**Extraction of rambutan peel and seed**

Extracts from rambutan peel and seed were obtained by using 50% aqueous ethanol as a solvent and each extraction was performed in triplicate. In test tubes, 0.5 g of sample was mixed with 20 mL of solvent to obtain a solid–liquid ratio of 40 mL/g. The test tubes were then incubated in water bath at different temperatures (25°C, 35°C and 45°C) at different times. The extraction kinetics was conducted during 180 min. The extracts were separated from rough particles after extraction by centrifugation (Multifuga 3 L-R Heraeus, Kendro Laboratory Products) for 5 min at 15,000 g (gravity force). The supernatants were kept at 4°C until further analysis.

**Total phenols content**

Total phenol content (TPC) of peels and seeds was determined according to the Folin-Ciocalteau procedure [9]. Briefly, the supernatants (1 mL) were mixed with 1N Folin-Ciocalteau reagent (1 mL) and 10% sodium carbonate (1 mL). Afterwards, the mixtures were incubated for 1 h at 35°C, and the absorbance was measured at 530 nm (Spectrophotometer UV-1601, Shimadzu Co., Ltd.). The TPC was expressed as gallic acid equivalents in milligrams per gram of dry material.

**DPPH assay**

The DPPH assay was conducted as reported by Braca et al. [10]. Briefly, 4.5 mL of 0.2% alcoholic solution of 1, 1-diphenyl-2-picrylhydrazyl was added to 0.5 mL of different concentrations (250, 500, 1000, and 2000 µg/mL) of samples and standard solutions separately, to have final concentrations of 25–200 µg/mL. The samples were kept in darkness at room temperature for 30 min, then the absorbance was measured at 517 nm. The results were expressed in gram of trolox equivalents (TE) per 100 grams of dry material.

**ABTS assay**

The ABTS assay was conducted as reported by Re et al. [11] with slight modifications. In brief, a stock solution was prepared by mixing 7.0 mM of ABTS’ solution with 2.45 mM of potassium persulphate solution. The resulting solution was allowed to stand for 12 h at room temperature in darkness. Afterwards, the working solution was prepared by diluting the stock solution with 90% methanol to obtain an absorbance of 0.7 ± 0.02 units at 734 nm. Then, 2 mL of working solution was added in an aliquot of the extracts (20 µL) and hold for 5 min in darkness at room temperature. Subsequently, the absorbance was measured at 734 nm, and results were expressed in gram of trolox equivalents (TE) per 100 grams of dry material.

**FRAP assay**

The ferric reducing antioxidant power (FRAP) assay was conducted as reported by Thaipong et al. [12] with slight modifications. In brief, a solution mixture containing 100 µL of extract, 300 µL of distilled water, 3 mL of FRAP reagent made
(freshly prepared) up to 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of TPTZ (10 mM TPTZ in 40 mM HCl), and 2.5 mL of FeCl₃ solution (20 mM FeCl₃, 6H₂O in water) was kept for 5 min in darkness at room temperature. Then the absorbance was measured at 593 nm, and results were expressed in gram of Fe⁷⁺ per 100 grams of dry material, using ferrous sulphate (FeSO₄·7H₂O) as standard.

**Kinetic modelling for the extraction methods**

In the present study, four mathematical models were used to fit the experimental data of TPC, DPPH, ABTS⁺ and FRAP analyses and evaluate the liquid-solid extraction process. The Power-law, Peleg, Second order and Page’s models, were compared in order to determine the best fit the experimental data.

The Power-law model explains the extraction mechanism by the diffusion of solute through a non-swelling material (Equation 1)

\[ q = Kt^n \]  \hspace{1cm} (1)

Where, \( K \) is the rate constant, and \( n \) are the diffusional exponent of the model. When the extraction is made from plant materials, \( n < 1 \), [13].

Peleg’s model (equation 2) also known as hyperbolic model which results from the second order rate is widely applied in food engineering science.

\[ q = \frac{K_1t}{1 + K_2t} \]  \hspace{1cm} (2)

Where, \( K_1 \) is the extraction rate at the beginning (min⁻¹) and \( K_2 \) is the constant related to the maximum extraction yield (min⁻¹), [13].

The Page model (equation 3) also known as exponential model is generally employed in solid-liquid extraction.

\[ q = \exp\left(-Kt^n\right) \]  \hspace{1cm} (3)

Where, \( K \) is the rate constant, and \( n \) are the diffusional exponent of the model. When the extraction is made from plant materials, \( n < 1 \), [13].

The second-order model (equation 4) has been used over years to model solvent extraction of numerous substances from leaves, seeds, and nuts.

\[ q = \frac{t}{1 + \frac{t}{h + C_0}} \]  \hspace{1cm} (4)

Where, \( h \) is the initial extraction rate, \( C_0 \) is the extraction capacity, and \( K \) is the second-order extraction rate constant which can be determined experimentally [14].

**Statistical analysis**

All the experiments were performed in triplicate. The correlation between the content of total phenols and antioxidant activities was studied by means of Pearson correlation was carried out using OriginPro 2020 software (OriginLab, North-ampton, USA). The parameters of the models were computed using non-linear regression analysis (Revenberg–Marquardt iteration algorithm) by means of OriginPro 2020 software (OriginLab, Northampton, USA). The chi square (\( \chi^2 \)), adjusted R-square (\( AdjR^2 \)), root mean square error (RMSE), and standard error (SE) were determined to assess the concordance between the modeled value and the experimental data. All the treatments and assays were performed in triplicate.

**Results and Discussion**

**Total phenols content**

Phenolic compounds found in plants play a role in promoting health and preventing disease such as oxidative damage, cardiovascular diseases, diabetes, arthritis, inflammatory disorders and neurodegenerative diseases, which arise due to the unfavorable imbalance between pro-oxidant and antioxidant species in the body [2, 3, 15, 16]. Phenolic compounds from plants enjoy an ever-increasing importance because of their structural diversities and abundance in vegetables, and their consumption is beneficial for human health since they possess both nutritive and medicinal values [17]. Screening for bioactive molecules in herbal products is commonly done by evaluating the total content of phenolic compounds [4, 7].

As discussed elsewhere by Razali et al. [18], the Folin-Ciocalteu assay is a simple, rapid, and reliable standardized test method for determining the total phenol content of samples. Several phenolic compounds have been documented to have antioxidant properties [19]. Phenolic compounds manifest their antioxidant activity by several mechanisms such as the donation of hydrogen atoms to free radicals and trapping of other reactive species such as \( \text{OH}^•, \text{NO}_2^+, \text{N}_2\text{O}_5^+, \text{ONO}_2^+ \) and \( \text{HOCl} \). Certain phenolic compounds can react with \( \text{O}_2^• \) or by binding to transition metal ions, in particular iron and copper, often leading to less active forms favoring free radical reactions. Additionally, phenolic compounds can also interfere with the absorption of bivalent metals such as iron, calcium, and magnesium from the diet [20, 21]. Therefore, TPC can serve as an indicator of total antioxidant capacity [22].

Rambutan peel and seeds were extracted with 50% aqueous ethanol as solvent at different temperatures (25 °C, 35 °C and 45°C). A rapid increase in the TPC of the extracts was observed at the beginning of the extraction process, which then slowed down with a further increase in time (Figure 1). The extraction process can be commonly split into three stages, a first stage is characterized by a rapid release of the bio-components due to the occurrence of solubilization and separation phenomena in which the bio-compounds are removed from the outer surface of the matrix at noticeably fast speed [7, 23, 24]. Then comes the second stage which is characterized by an intermediate phase of transition to diffusion, where mass transfer resistance begins to appear in the solid-liquid interface, at which time mass transfer by diffusion and convection prevails. Then, in the last step, the solute overcomes the interactions binding it to the matrix so that it can diffuse into the extraction solvent. This stage is irreversible during the extraction process, it is generally considered as the frontier stage of the process, in which the extraction rate is low due to the elimination of the extract by the diffusion mechanism [25].
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Figure 1: Variation of TPC—Total phenolic content and DPPH—1,1-diphenyl-2-picrylhydrazyl during ethanolic extraction of rambutan peel and seed.

Figure 2: Variation of ABTS—2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) and FRAP—ferric reducing antioxidant power during ethanolic extraction of rambutan peel and seed.
The diffusion rate decreases as the extraction time increases due to the high concentration of solute in solution at the end. However, the outcomes showed that the impact of temperature (25 °C, 35 °C and 45 °C) in the process of extracting TPC from rambutan peel and seed extracted performed using 50% (Figure 1) be split in two stages. Two phenomena were verified, which are the processes that occur during the liquid-solid of rambutan peel and seeds during which maximum extraction takes place. The first stage corresponds to an intense dissolution of the matrix while the second stage consists in releasing the soluble molecules from the internal part of the matrix. This second step is slow due to the problems of transferring other molecules and modifying the solid structure. This step essentially corresponds to an external diffusion which concerns the rest of the soluble matter. In this study, the first stage occurring during the first 40 min is characterized by a swift increase in TPC which could be attributed to the increase in solubility and mass transfer rate with time while, further increase in extraction time resulted in slight phenolic extraction. As reported by Zhang et al. [26], increasing the extraction time has a positive effect on the extraction of phenolic compounds until solute equilibrium is reached inside and outside the matrix. Consistent with this statement, the TPC of rambutan peel increased rapidly to approximately 18 mg GAE/g DM as a function of time during the first 50 min for all temperatures, thereafter trending towards a constant value. The same observation was obtained for rambutan seed with an increase in TPC up to about 0.25 mg GAE/g DM. According to Fick’s law, at the beginning of an extraction process, a high concentration gradient between the solid phase and the liquid phase (solvent) leads to a high diffusion of the polyphenolic compounds in the solvent, as the extraction continues, the concentration gradient decreases; thus, increasing the extraction yield until the peak point is reached [27]. Furthermore, it was not a reduction in TPC after 160 min. This may be attributed to the thermal degradation of polyphenolic compounds. As stated by Narayana Namasivayam et al. [28], phenolic are mostly thermolabile compounds which could be destroy under oxidation and decomposition when exposed to high temperature.

**Antioxidant activity**

The antioxidant potential of rambutan peel and seeds was evaluated using assays such as DPPH, FRAP and ABTS. It was found that the antioxidant capacity of the extracts follows the extraction trend of TPC (Figures 1 and 2). Higher amount of antioxidants from rambutan peel and seed extracts were obtained at 45 °C compared to their counterparts at 25 °C and 35 °C. The results obtained show a considerable impact of temperature as an extraction parameter with respect to TPC and rambutan antioxidants (Figures 1 and 2). In line with previous research [29, 30], a strong significant Pearson relationship (Table 1) was found between TPC and antioxidant activities, suggesting that phenolic compounds from rambutan peel and seeds contribute significantly to their antioxidant activity.

The dynamics of antioxidant and TPC extraction were further analyzed using second-order, Peleg’s, Page’s and power’s law models. The ranking model app of Originpro which allow the comparison of multiple models based on four chi square ($\chi^2$, Adj-$R^2$, RMSE and SE) statistical measures was used to select the model which best fitted the extraction experimental data. From the results in Table 2 and 3, it was found that the most suitable model to describe the antioxidant components extraction release kinetics of rambutan was the Peleg model. This finding is consistent with that of Tusek et al. [31], in which the Peleg model was the best suited kinetic model for describing the solid-liquid extraction of bio compounds from plants of the family Asteraceae. As mentioned above, the Peleg’s model parameters $K_p$ represent the rate constant associated with the extraction rate at the beginning of the extraction process, while $K_s$ depicts the Peleg’s capacity constant associated with the maximum TPC content and antioxidants during the extraction process. For all plant parts, the same trend is noted, in fact the value of $K_p$ decreases with the increase in extraction temperatures, thus highlighting a high initial extraction rate. Therefore, the initial values of seed extraction rate were found to be higher than those of peel, for about 150 times for TPC, 27 times for DPPH, 341 times for ABTS and 150 times for FRAP, at 25 °C. Whereas at 45 °C, the initial extraction rate decreases up to 90 times for TPC, 14 times for DPPH, 120 times for ABTS and 130 times for FRAP. The positive impact of increasing the extraction temperature on the maximum content of extracted phenolic compounds and antioxidants represented by the parameter $K_s$ was also observed. This impact was particularly evident for the peel extract where the associated peak extraction yield was found to be approximately 1.2 times higher at 45 °C than at 25 °C.

**Conclusion**

The extraction kinetics of phenolic compounds and antioxidants from rambutan (*Nephelium lappaceum* L.) peel and seeds were influenced by the extraction temperature. Four kinetic models (empirical and semi-empirical) of solid-liquid extraction were evaluated using experimental data. All calculated models were found to be effective in modeling phenolic compounds and liberating antioxidants under the extraction conditions used, which was proven by a good high linear correlation coefficient (Adj-$R^2$ > 0.7). The Peleg model gave the best fit to describe the antioxidant components and the kinetics of total polyphenols. This model (with the highest Adj-$R^2$) fitted to experimental data slightly better than the best physical model (second-order model) based on diffusion.
### Table 2: Statistical tests of proposed models for total phenolic content and antioxidant capacity of the aqueous - ethanolic extraction of rambutan seed and peel.

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**TPC** - Total phenolic content, **DPPH** - 1,1-diphenyl-2-picrylhydrazyl, **ABTS** - 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), **FRAP** - ferric reducing antioxidant power, $\chi^2$ - chi square, $AdjR^2$ - adjusted R-square, RMSE - Root mean square error, SE - Standard error
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TPC - Total phenolic content, DPPH - 1,1-diphenyl - 2-picrylhydrazyl, ABTS - 2,2’-azino - bis - (3-ethylbenzothiazoline - 6-sulfonic acid), FRAP - ferric reducing antioxidant power, \( K \) - rate constant, \( n \) - diffusional exponent of the model, \( \ln \) - natural logarithm, \( R^2 \) - coefficient of determination, \( C_s \) - extraction capacity.

### Table 3: Kinetic parameters obtained from the aqueous - ethanolic extraction of total phenolic content and antioxidant capacity of rambutan seed and peel.

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### Table 3 continued

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theory. The highest extraction efficiency of antioxidant and total polyphenol content was obtained at the highest extraction temperature of 45 °C for both plant parts used. In this study, the extraction process was found to be more effective for seeds than the peels. Most of the polyphenols and antioxidants were released from the plant material to the solvent within the first 40 min of the extraction process. The outcomes of the present study can serve as a basis for the optimization and simulation of the kinetics of extraction of antioxidant components from the by-products of rambutan (Nephelium lappaceum).

Conflict of Interest

The authors declare that they have no conflict of interest, and are responsible of the content of the manuscript.

References
