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Ultrasound-Assisted Extraction of *L*-Tryptophan from Chamomile Flower: Method Development and Application for Flower Parts Characterization and Varietal Difference

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Abstract

Chamomile has been widely used as a functional tea due to its effects on several neurohormones, some of which are related to tryptophan. An analytical ultrasound-assisted extraction (UAE) technique was successfully optimized and validated to determine tryptophan levels in chamomile flowers. A Box-Behnken design, in conjunction with response surface methodology, was conducted based on 3 factors and 3 levels: Temperature (x_1; 30, 50, and 70 °C), solvent composition (x_2; 0, 40, and 80% methanol in water), and ultrasonic power (x_3; 20, 60, and 100%). The main (x_2) and quadratic effect of solvent (x_2x_2); quadratic of temperature (x_1x_1) and ultrasonic power (x_3x_3); also, the interaction of solvent-ultrasonic power (x_2x_3) and temperature-ultrasonic power (x_1x_3), significantly affected ($p < 0.05$) the level of *L*-tryptophan in the extracts. Optimum extraction conditions were achieved by applying a temperature of 54 °C for 15 min using 17% methanol in water and 69% ultrasonic power. A high recovery (94.26%) was achieved with 2 extraction cycles. The coefficient of variation (CV) demonstrated that the developed method had a satisfactory level of precision (CV < 5%) for repeatability and intermediate precision. To check the applicability of the method, the parts of 2 types of chamomile flowers (German and Roman) were evaluated. This method was successfully applied to characterize chamomile flowers based on *L*-tryptophan levels.

Keywords: Tryptophan, Optimization, Edible flower, Box-Behnken design, Ray florets, Disc florets

Introduction

Tryptophan is an essential amino acid that cannot be synthesized by the human body and the level is the lowest compared with other amino acids. Hence, the compound must be supplied from the diet. Only the *L* isomer is used in protein synthesis and passes through the blood-brain barrier, restoring the usefulness of tryptophan [1]. In humans and animals, tryptophan acts as a precursor for several important neurohormones, including serotonin, kynurenine [2], tryptamine [3], and melatonin [4]. While in plants, tryptophan is essential for growth by acting as a precursor to auxin [5].

Dietary tryptophan and its derivatives provide activities in preventing and curing several diseases, such as inflammatory bowel disease [6], microbial infections [7], Alzheimer's, and Parkinson's [8]. The compounds are present in a wide range of natural sources, including edible flowers. Chamomile (*Matricaria chamomilla* L.) tea, in combination with saffron, can elevate the availability of tryptophan in the brain by decreasing the plasma concentration of tryptophan, which later induces the formation of serotonin [9]. Moreover, consuming chamomile extract (200 mg) can improve sleep quality compared to those who consumed a placebo ($p < 0.05$) in older adults (n = 60) [10]. This sleep-awake system in the human body is controlled by melatonin, which is transformed from tryptophan [11]. Due to these health benefits, the determination of tryptophan in chamomile flowers is necessary to provide a complete database for further utilization.

Several studies have reported the analytical procedure to extract the tryptophan compound from the edible flower matrix using conventional methods. Parraga *et al.* [12] conducted acid hydrolysis and amino acid derivatization to detect tryptophan from several parts of stove-dried *Erythrina edulis* . Whereas, Sayed *et al.* [13] used solid-liquid maceration followed by derivatization of sun-dried *Celosia eristanta* Linn. A reflux of 2 h extraction procedure for tryptophan from chamomile capitulum was proposed by Qureshi

et al. [14]. These conventional methods provide several drawbacks as require massive amounts of solvents and are time-consuming. Therefore, applying green chemistry principles, ultrasound-assisted extraction (UAE) technology appears as an option to improve the extraction efficiency. This approach has been successfully used for the extraction of flavonoids from chamomile flowers using a continuous counter-current ultrasound wave [15].

In spite of using a lower solvent consumption, a higher recovery can be achieved by UAE due to the employment of ultrasonic waves that rupture the sample cell wall so the analyte can be easily extracted into the solvent. Several disclosures have compared the benefits of UAE and conventional extraction method in recovering bioactive compounds. The level of total phenolic compounds in the extract recovered by UAE was significantly higher (15.27 %) than by a shaking water bath. Moreover, the result from CUPRAC and ABTS assays showed that antioxidant capacity of the extract obtained from UAE was significantly higher compared to those using conventional method. The UAE method also performed faster than the conventional method. The extraction of the phenolic compounds from grape pomace using UAE allowed a reduction in energy cost since it was 8 times faster compared to maceration at a temperature of 50 °C [16]. Melatonin, a compound derived from tryptophan, was extracted from rice grain in 10 min using UAE [17], whilst conventional extraction methods required over than 30 min to reach similar recoveries. UAE also successfully applied in the extraction of tryptophan from rice grains matrix [18].

The efficiency of the UAE method is influenced by a matrix of the sample and the extraction condition. Variables related to ultrasound setting includes ultrasound power, frequency, pulse duty of cycle, and extraction temperature. Meanwhile, other variables include extraction solvent, pH, solid-to-solvent, and ratio are depended on the characteristic of the targeted analyte, such as solubility and stability [19,20]. Hence, a simultaneous optimization of the aforementioned extraction variables is necessary to achieve the complete recovery.

In this study, a Box-Behnken design (BBD) was applied to assist the optimization of the extraction variables because this approach has been proven suitable for UAE optimization [17,18,21]. The BBD is compatible with the response surface methodology (RSM) because it enables the estimation of the quadratic model's parameters, sequential design construction, discovery of model lack of fit, and prediction of the ideal extraction condition [22]. The aim of this study was to optimize the UAE method for extracting tryptophan from chamomile flowers using BBD in conjunction with RSM.

Moreover, a database that includes the level of tryptophan from several parts of the chamomile flowers is unavailable. The previous study only reported the level of tryptophan from the ray and disc florets of sunflower (*Helianthus annuus* L.) [23]. The evaluation of tryptophan from ray and disc florets of chamomile is essential to determine the levels of this compound for further utilization. Hence, the optimized extraction method proposed in this study could be further applied to quantify the tryptophan level in different parts of chamomile flowers and can be considered for choosing the raw material for developing a functional drink based on chamomile flowers.

Materials and methods

Plant material

Two varieties of chamomiles were studied: Roman (*Chamaemelum nobile* L.) and German (*Matricaria recutita* L.). Fresh chamomile samples were purchased from a local farmer in West Dieng, Banjarnegara, Central Java, Indonesia. The flowers were harvested during the blooming stage after 3 months of growth. Immediately after harvesting, the flowers were lyophilized (Labcoco FreeZone series, Benchtop, MO, USA) at –45 °C and 0.099 bar for 24 h until dry then ground using a grinder (KLAZ, Guangdong, China) for 30 and 30 s off until the particles passed through a 40-mesh sieve. For method development, the sample was a mixture of capitulum of the Roman and German chamomile (1:1), whereas for the real sample application, the ray and disc florets were studied separately. The parts of the chamomile flowers are presented in **Figure 1**. The ground sample was then stored in an airtight container at –24 °C until further analysis.

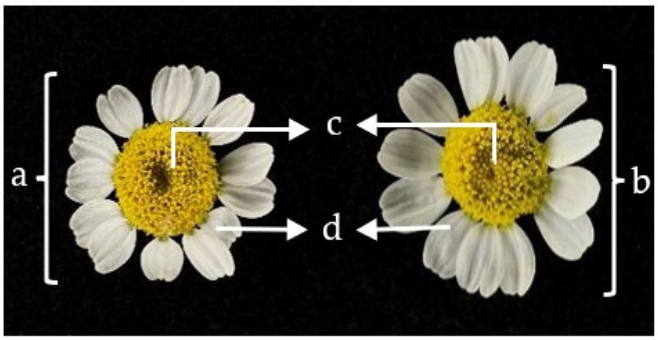


Figure 1 German (a) and Roman (b) chamomile capitulum, disc florets (c), and ray florets (d).

Chemical and reagents

Standard compounds of *L*-tryptophan were purchased from Sigma Aldrich (MO, USA) with the highest purity (> 98%). Methanol, acetic acid (Merck KGaA; Darmstadt, Germany), and water (PT. Ikapharmindo Putramas, East Jakarta, Indonesia) were analytical and HPLC grade.

Overview of experimental analysis and design

In order to develop and validate a new UAE method for extracting tryptophan from chamomile flowers, some stages of studies have been conducted (**Figure 2**).

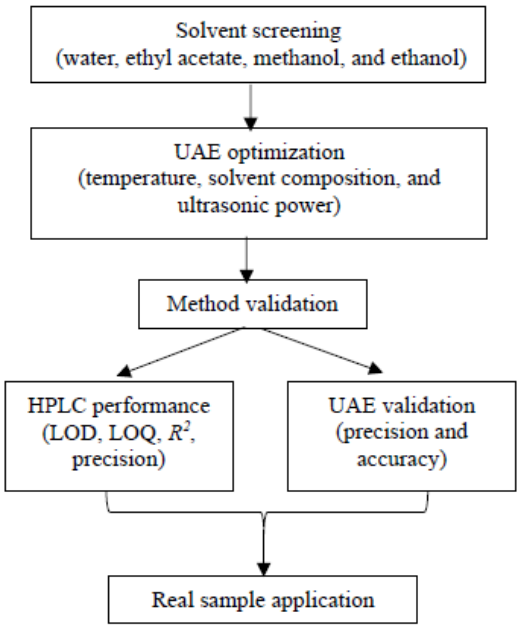


Figure 2 Experimental stages in developing an ultrasound-assisted extraction method.

Ultrasound-assisted extraction (UAE)

The lyophilized flowers were extracted using an UP200St ultrasonic system with a probe diameter of 7 mm, frequency of 26 kHz and power of 200 W (Hielscher Ultrasonics GmbH, Teltow, Germany). Temperature was controlled using a water bath (J.P. Selecta, Barcelona, Spain). The extraction conditions for the temperature, solvent composition, and ultrasonic power were set based on the experimental design. The solid-to-solvent ratio was fixed at 1:10 and the pulse duty of the cycle at 1 s^{-1}, as previously reported, did not affect amino acid extraction by UAE [24]. After extraction, the pellet and extract were separated using a centrifuge (Sorval ST-8R, Thermo Fisher Scientific, Langenselbold, Germany) at 4,500 rpm for 15 min. The volume of the extract was adjusted to 10 mL using a fresh solvent and stored in closed vials at 4 °C until chromatographic analysis.

Determination of *L*-tryptophan using HPLC-DAD

The separation of the compounds was elucidated using a C18 reverse-phase column (5 µm, 4.6×150 mm^2, Shimadzu Corp., Kyoto, Japan) at 30 °C using high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) (Shimadzu Corp., Kyoto, Japan) system. The mobile phase consisted of a binary phase, phase A (2% acetic acid and 5% methanol in water) and phase B (2% acetic acid and 88% methanol in water). The gradient of elution (time, phase B): 0 min, 20%; 15 min, 100%; 18 min, 100%. Before injection, the sample was filtered using a 0.45 µm nylon filter. The injection volume used was 20 µL. The chromatograms were processed using LabSolutions CS (Shimadzu Corp., Kyoto, Japan). *L*-tryptophan was identified by comparing the retention time and the UV full spectra (190 - 400 nm) of the sample peak with the tryptophan standard (**Figure 3**). Additionally, identification was confirmed using a spiking procedure. A specific wavelength was chosen based on the maximum absorbance of the *L*-tryptophan standard (280 nm) for the quantification [25,26].

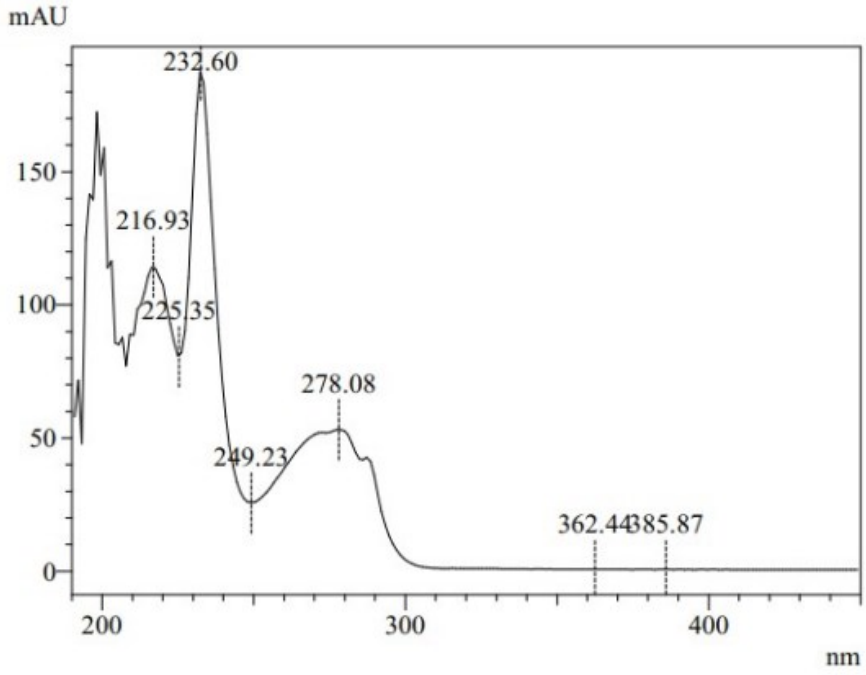


Figure 3 UV absorption of tryptophan standard.

Experimental design and statistical analysis

Solvent screening (methanol, ethanol, water, and ethyl acetate) and a literature review were conducted prior to the optimization of temperature (x_1), solvent composition (x_2), and ultrasonic power (x_3). The effect of UAE variables on the level of extracted *L*-tryptophan was evaluated simultaneously using a Box-Behnken design (BBD). The BBD with 3 levels of variables (−1, 0, and 1) employed 15 randomly run experiments with 3 repetitions of the central point (**Table 1**), and the actual values of the coded independent variables are shown in **Table 2**.

Table 1 Independent variable coding levels and real values for Box-Behnken design.

Independent variables	Symbols	Coded levels		
		−1	**0**	**1**
Temperature (°C)	x_1	30	50	70
Solvent composition (% methanol in water)	x_2	0	40	80
Ultrasound power (%)	x_3	20	60	100

Table 2 Matrix of Box-Behnken design with normalized observed response and the prediction error.

Run	Variables			Responses* (%)		Error (%)
	x_1	x_2	x_3	Observed	Predicted	
1	1	1	0	63.65	63.27	0.60
2	0	1	1	55.51	54.16	2.43
3	−1	0	−1	80.19	78.45	2.17
4	0	0	0	97.43	96.39	1.07
5	0	1	−1	61.93	65.11	5.13
6	−1	−1	0	89.55	89.94	0.44
7	1	0	−1	94.78	91.98	2.95
8	1	0	1	81.75	83.49	2.13
9	0	−1	1	100.00	96.82	3.18
10	−1	1	0	63.08	61.64	2.28
11	0	0	0	97.14	96.39	0.77
12	−1	0	1	86.47	89.27	3.24
13	0	−1	−1	82.19	83.54	1.64
14	0	0	0	94.61	96.39	1.88
15	1	−1	0	94.61	96.06	1.53

*The relative value to the highest chromatographic response (%) of the *L*-tryptophan in the samples.

The terms of main, interaction, and quadratic effects were then included the response surface methodology to obtain Eq. (1);

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_1^2 + \sum_{i=1}^{k} \sum_{j=1, j \neq i}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where x_1, x_2, …, x_k is the UAE factor affecting the efficiency extraction process, *y*; β_0, β_{ii} (*i* = 1, 2, …, *k*), β_{ij} (*i* = 1, 2, …,*k*; *j* = 1, 2, 3, …,*k*) are unknown parameters; *k* is the number of studied independent factors, and ε represents random error.

Method validation

Before detecting and quantifying the level of *L*-tryptophan in the extract, the performance of HPLC was evaluated. It covered linearity, limit of detection (LOD), limit of quantification (LOQ), and precision. The optimized analytical method was then validated based on the ICH Guideline Q2 (R1) [27], which covered accuracy and precision. The precision was elucidated at 2 levels, repeatability and intermediate precision, which were expressed as coefficients of variation (CV, %). For intermediate precision, 3 replicates were conducted within 3 consecutive days (n = 3×3). Repeatability was evaluated using 9 replicates within the same day (n = 9). According to the AOAC manual, the accepted CV maximum for the Peer-Verified Method program is ± 10% [28]. For the accuracy, the recovery (R, %) of tryptophan extracted by multi-cycle extraction was measured. At the optimum UAE conditions, extraction was performed for up to 7 cycles, with each cycle using a fresh solvent. The level of *L*-tryptophan in the extract resulting from each extraction cycle was then measured.

Real sample application

To ensure the method applicability, the optimized UAE method was applied to extract *L*-tryptophan from the most consumed chamomile flowers: Roman and German varieties. Ray and disc florets were separated to check the distribution of L-tryptophan in the flowers.

Data analysis

The construction and analysis of the experimental design, response surface, and desirability functions to achieve optimum conditions were performed using Statgraphics Centurion XIX (Statpoint Technologies Inc., Warrenton, VA, USA).

Results and discussion

Solvent screening

Previously, a screening study was conducted to evaluate the suitability of different solvents to extract *L*-tryptophan compound. Four solvents (water, ethyl acetate, methanol, and ethanol) were used in triplicate for the extraction at 40 °C with a pulse duty cycle of 0.5 s^{-1} and a solvent-to-sample ratio of 20:1. The results revealed that *L*-tryptophan was only detected in the extract sample which utilized the most polar solvents, i.e. water and methanol. Whereas the highest concentration was obtained in the extraction using water (11.48 ± 0.28 µg g^{-1}). Although methanol provided low concentration of extracted *L*-tryptophan (3.02 ± 0.41 µg g^{-1}), mixtures of methanol in water should be evaluated. This is in agreement with a previous finding by Diraz-Yildirim *et al.* (2021), who succeeded in extracting tryptophan from leaves and flowers of several *Tanacetum taxa* plants using a mixture of methanol/water. However, a suitable mixture percentage of the solvent required further optimization.

The sequence from the highest to the lowest solubility for tryptophan in the 4 studied solvents was water, methanol, ethanol, and ethyl acetate. Because *L*-tryptophan has a pair of unshared electrons on the nitrogen atom, it has an affinity for basic protons as well as water. Ethanol and ethyl acetate are less soluble in *L*-tryptophan compared to water and methanol [30]. The result disclosed that no *L*-tryptophan was detected in the ethanol or ethyl acetate extracts (below the LOD).

Effects of UAE variables

By using a 3-level Box-Behnken design with triplicate central points, the factors influencing UAE in extracting *L*-tryptophan from chamomile were assessed. The effects of temperature (x_1), solvent composition (x_2), and ultrasonic power (x_3) were calculated according to the analysis of variance (ANOVA). Meanwhile, response surface analysis was used to optimize the UAE conditions. It showed the main, quadratic, and interaction effects between these factors on the level of *L*-tryptophan in the extract. The obtained effect is shown in the Pareto chart in **Figure 4**. A bar crossing the vertical line corresponded to factors that have a significant effect on the response ($p < 0.05$). Hence, the sole influencing main factor in this research was the solvent composition (x_2) for extracting *L*-tryptophan from chamomile flowers, whilst temperature (x_1) and ultrasonic power (x_3) provided no effect on the extraction yields.

The solvent composition showed a negative effect, indicating a decrease in the percentage of methanol in the solvent provided a higher level of tryptophan in the extract. The same effect was observed for the quadratic of solvent composition (x_2x_2). *L*-tryptophan is categorized as a nonpolar amino acid. However, according to its chemical structure, *L*-tryptophan has 2 unshared lone pairs of electrons in the nitrogen atom of the amino group, which allows *L*-tryptophan to act as a Lewis acid to create a hydrogen bond with a proton solvent, such as water, because the amino group has an affinity for a basic proton. This interaction also occurs with methanol; however, it is stronger with the water molecules. Moreover, compared to other solvents, water has a smaller structure but a higher in dielectric constant (78.514 at 298.15 K), thus endorsing the solubility of *L*-tryptophan during extraction.

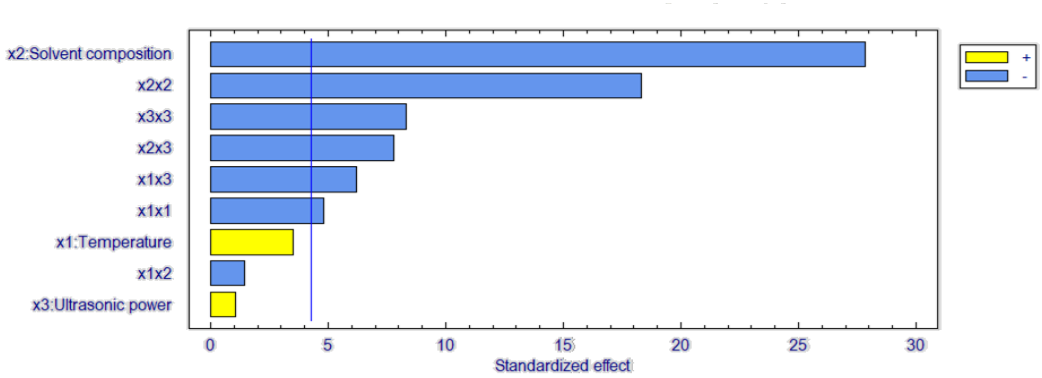


Figure 4 Pareto chart for standardized effects. The vertical line across the bars indicates that the corresponding factor significantly influences the response at the 95 % confidence level (shows positive effects and shows negative effects.

Additionally, a mixture of water and methanol can cause plant cells to dry out and collapse, as well as denature the protein in the cell wall, which makes the extraction process more effective. By using hydro-

alcoholic mixtures, a synergetic effect between the solvents can be achieved. Water acts as a swelling agent of the plant matrix, increasing the contact surface, and besides, alcoholic solvent induces the rupture of the bond between the solutes and the matrix [31]. However, due to the cavity, dipole-dipole, and dispersion interactions, a mixed solvent of water and methanol can reduce the solubility of *L*-tryptophan by increasing the methanol content [30]. A previous study showed that *L*-tryptophan in the rice matrix was successfully extracted using a mixed solvent, that is 8 % methanol in water [18].

Although the other main effects (temperature (x_1) and ultrasonic power (x_3)) did not have a significant influence, the quadratic terms had a positive effect on the level of extracted *L*-tryptophan. The yield of UAE enhanced with increasing ultrasonic power, but then decreased after reaching the optimum yield [20]. It is relevant to the result, that the quadratic effect of the ultrasound power (x_3x_3) delivered a negative impact and indicated that if the power is very high, it will stimulate the destruction of *L*-tryptophan in the sample. Consequently, the yield of the target analytes decreased.

The positive effect of temperature is relevant to the previous finding by Liu *et al.* [32] that the solubility of *L*-tryptophan increases with increasing temperature. Increasing temperature reduces the viscosity and superficial tension of the solvent by enhancing the vapor pressure, which then initiates the mass transfer rates and diffusivity in the plant cellular tissues [33]. UAE at a higher temperature showed an elevated yield of total phenolic compounds from wild garlic, while raising the temperature from 40 to 80 °C. This phenomenon can be linked to an increase in the number of cavitation bubbles as well as increased solid-solvent interaction, increased solvent diffusivity, and improved desorption and solubility of the targeted compound [34]. Meanwhile, the quadratic temperature factor (x_1x_1) gave a significant negative effect. A very high temperature of UAE produces higher efficiency in the extraction due to enhancing the number of cavitation bubbles in the surface contact area but then decreases when the temperature is near the solvent's boiling point [19]. Additionally, the extraction process was not performed under vacuum conditions, which led to contact with atmospheric oxygen (O_2). In the presence of oxygen or hydrogen peroxide, tryptophan is susceptible to degradation [35].

Moreover, the interaction between the temperature and power variables (x_1x_3) was proved to have a significant negative effect. The high temperature and power after reaching the turning point decreased the extraction efficiency. Therefore, extensive heat exposure during UAE decreases the yield of the target analyte. The interaction between the solvent composition and power (x_2x_3) also showed a significant negative effect on the extraction yield. As mentioned before, the higher the ultrasonic power, the higher the extracted yield. Ultrasonic power ruptures the cell wall and destroy the intra- and intermolecular bonds by acoustic action. Consequently, the solvent easily penetrates into cell wall and the solubility of the target analyte increases upon contact with the appropriate extraction solvent. However, a further increase in ultrasound power leads to a decrease in the extracted yield after reaching the turning point [36].

The contribution of all terms was used to evaluate the fitting properties for the model Eq. (2);

$$y = 96.39 + 1.94x_1 + 15.27x_2 + 0.58x_3 - 3.89x_1^2 - 1.23x_1x_2 - 4.83x_1x_3 - 14.78x_2^2 - 6.06x_2x_3 - 6.71x_3^2 \quad (2)$$

where, y is the level of *L*-tryptophan in the extract and x_i are the extraction variables (x_1, temperature; x_2, solvent composition; and x_3, ultrasonic power).

To determine whether the chosen model adequately explained the observed data or whether a more complicated model was required, a lack-of-fit test was carried out. The test compares the variance of the residuals of the current model to the variance of the observations made in the replicate setting for the factors. Using ANOVA, the derived *p*-value for the lack-of-fit was 0.1286. The model was found to be satisfactory for the observed data at 95% confidence level when the value was greater than 0.05.

Based on the experimental results, the mean absolute error between the real and predicted values was 2.10% and the applicability of the model was confirmed by the high coefficient of determination (R^2) obtained from the regression statistics. The obtained R^2 was 0.9825, indicating that the model fitted with the variability of the extraction yield (**Table 2**); therefore, a very good agreement between the experimental values and the results of the model was obtained. Consequently, the model can be used to predict the response for optimization purposes.

Response optimization

Significant independent factors are necessary to achieve the highest extraction yield when optimizing this method. Three-dimensional surface plots were constructed to predict the effects of independent factors on the response. According to the response surface methodology (RSM) graph (**Figure 5**), the optimum UAE conditions to achieve the highest level of *L*-tryptophan from chamomile flower were set as follows:

Temperature 54 °C, solvent composition 17% methanol in water, and ultrasonic power 69% for 15 min of extraction time. The obtained verification response was in agreement with the predicted value. The difference between the results was only 1.22%. Therefore, it can be concluded that the optimum UAE conditions can be used to predict the extraction response.

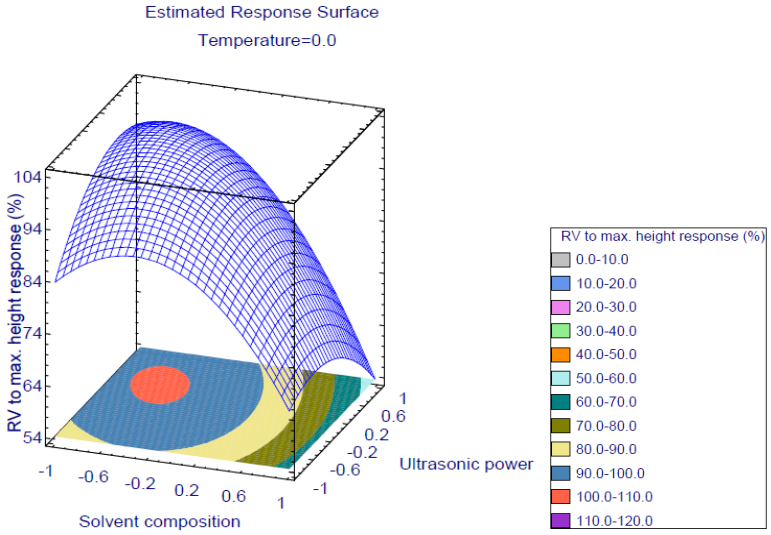


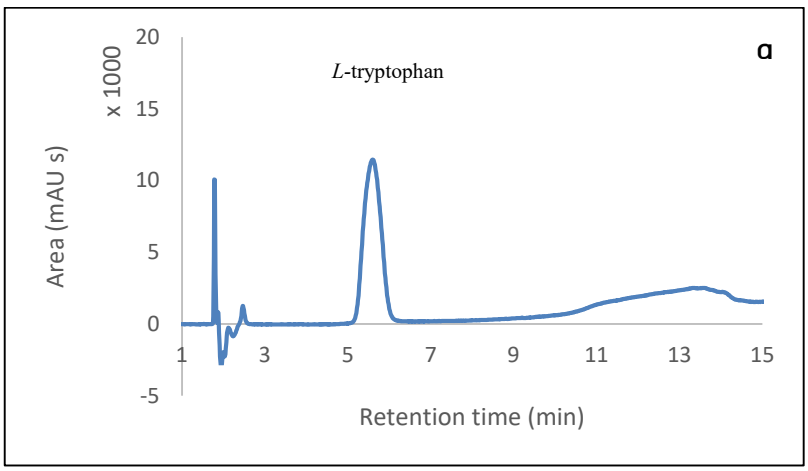
Figure 5 Response surfaced plots displaying the effects of UAE factors on the level of *L*-tryptophan in the extract from chamomile flowers.

Method validation

HPLC performance

An HPLC-DAD chromatographic system as used to determine *L*-tryptophan in the extract sample. Before detecting and quantifying *L*-tryptophan, the chromatographic method was validated in accordance with the ICH Guideline Q2 (R1) [27]. A series of dilutions of the *L*-tryptophan standard solution that covered the concentration range of 1 - 40 ppm was utilized to create the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were evaluated using the resulting standard deviations and regression slopes. The coefficient of determination (R^2) was 0.99, indicating excellent linearity for predicting the concentration of the target analyte within the studied range. The LOD (1.78 ppm) and LOQ (5.38 ppm) were lower than the concentration found in the extracts. This demonstrates that the HPLC method is reliable for quantifying *L*-tryptophan from the sample at a low concentration of the analyte of interest. Furthermore, the precision showed a satisfactory result, with a CV of repeatability and intermediate precision of < 5%. This was below the acceptable limit set by the AOAC (< 10%).

During HPLC analysis, the detected peak of *L*-tryptophan in the standard solution and extract sample appeared at the 5 min, as shown in **Figure 6**.



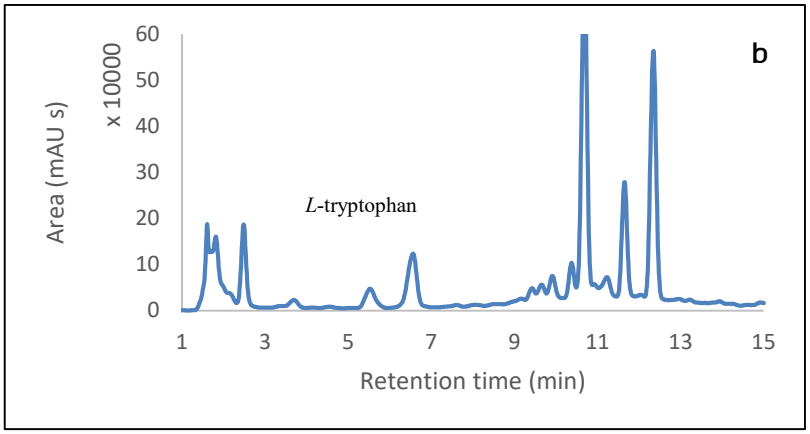


Figure 6 Chromatograms of *L*-tryptophan in standard solution (a), and sample (b).

UAE validation

UAE validation covered the analysis of precision and accuracy. The precision of the method was determined using 2 levels: Repeatability and intermediate precision. The precision, expressed as CV, of the developed method was 3.27% for repeatability (n = 9) and 3.19% for intermediate precision (n = 3×3). The CV values for precision were below the acceptable limit referring to AOAC (± 10 %) [28]. To evaluate the accuracy, the sample was extracted for up to 7 cycles using fresh solvent according to the optimum conditions (17% methanol in water). *L*-tryptophan extraction required 2 extraction cycles to achieve a recovery of 94.26%. Based on AOAC recommendations, the recovery of *L*-tryptophan from chamomile flowers was within the acceptable range (90 - 107%) [28].

Real sample application

To assess the applicability of the method, it was used to extract some real samples. Using the working conditions of the developed UAE method, 3 components (flower capitulum, ray, and disc) of the 2 most popular chamomile (Roman and German) flowers (**Figure 1**) were evaluated for *L*-tryptophan levels. The detection of *L*-tryptophan in the capitulum part of the chamomile was previously studied by Qureshi *et al.* using a reflux extraction method for 2 h and utilizing a water solvent [14]. They revealed that the concentration of this analyte was 172 µg g^{-1} dry basis (db). The level of *L*-tryptophan is presented in **Table 3** in which disc florets and capitulum have the highest content of *L*-tryptophan compared with the ray florets in either the Roman or German chamomiles.

Table 3 *L*-tryptophan level in chamomile flowers.

Flower	Part of flower	*L*-tryptophan (µg g^{-1} db)
Roman chamomile	Capitulum	435.43 ± 2.16
	Disc florets	443.78 ± 3.15
	Ray florets	249.65 ± 4.05
German chamomile	Capitulum	356.96 ± 5.38
	Disc florets	385.11 ± 4.74
	Ray florets	225.43 ± 0.50

The higher level of *L*-tryptophan in the disc than in the ray floret can be indicated by the growth pattern of chamomile that needs tryptophan for the maturation process. The growth of the ray florets on the chamomile starts from the disc florets, which later *L*-tryptophan transforms into auxin and regulates the floral organ initiation, growth, and patterning into a mature flower [37,38]. The detection of tryptophan in ray and disc florets of another Asteraceae family was only performed by Liang *et al.* [23]. They studied the levels of several individual amino acids, including tryptophan, in ray and disc florets of *Helianthus annuus L.* (sunflower). The study revealed that the levels of amino acids in the rays and discs varied. However, the

tryptophan levels were equal between ray and disc florets. Although chamomile and sunflower belong to the same family, the levels of tryptophan in their parts of the flower can differ.

Conclusions

Using a Box-Behnken design and response surface methodology, the ultrasound-assisted extraction method for the extraction of *L*-tryptophan from chamomile flowers was successfully optimized. The optimum extraction conditions involved the use of a certain amount of methanol (17%) in water at a temperature of 54 °C using an ultrasound power of 69% for 15 min extraction time. Complete recovery was achieved after 2 cycles of extraction. Moreover, this method allows for the determination of tryptophan in different parts of chamomile flowers. The lowest level was found for ray florets, approximately 55 - 60% of the levels found in the capitulum, either in the Roman or German chamomiles. This *L*-tryptophan characterization finding would be very useful for further utilization in developing a functional beverage using chamomile flowers, in which Roman chamomile is more favorable as the raw material because it has a higher content of *L*-tryptophan than German chamomile. However, the effect of the drying method should be further studied because dehydrated chamomile flowers are more common in the market than the freeze-dried flowers.

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