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ULTRASOUND-ENZYME ASSISTED EXTRACTION OF POLYPHENOL AND AMYLASE ENZYME FROM VIETNAMESE RAMIE LEAVES: PREDICTIVE MODELING DEVELOPMENT AND OPTIMIZATION STUDY

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ABSTRACT

Ramie leaves are rich in antioxidant polyphenols and amylase enzyme, which could be a good source for food applications. This study investigated the impact of combined ultrasound-enzyme treatment (U-EAT), including ultrasound intensity (10-30%), content of Viscozyme L. enzyme addition (0.5-1%) and extraction time (5-15 min) on polyphenol recovery, antioxidant activity, and amylase activity. The result revealed that U-EAT could enhance the efficiency of extraction, with a significantly higher yield of bioactive compounds and antioxidant capacity, and amylase enzyme activity compared to an untreated sample. Multiple regression analysis was applied to predict the efficacy of extraction through the values of total phenolic content, antioxidant activity and enzyme activity. The established models shown high accuracy in predicting the effect of the extracted conditions on the yield of the extraction of antioxidant and enzyme amylase. It was found that the maximum yield of extraction could be obtained when the operation conditions were ultrasound intensity of 26.5%, enzyme concentration of 0.87% and extraction time of 10.88 min. Under these optimal conditions, the highest values were found (TPC of 99.047 mgGAE/100 g, antioxidant activity of 65.02% and amylase activity of 45.36 U/mL). In summary, the findings indicate that the utilization of ultrasound combined with the enzymatic extraction process of polyphenol and amylase enzyme from ramie leaves holds potential for enhancing both the extraction yield and bioactivity of the extract. Further study should be concerned with the process of producing food ingredients or products from this extract.

Keywords: sustainable technology, extraction, polyphenol, amylase, ramie leaves

INTRODUCTION

Ramie leaf (*Boehmeria nivea* L.) is a species of nettle in the Urticaceae family and grows widely in Southeast Asia **(Lee et al., 2009)**. In Vietnam, ramie leaves are grown in the Central region, especially in Binh Dinh. Ramie leaves contain a lot of vitamin C and chlorogenic acid - a strong antioxidant. Chlorogenic acid is 10 times stronger than vitamin E, so it has a very good disease prevention effect. In addition, ramie leaves also contain triterpenes, polyphenols, flavonoids and quercetin, ursolic acid **(Habibie et al., 2021)**. **Rehman et al. (2019)** reported that ramie leaves also contain anticancer, antiviral, antibacterial, hepatoprotective, glucose-lowering and antioxidant effects. Recently, β-amylase has been found in spiny leaves **(Niittylä et al., 2004)**. Taking advantage of the presence of enzymes available in ramie leaves to improve the structure and sensory value of bread is necessary. Fortification/substitution is one of the most common methods to achieve a product with the desired value while also supporting health promotion **(Świeca et al., 2017)**. The aforementioned procedure entails the incorporation of one or more functional constituents into certain food items, hence mitigating any potential deficiencies and/or bestowing supplementary advantages **(Dziki et al., 2014)**. The practice of incorporating additional or partially substituting ingredients is a prevalent method employed in the production of frequently consumed goods such as bread products, pasta, and juices. This approach not only enhances the efficiency of the manufacturing process but also enables these products to cater to a larger consumer base **(Dziki et al., 2014; Świeca et al., 2017)**. Furthermore, the implementation of targeted and market-driven fortification strategies enables the development of novel food products that frequently align with the characteristics of "functional foods". **(Dahiya et al., 2020; Siro et al., 2008; Świeca et al., 2017)**. However, in order for agricultural goods to be utilized as raw materials for the creation of high-value products, they must first be transformed into their proper form. Extraction is a commonly employed technique for the utilization of ramile leaves.

A recent study was conducted by **Martins et al. (2023)**, a number of ecologically sustainable solutions have been identified as effective in mitigating the adverse impacts of extraction conditions on the bioactivity of bioactive substances. Hence, in order to achieve more efficient extraction of important chemicals from plant processing, alternative technologies such as ultrasound-assisted extraction (UAE) and enzyme-assisted extraction (EAE) have been implemented. The efficiency of the UAE might be linked to the phenomenon of sonic cavitation, which results in enhanced mass transfer across cell membranes **(Nicolescu et al., 2022; Shen et al., 2023)**. In contrast, the EAE method relies on the enzymatic capacity of a specific enzyme to break down the cellular barrier and liberate the intracellular constituents **(Huynh et al., 2014)**. Enzyme or ultrasonic treatments have been extensively employed as environmentally friendly alternatives to industrial extraction processes, with the aim of enhancing yield, phenolic content, and antioxidant activity **(Le & Le, 2012; Łubek-Nguyen et al., 2022)**. Nevertheless, it should be noted that the enzymatic liquefaction process can be both time-consuming and energy-intensive, as highlighted by **Wang et al. (2019)**. Additionally, it has been reported that an extended ultrasonic treatment might lead to quality alterations and the destruction of phenolic compounds, as discussed by **Nicolescu et al. (2022)**. Recent research has investigated the potential synergistic impact of utilizing both ultrasound and enzymatic treatment as a viable approach to enhance the efficiency of the extraction and recovery of bioactive compounds. This combination of techniques offers advantages derived from both extraction methods, as demonstrated in recent studies conducted using these methods **(Liao et al., 2015; Nicolescu et al., 2022; Tchabo et al., 2015; Wang et al., 2019)**. Nevertheless, the utilization of this integrated approach has not yet been implemented in the extraction procedure of ramie leaves. Hence, this study was conducted to examine the efficacy of enzyme and ultrasound-aided extraction (U-EAT) in enhancing the extraction efficiency of polyphenols and amylase activity from ramie leaves. This work would not only yield practical implications for enhancing bioactive substances in ramie extract, but also for optimizing the activity of the amylase enzyme in various industrial processing applications.

MATERIAL AND METHODS

Materials

The collection of recently harvested ramie leaves took place at a farm located in Vietnam. The ramie leaf was subjected to freezing at a temperature of -60°C using a deep freezer immediately after being harvested, followed by the process of freeze-drying. The leaves were subjected to freeze-drying, followed by grinding and subsequent sieving using 100-mesh screens. The resulting material was then kept in a vacuum desiccator.

Chemicals

The chemicals used during the experiment were of analytical grade and were supplied by Merck Chemical Company, located in Darmstadt, Germany. For the analysis of antioxidant properties and enzyme activity, the following substances were used: Viscozyme L, a cellulolytic enzyme mixture containing arabinase, cellulase, β-glucanase, hemicellulase, and xylanase at a concentration of 100 FBGU/g; Folin-Ciocalteu reagent; 2,2-diphenyl-1-picrylhydrazyl (DPPH); maltose; 3,5-dinitrosalicylic acid (DNS); and methanol.

Ultrasound-enzyme asssited extraction

A mixture was prepared by combining 5 grams of powdered ramie leaves with water at a solid-to-water ratio of 1:10 (w/v). Viscozyme L was employed in solutions with concentrations of 0.5%, 1%, and 1.5%. The incubation process was conducted for a duration of 30 minutes at a temperature of 50°C using a water bath shaker operating at a speed of 120 rpm. Following a period of incubation, ultrasonic-assisted extraction (UAE) was conducted with an ultrasonic intensity ranging from 10% to 30% of the maximum power (300 W) for extraction durations spanning 5 to 15 minutes. Subsequently, the mixture underwent filtration using Whatman No. 1 filter paper, and the resulting filtrate was subjected to centrifugation at a speed of 4,000 g per minute for a duration of 10 minutes. The liquid portion was collected and thereafter stored at a temperature of 4°C for subsequent examination.

Determination of total phenolic content

The Folin-Ciocalteu test was employed to analyze the total phenolic content (TPC), as per the methodology outlined by **Wanyo et al. (2014)**. In this experiment, a volume of 300 µL of extract was subjected to a reaction with 2.25 mL of a Folin–Ciocalteu reagent solution with a concentration of 10%. The resulting mixture was then left to stand at room temperature for a duration of 5 minutes. Subsequently, 2.25 mL of a sodium carbonate solution with a concentration of 60 g/L was introduced into the mixture. The absorbance of the reaction was measured at a wavelength of 725 nm after being kept at room temperature for a duration of 90 minutes. The TPC of the extract was determined by utilizing the standard curve of gallic acid for calculating purposes.

Determination of antioxidant activity

A total volume of 20 µL of the extract was subjected to a reaction with 180 µL of a DPPH solution, which had a concentration of 0.1 mM and was dissolved in methanol at an 80% concentration. The absorbance of the combination was subsequently measured at a wavelength of 517 nm. The calculation of the inhibition percentage was performed by **Tabaraki and Nateghi (2011)**.

Determination of amylase enzyme activity

The measurement of α-amylase activity is conducted via a colorimetric technique, including the employment of 3,5-dinitrosalicylic acid (DNS) reagent **(Zin et al., 2022)**. In this experimental procedure, starch is enzymatically hydrolyzed by α-amylase, resulting in the conversion of starch into maltose. The quantification of maltose liberated from starch is accomplished through the utilization of the reduction reaction involving 3,5-dinitrosalicylic acid. Briefly, a volume of 0.5 milliliters of appropriately diluted crude enzyme, prepared in an acetic acid buffer solution with a pH of 4.9, is subjected to incubation for a duration of 15 minutes at a temperature of 40ºC. Simultaneously, a volume of 0.5 milliliters of a soluble starch solution with a concentration of 1% weight/volume is added to the mixture. Subsequently, the quantification of reducing sugars liberated from starch is conducted in accordance with the previously outlined methodology **(Zin et al., 2022)**. The unit of activity (U/mL) for the enzyme a-amylase is defined as the amount of enzyme necessary to produce 1 µmole of maltose within a 1-minute incubation period, when the enzyme and substrate are subjected to a pH of 4.9 and a temperature of 40°C.

Data analysis

The values were reported as the mean plus or minus the standard deviation (mean±SD). The parameters of the selected model were evaluated and the ideal value was measured using the statistical software Statgraphics Centurion XV.I (USA) through the application of multiple regression analysis. The major criteria utilized for selecting the optimal equation were the coefficient of determination (R^2), lack of fit test and the p-value of the model. These metrics were employed as the primary basis for determining and selecting the most suitable equation.

RESULTS AND DISCUSSION

Experimental design and results

To ultilize the antioxidant compounds and the functional properties of different sources of plants, the initial stage is investigation of these bioactive phenolic compounds, which involves their extraction conditions. Generally, the extraction of phenolic chemicals from plant materials has predominantly relied on conventional techniques such as heating, boiling, or refluxing. Nevertheless, a significant limitation of these methodologies lies in the potential degradation of phenolic compounds, their associated bioactivities, as well as some endogenous enzymes such as amylase enzyme. This degradation is mostly attributed to oxidation, ionization, and hydrolysis, which are induced by prolonged extraction durations and elevated extraction temperatures **(Odabaş & Koca, 2016)**. Within this particular context, various alternative innovative methods for extraction have been invented. Some of these emerging and "green" methods encompass ultrasonic assisted extraction (UAE) and enzymatic assisted extraction (EAE), as documented in **Shen et al. (2023)** and **Łubek-Nguyen et al. (2022)**. Ramie leaf was known to be high in antioxidants and amylase enzyme **(Le Loan et al., 2021)**. In this study, it could be seen from Table 1 that the combination of ultrasound and enzymatic methods affected the yield of antioxidant compounds, their activitiy and amylase activity of extract from ramie leaves. The control sample (without assisted by ultrasound and enzyme) had 45.34 mgGAE/g of TPC, 40.34% of DPPH inhibition, and 32 U/mL of amylase activity. While, the enhance of these values were found when applying ultrasound-enzyme assisted extraction. Table 1 shows that the total phenolic compound, percentage of DPPH inhibition, and enzyme activity ranged from 89.16-99.52 mgGAE/g, 54.23-65.40%, 34.20-45.63 U/mL, respectively. One of the extraction methods that is often regarded as mild, efficient, and environmentally friendly is EAE. It has been demonstrated to be successful in enhancing the production of desired chemicals **(Huynh et al., 2014)**. Nevertheless, it is commonly seen that EAE is correlated with extended extraction duration, hence potentially leading to an escalation in processing expenses **(Le & Le, 2012)**. The UAE has demonstrated itself as a viable option that offers speedy, efficient, straightforward, and cost-effective techniques. These techniques have the potential to enhance repeatability, increase extraction yields, improve the purity of the final product, and have a minimal impact on the bioactivities of the products **(Shen et al., 2023)**. Therefore, the combination of EAE and ultrasound irradiation has shown potential as a viable technique for extracting target chemicals. Several studies have shown favorable outcomes in the context of ultrasonic-assisted enzymatic extraction of these substances **(Liao et al., 2015; Odabaş & Koca, 2016; Tchabo et al., 2015)**.

Table 1 Effect of extraction conditions on total phenolic compounds, antioxidant activity and enzyme activity

X_1	X_2	X_3	TPC (mgGAE/100 g)	DPPH (%)	EA (U/mL)
10	5	0.5	89.16±0.52	54.23±0.72	34.49±0.22
20	5	0.5	90.25±0.11	56.35±0.11	36.56±0.19
30	5	0.5	90.34±0.29	56.66±0.28	36.68±0.12
10	10	0.5	94.11±0.52	58.45±0.01	35.59±0.23
20	10	0.5	96.51±0.20	60.28±9,16	38.53±0.06
30	10	0.5	96.27±0.06	60.42±0.23	38.81±0.13
10	15	0.5	95.27±0.40	59.45±0.21	37.68±0.22
20	15	0.5	95.30±0.66	60.31±0.17	40.41±0.06
30	15	0.5	96.32±0.13	60.65±0.09	41.57±0.11
10	5	0.75	91.23±0.58	54.34±0.22	36.66±0.12
20	5	0.75	96.01±0.59	62.55±0.26	38.53±0.06
30	5	0.75	97.05±0.52	62.60±0.13	39.60±0.06
10	10	0.75	94.30±0.22	58.29±0.71	40.34±0.11
20	10	0.75	97.64±0.23	65.40±0.46	45.42±0.23
30	10	0.75	99.52±0.07	65.27±0.56	45.63±0.27
10	15	0.75	94.35±0.29	61.86±0.43	40.45±0.01
20	15	0.75	97.91±0.49	63.29±0.63	45.48±0.26
30	15	0.75	97.97±0.45	63.23±0.57	45.59±0.31
10	5	1.0	92.05±0.53	56.65±0.27	34.20±0.64
20	5	1.0	95.59±0.22	62.85±0.62	38.51±0.07
30	5	1.0	96.38±0.06	62.83±0.65	38.42±0.06
10	10	1.0	95.24±0.52	61.50±0.06	40.35±0.10
20	10	1.0	97.26±0.72	63.42±0.23	44.34±0.20
30	10	1.0	97.49±1.11	63.53±0.07	44.12±0.51
10	15	1.0	95.05±0.42	61.53±0.12	40.66±0.08
20	15	1.0	96.49±0.28	62.42±0.05	43.12±0.59
30	15	1.0	97.19±0.55	62.57±0.01	42.98±0.57

X_1 is ultrasound power (%); X_2 is extraction time (min); X_3 is concentration of enzyme (%)

Model fitting

In addition, Table 2 shows an analysis of variance for the determination of optimization model fit and regression coefficient values of the experimental variables. The regression coefficients were determined using the least squares approach in order to construct second-order quadratic polynomial models. It shows all the responses analysed provided significant models with a coefficient of determination (R^2) above 90% indicating that the relationship between independence process variables and responses was significant. Good reliability and high degrees of precision in the conducted experiments were implemented by the low value of the coefficient of variance. Besides, significance was found when the P-value of the models was lower than 0.05. In addition, the lack-of-fit test was not significantly different (P-value > 0.05), which could confirm that the established models could be enough for predicting the extraction yield of antioxidant compounds, antioxidant activities and amylase activity of ramie leaf extract.

Table 2 Analysis of variance and regression coefficients of the predicted model for response variables

Model parameters	TPC (mgGAE/g)	DPPH (%)	Amylase activity (U/mL)
Intercept	59.288*	17.678*	-3.804*
Linear			
X_1	0.491*	0.964*	0.747*
X_2	2.537*	2.461*	1.780*
X_3	43.951*	49.778*	67.299*
Interaction			
X_1X_2	-0.008*	- 0.022*	0.003
X_1X_3	0.144*	0.122*	0.036
X_2X_3	-0.827*	- 0.599	0.247
Quadratic			
X_1^2	-0.009*	-0.016*	-0.016*
X_2^2	- 0.073*	- 0.064*	-0.077*
X_3^2	-22.863*	-26.295*	- 43.058*
R^2	88.39	87.56	92.21
Adjusted R^2	86.92	85.98	91.22
P-value	0.0000	0.0001	0.0000
P-value of Lack-of-fit	0.3703	0.1512	0.5054

** P-value < 0.05*

Multiple regression was employed to identify terms that were statistically significant ($p < 0.05$) and subsequently eliminate them from the model equation. The significant difference in the p-value of the model denotes the determination of the deviation in the output or response that can be pointed out by regression model equations **(Thuy et al., 2022b; Van Tai et al., 2021)**. The final regression model is presented in Table 3.

Table 3 The suggested models betweeen response versus extraction variables

Response	Regression model
Total phenolic content (mgGAE/100 g)	$TPC = 59.288 + 0.491X_1 + 2.537X_2 + 43.951X_3 – 0.009X_1^2 – 0.008X_1X_2 + 0.144X_1X_3 – 0.073X_2^2 – 0.827X_2X_3 – 22.863X_3^2$
Antioxidant activity	$DPPH = 17.678 + 0.964X_1 + 2.461X_2 + 49.778X_3 – 0.016X_1^2 – 0.022X_1X_2 + 0.122X_1X_3 – 0.064X_2^2 – 26.295X_3^2$
Enzyme amylase activity (U/mL)	$EA = -3.804 + 0.747X_1 + 1.780X_2 + 67.299X_3 – 0.016X_1^2 – 0.077X_2^2 – 43.058X_3^2$

X_1 is ultrasound power (%); X_2 is extraction time (min); X_3 is concentration of enzyme (%)

The figures presented in this study depict 3D response surface plots that illustrate the correlation between extraction parameters, specifically ultrasound intensity, enzyme concentration and extraction time, and the corresponding responses: TPC, antoxidant activity, and enzyme activity (Figures 1-3, respectively). The data presented in the figures illustrates a positive correlation between ultrasound power and the levels of total phenolic compounds, DPPH values, and amylase enzyme activities. Specifically, it is observed that as ultrasonic power increases, there is a corresponding increase in these parameters. Furthermore, it is noteworthy that the peak values for these parameters are achieved at an ultrasound intensity ranging from 18% to 22%. Following this particular number, there was a tendency observed towards stability or a tiny drop in value. Ultrasound intensity induces various material alterations through the generation of acoustical cavitation, which manifests as the disruption of physical integrity or acceleration of specific chemical reactions **(Gadioli Tarone et al., 2021)**. The generation of cavitation microbubbles occurs as ultrasonic waves propagate, leading to subsequent implosions that result in the release of significant energy. This energy release induces the production of microjets, which are directed towards the solid surface and can attain high velocities, hence boosting the occurrence of shear stress. The aforementioned forces possess sufficient strength to induce the breakdown of cell walls, hence enhancing the penetration of the solvent and facilitating the rapid exudation of intracellular components. Consequently, this process intensifies the transfer of analyte mass from the solid phase to the liquid phase **(Gadioli Tarone et al., 2021; Thuy et al., 2022a)**. The present study demonstrates that the extraction of organic compounds from plant material is notably enhanced through the utilization of ultrasound, with a positive correlation shown between ultrasonic intensity and extraction efficiency. Increasing the sonication power can result in a more intense turbulent flow, leading to enhanced mass transfer and ultimately yielding a higher extraction efficiency **(Yao et al., 2023)**. However, a higher intensity of sonication could = discruption the functional compounds **(Hussain et al., 2023)**.

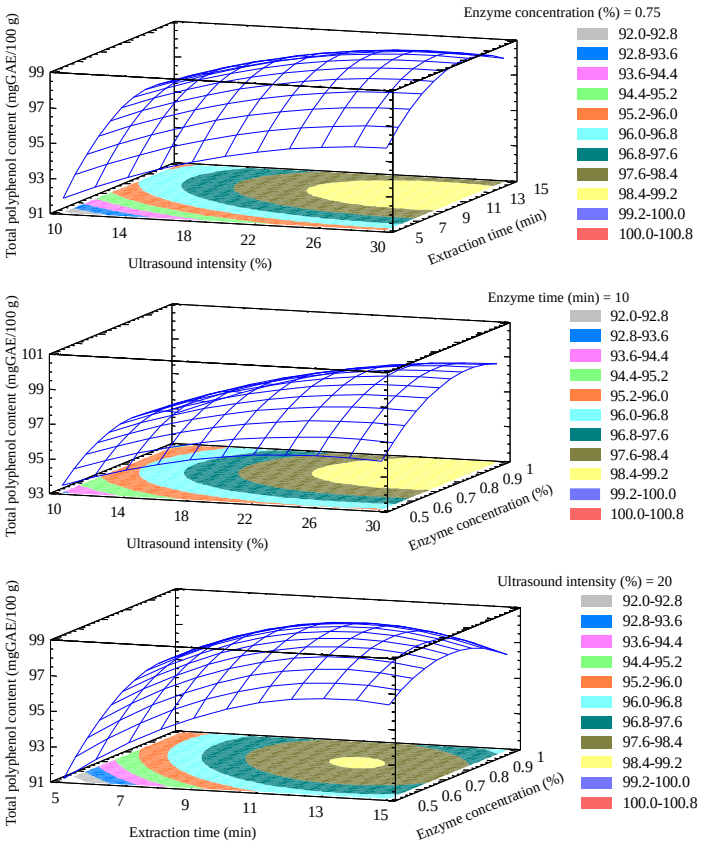


Figure 1 Response surface plots for effect of extraction conditions on total polyphenol content of extract

The concentration of the enzyme used also had a main effect on the recovery of compounds from ramie leaves.The enhance of enzyme concentration could increase the yield of extraction. The peak region of extraction yield was found at approximately 0.6-0.8% of enzyme. Enzymes exhibit a high degree of specificity under suitable environmental circumstances. Enzymatic reactions exhibit optimal efficiency when conducted under conditions characterized by lower temperatures, moderate pH levels, and shorter durations (often within a few hours). Moreover, these reactions do not necessitate the use of costly equipment. The extended duration and favorable environmental conditions facilitate the reduction of degradation or isomerization of active compounds **(Nicolescu et al., 2022)**. Regardless of the specific extraction technique employed, there are inherent mechanical obstacles that impede the extraction process. The aforementioned structures pertain to cellular components that must be surmounted in order to extract the desired metabolites. The presence of many constituents in the cell wall, such as lignins, celluloses, and certain proteins, contributes to cellular strength. However, these components can present challenges when attempting to extract bioactive compounds. Certain active metabolites can be found in the cytoplasm, while others are kept in vacuoles or plastids. Additionally, some active metabolites are bound inside a polysaccharide-lignin network by hydrogen bonds, which restricts their availability for the EAE method in all instances facilitates the reduction of the resistance exhibited by natural substances. The EAE methodology relies on the utilization of enzymes that facilitate the hydrolysis of covalent bonds in the presence of water. The process under consideration leads to the breakdown of cellular structures and enhances the material's permeability. As above-mentioned, the combination the aid of ultrasound and enzyme could greatly enhance the extraction yield of the sample **(Tchabo et al., 2015)**.

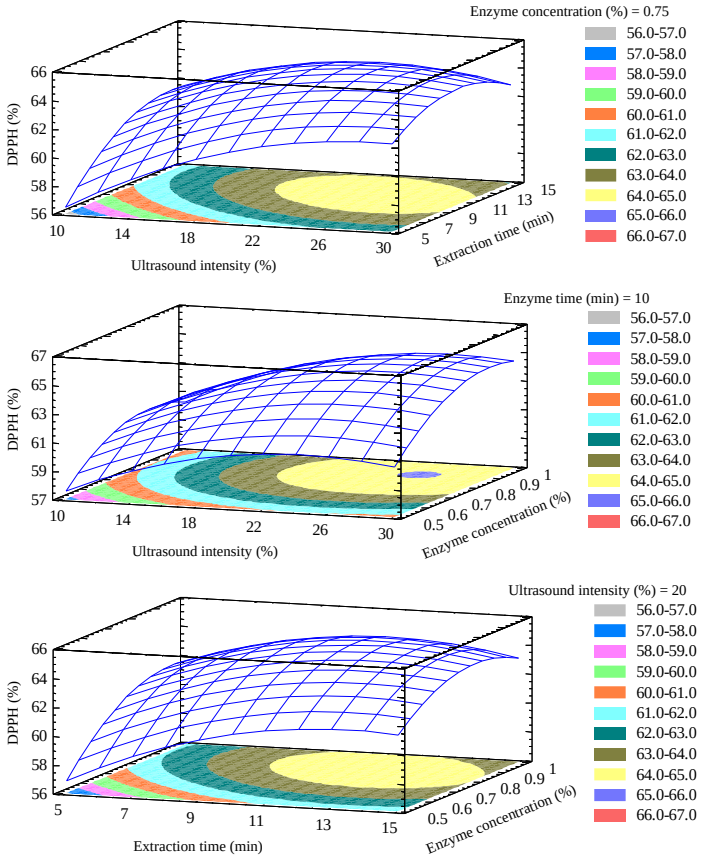
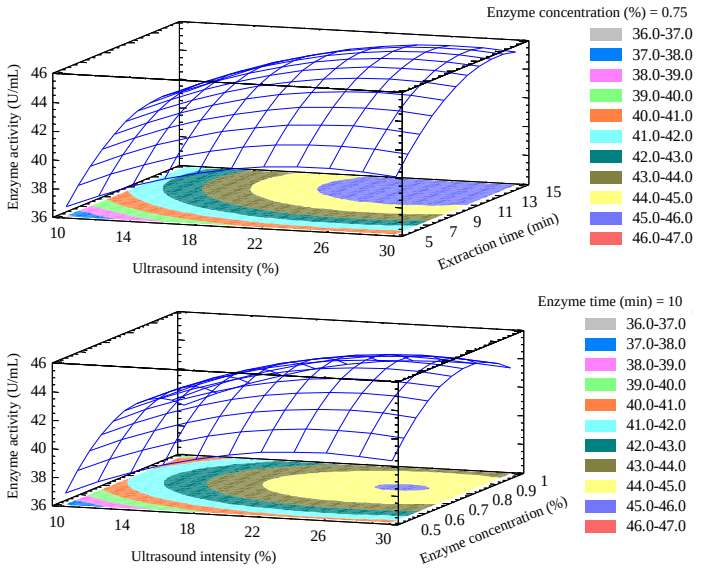


Figure 2. Response surface plots for effect of extraction conditions on antioxidant activity of the extract

The duration of the extraction process is a critical factor that has the potential to impact the efficiency of the extraction. Several investigations have provided evidence that an extended duration of extraction has a positive impact on the synthesis of phenolic compounds **(Heleno et al., 2016)**. Nevertheless, prolonged extraction time can potentially lead to alterations in the molecular structure and bioactivities of phenolic compounds due to processes such as hydrolysis or oxidation **(Ghafoor et al., 2009)**. The objective of this study was to examine the impact of varied ultrasonic-enzymatic treatment durations on the extraction of total phenolic compounds (TPC), antioxidant activity, and amylase activity. Specifically, ultrasonic periods ranging from 5 to 15 minutes were explored. As depicted in the figures, there was a clear and noticeable rise in the extraction yield as the duration of the extraction process rose from 5 to 15 minutes. The region of maximum yield was achieved when the extraction period exceeded 11 minutes. Subsequently, any additional extension of the extraction time resulted in either a sustained yield or a minor drop in yield. The observed phenomena could perhaps be attributed to the degradation of the extracted chemicals, which may occur as a result of overly prolonged extraction time **(Xu et al., 2016)**.



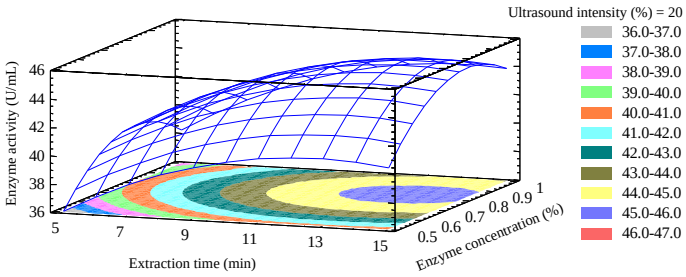


Figure 3 Response surface plots for effect of extraction conditions on amylase activity of the extract

The optimum U-EATE condition of ramie leaves must be selected to obtain maximum phenolic, activity of antioxidants and amylase enzyme. These optimized conditions were ultrasound intensity of 26.5%, enzyme concentration of 0.87% and extraction time of 10.88 min. Under these optimal conditions, the highest value TPC was 99.047 mgGAE/100 g, antioxidant activity was 65.02% inhibition of DPPH and amylase activity was 45.36 U/mL (Figure 4). Moreover, overall desirability was reached at value of 0.97.

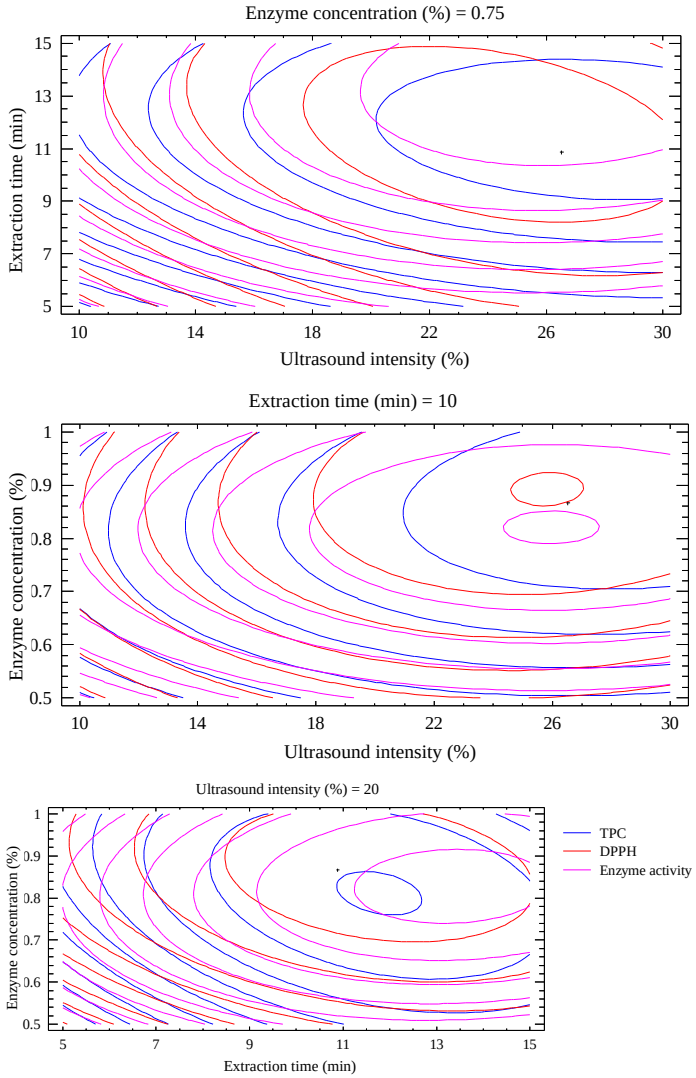


Figure 4 Contour plots for selecting optimal extraction conditions based on multi-objective optimization approach

Verification Experiments

Based on the established mathematical models and in order to validate the appropriateness of the model equation for predicting the optimal responses, three experiments were conducted under optimized conditions with minor adjustments made to account for the challenges encountered during the actual extraction experiments. These adjustments included an ultrasound intensity of 26%, an enzyme concentration of 0.87%, and an extraction time of 10.88 minutes. The testing results yielded the following values: total phenolic content (TPC) was determined to be 102.047±1.34 mgGAE/100 g, antioxidant activity exhibited a value of 64.02 ± 0.34% inhibition of DPPH, and amylase activity was measured at

42.36±1.34 U/mL. The anticipated outcomes demonstrated a strong agreement with the experimental data acquired under optimal extraction conditions. This agreement serves to validate the response surface technique model, as evidenced by a high correlation coefficient ($R^2 > 0.95$). Consequently, the models were deemed to possess a high degree of accuracy and reliability in forecasting the phytochemical components and enzyme characteristics of ramie leaf extract, particularly for the U-EAT. This finding serves to validate the suitability of the response model for effectively representing the anticipated optimization.

CONCLUSION

The effect of ultrasound-enzyme assisted treatment on the extraction efficiency of polyphenol and amylase enzyme from ramie was evaluated. It could be seen that the extraction efficiency was higher than the non-treated sample. Regression analysis showed the potential to predict the value of total phenolic content, DPPH and enzyme activity on the extract effect by extraction conditions. The accuracy and reliability of the models in predicting the phytochemical components and enzyme characteristics of ramie leaves extract were deemed essential for the U-EAT. This assessment indicated that the response model adequately reflected the predicted optimization. The optimum parameters for this experiment include an ultrasonic intensity of 26.5%, enzyme concentration of 0.87%, and an extraction time of 10.88 minutes. The results obtained under the ideal conditions indicated that the greatest values for total phenolic content (TPC) were 99.047 mgGAE/100 g, antioxidant activity showed 65.02% inhibition of DPPH, and amylase activity was measured at 45.36 U/mL. This extract could be potential used as the functional ingredients for developing the nutraceutical foods, especially in bakery industry.

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