Research Article

The Evaluation of Tea Extracted from the Dried Roots of *Asparagus officinalis* L. using Cellulase

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Abstract | *Asparagus officinalis* L. emerges as a beneficial herbaceous derivative, offering great feasibility for creating high-value food products based on its notable nutritional properties and distinct flavor and aroma. To maintain its quality for producing high-value products, particularly in the realm of tea, various extraction methods were explored. The main objective of this study was specifically enhancing the efficiency of enzyme-assisted extraction (EAE) on dried asparagus roots by utilizing cellulase. The investigation examined cellulase enzyme concentrations at 1, 2, 3, and 4% (v/w, compared with raw material) and pH levels at 4.5, 5.0, 5.5 and 6.0 to evaluate the quality of the extracted tea solution. Parameters of pH 5.5 and 3% enzyme were selected as optimal condition for the extraction. The extract from dried asparagus roots derived under this condition demonstrated significant nutritional content, including sucrose (1.49 g), vitamin C (1.10 g), total phenolic content (0.58 g TAE), flavonoid content (0.15 g QE), and saponin content (1.39 g SE) per 100 g of dry matter. Additionally, the extract exhibited notable antioxidant activity, with DPPH radical scavenging capacity of 63.72% and ferric reducing antioxidant power (FRAP) equivalent to 1.15 M Fe^{2+}, achieving the highest sensory preference score.

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Introduction

Asparagus (*Asparagus officinalis* L.) ranks among the world's 20 most valuable crops, renowned for its outstanding nutritional composition and distinct flavor derived from pyrazines and sulfur-containing volatile compounds (Pegiou *et al.*, 2020; Viera-Alcaide *et al.*, 2022). Its green and white varieties are widely consumed annually, offering health benefits due to low calorie content, high fiber levels, and bioactive compounds such as fructans, flavonoids, vitamins, saponins, and cinnamic acids, which exhibit antioxidant, antitumor, immunomodulatory, and other therapeutic properties (Ku *et al.*, 2018; Araceli *et al.*, 2023; Guo *et al.*, 2020). Asparagus production and processing result in significant residues, including

discarded stems, leaves, fruits, and roots. They pose challenges for producers in agronomy, economy, environment and sustainability. Recent studies have highlighted the nutritional potential of these residues, especially roots rich in dietary fiber, fructans, saponins, and phenolics, which can be utilized to create valuable food ingredients, promoting sustainability and industrial innovation (Fuentes-Alventosa *et al.*, 2013; Witzel and Matros, 2020; Araceli *et al.*, 2023). By 2020, green asparagus cultivation in Vietnam has expanded to over 500 hectares, Ninh Thuận Province and the Mekong Delta region have led in production. However, asparagus is mainly cultivated for its edible shoots, which are consumed fresh or processed into various food products, while the roots are typically discarded and lack practical utility. Among commonly high value goods, herbal tea from asparagus roots still remains limited (Nguyen *et al.*, 2023).

The extraction process is essential in deriving preferred natural compounds from crude resources, utilizing techniques like extraction using solvent, mechanical pressing, distillation and sublimation, depending on the underlying extraction principles. The enzyme-assisted extraction (EAE) stands out for its efficiency, utilizing the hydrolytic activity of enzymes to break down cell wall and membrane components, along with macromolecules within the cell (Zhang *et al.*, 2018). This process promotes the release of natural compounds, with cellulases playing a crucial role. Cellulases includes three main categories: EC 3.2.1.4 (endo-(1,4)-β-d-glucanase), EC 3.2.1.91 (exo-(1,4)-β-d-glucanase), and EC 3.2.1.21 (β-glucosidase), which possess significant biotechnological applications across various industries. In the food sector, cellulases facilitate the extraction of bioactive compounds from many types of veggies and fruits, while in beverage industry, they aid in maceration and juice filtration, improving clarity, stability, and overall wine quality (Guadalupe *et al.*, 2007; Subhojit *et al.*, 2016). Cellulases are key components of the macerating enzyme complex (including cellulase, xylanase, and pectinase), which is extensively used for extracting and clarifying juices and wine. This enzymatic complex enhances juice yield, cloud stability, and viscosity reduction, particularly in tropical fruits (De Carvalho *et al.*, 2008; Singh *et al.*, 2019). The increasing need for innovative biocatalysts in the juice industry has driven enzyme production from diverse sources. Enzymes have been utilized to extract antioxidant compounds from various by-products, including pumpkin peels (Wu *et al.*, 2014), rice bran (Kim and Lim, 2016), and oyster mushrooms (Nguyen and Nguyen, 2021). As biological catalysts, enzymes exhibit high substrate specificity and adaptability to a wide range of pH and temperature conditions. The enzymatic breakdown of biomaterials is influenced by factors such as incubation duration, temperature, enzyme level, agitation, pH, and type of substrate within the reaction system (Zhang *et al.*, 2018; Singh *et al.*, 2019).

Base on the on-going demand, this study focused on analyzing the effectiveness of cellulase on the extraction of dried asparagus roots intended for tea production as a new added-value product of asparagus's by-product.

Materials and Methods

Materials

Fresh, high-quality and free from physical damage and pest infestation of green asparagus roots were collected from local produce supplier in Long Xuyen city, An Giang province, Vietnam.

Cellulase enzyme synthesized from *Aspergillus niger*, with an activity of 150 U/mL. The activity unit is defined as the amount of enzyme that hydrolyzes cellulose to release reducing sugars equivalent to 1 µg of glucose per minute under pH 5.0 and 40 °C. This enzyme is supplied by Tien Phong Import-Export Company, Vietnam.

Experimental design

Two kilograms of raw material were cut into pieces with 1 cm length. The pieces of roots were first sorted and washed before undergoing a blanching process, where they were immersed in hot water (85°C) for 2 minutes. After blanching, the samples were dried at 70°C with 1 m/s airflow speed using a Forced Convection Oven (ESCO, OFA-110-8, Indonesia). After that, dried sample was ground and strained through a 1 mm-diameter sieve (Nguyen *et al.*, 2023). For extraction, 100 g of powdery sample was placed in a flask, water was added with a ratio of 20:1 (v/w). The cellulase concentration was gradually increased (1%, 2%, 3%, and 4%), and the extraction process was conducted under varying pH levels (4.5, 5.0, 5.5, and 6.0). The resulting extract was used for further analysis.

Determination of color

The color of the extract was measured using a colorimeter (Konica Minolta CR400) by recording the L, a, and b figures.

Determination of total soluble solid (TSS) content

The TSS in the extract was recorded using a digital handheld refractometer (Atago, Japan) with the refractive index in range of 0-53.

Determination of saccharose

Saccharose were indicated using Dinitrosalicylic acid (DNS), which adjusts the color of the solution during the reaction (Nielsen, 2010). One mL of sample was mixed with 2 mL of reagent DNS in the test tube. The blank sample was the mixture of standard glucose and samples that was put in boiling water for 10 minutes. Before analysing with a UV-visible spectrophotometer (V730, Jasco, Japan) at an absorption of 575 nm, 7 mL of distilled water was added. The concentration of saccharose was based on a standard curve of glucose, y =23.885x + 0.126 (R^2=0.9999) and equation 1, where y represents the absorbance, while x denotes the solution concentration in the tube.

$$X\,(g/L) = \frac{x * \text{dilution} * 1000}{V_{sample}} \quad \ldots (1)$$

Determination of vitamin C

Ascorbic acid was determined using a colorimetric method based on its reaction with 2,4-dinitrophenyl hydrazine (Sharaa and Mussa, 2019). In summary, 1 mL of the extracted solution was mixed with 5 mL of a solution consisting of 3% metaphosphoric acid (HPO_3) (w/v) and 8% acetic acid (CH_3COOH) (v/v) in a centrifuge tube (15 mL). The mixture was shaken for an hour using a reciprocating shaker (Stuart, UK) and centrifuged. After centrifugation, 1 mL of the upper phase was added with 500 µL of 3% bromine solution, 250 µL of 10% thiourea, and 250 µL of 2,4-dinitrophenyl hydrazine. The mixed solution then was incubated (37°C for 3 hours). Then, to develop a red complex, 10 mL of 85% sulfuric acid (H_2SO_4) was used, the mixed solution was then cooled to room temperature, and its absorbance was measured at 521 nm using a UV-visible spectrophotometer (V730, Jasco, Japan). The vitamin C concentration was indicated using a standard ascorbic acid calibration curve, which followed the method outlined by Nguyen *et al.* (2023), y= 0.2253x + 0.0024 (R^2 = 0.9999), where y represents the absorbance, while x denotes the solution concentration in the tube.

Determination of phenolic

Phenolic compounds (g TAE per 100 g of dry matter) were detected following the procedure outlined by Sumaiyah *et al.* (2015). In summary, 150 µL of the extracted solution was mixed with 450 µL of sodium carbonate (Na_2CO_3) solution 5% (w/v) and 1.2 mL of distilled water in a test tube. Then, 100 µL of Folin-Ciocalteu solution was added, and the mixture was allowed to rest at room temperature for one and a half hours. In an alkaline medium, the phenolic compounds interacted with the reagent, create a blue phosphomolybdenum complex. The phenolic content was quantified based on the absorbance value of the solution, which was measured at 750 nm, using a standard tannic acid curve (TAE) that followed the method outlined by Nguyen *et al.* (2023), y = 0.0021x + 0.0064 (R^2= 0.9999), where y represents the absorbance, while x denotes the solution concentration in the tube.

Determination of flavonoid

Flavonoid compounds were assessed through a reaction with aluminum chloride, following a modified method by Sumaiyah *et al.* (2015). A stable acidic complex was created between $AlCl_3$ and the C-4 keto groups, along with the hydroxyl groups at either C-3 or C-5 positions of flavones and flavonols. In summary, 100 µL of the extracted solution was combined with 30 µL sodium nitrite ($NaNO_2$) 5% (w/v) and 1.2 mL of distilled water. After 5 minutes, the mixture was added with 60 µL of 10% (w/v) aluminum chloride ($AlCl_3 \cdot H_2O$), 200 µL of 1 M sodium hydroxide (NaOH), and 110 µL of distilled water. The absorbance of the obtained solution was read at 510 nm. Flavonoid concentration was quantified based on the standard quercetin curve (QE), which followed the method outlined by Nguyen *et al.* (2023), y= 8.2634x + 0.0182 (R^2 = 0.9999), where y represents the absorbance, while x denotes the solution concentration in the tube.

Determination of saponin

Saponin content was measured using the vanillin-sulfuric acid technique outlined by Le *et al.* (2018). Briefly, triterpene saponins are oxidized by vanillin and sulfuric acid, resulting a red-violet color reaction. The incubation of the combination of 250 µL of 8% vanillin in 96% ethanol with 250 µL of the sample and 2.5 mL of 72% sulfuric acid (H_2SO_4) at 60°C for 30 minutes. The mixture was cooled to

room temperature. The absorbance of the obtained solution was read at 510 nm and saponin content was quantified by a standard saponin curve (SE), which followed the method outlined by Nguyen *et al.* (2023), y= 0.1348x + 0.0075 (R^2 = 0.9999), where y represents the absorbance, while x denotes the solution concentration in the tube.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The antioxidant capability of the extracted solution was assessed by evaluating its ability to capture free radicals using the DPPH assay, which described by Molyneux (2004) with minor modifications. A 1.5 mL sample was mixed with an equal volume of DPPH solution (1:1, v/v). Briefly, a reaction of electron transfers results in a purple color in ethanol, its absorbance was read at 517 nm. The effectiveness of inhibiting free radicals was then calculated using Equation 2.

$$\text{Inhibition of DPPH radical}(\%) = 100 \times (A_c - A_s)/A_c \quad \ldots(2)$$

Where: A_c presents the absorbance of the control and A_s presents the absorbance of the extracted sample.

Determination of ferric reducing antioxidant power (FRAP)

This assay (expressed in mM $FeSO_4$ per gram of dry matter) was performed according to the protocol of Sudha *et al.* (2012). The reaction is dependent on the reduction of the ferric-tripyridyltriazine complex ($Fe(TPTZ)^{3+}$) to its ferrous form ($Fe(TPTZ)^{2+}$), resulting in a blue color in an acidic environment. Briefly, mixing 100 mL of 200 mM acetate buffer at pH 3.6, 10 mL of 10 mM TPTZ in 40 mM HCl and 10 mL of 20 mM $FeCl_3 \cdot 6H_2O$ to made FRAP reagent. For the assay, 50 µL of the extracted sample was added with 1.5 mL of the reagent and 150 µL of distilled water. The combined solution was incubated at 37°C for 8 minutes, and the absorbance was measured at 593 nm.

Sensory characteristics

Sensory evaluation played a crucial role in aligning technical product development with consumer satisfaction, ensuring the final product was both high-quality and appealing. The sensory characteristics of dried asparagus root were evaluated based on attributes such as color, flavor, and overall preference. The Quantitative Descriptive Analysis (QDA) method was utilized for this assessment. A sensory panel of 30 participants was instructed to evaluate the color and flavor of the dried asparagus root extract using a descriptive scale ranging from 1 (poor) to 5 (excellent) (Thuy *et al.*, 2012). Preference levels were assessed using a hedonic scale, with scores spanning from 1 (extreme dislike) to 9 (extreme like).

Data analysis

The data was gathered, processed, and analyzed using the Statgraphics Centurion XVI (USA) software for variance analysis (ANOVA), the LSD test to identify differences between trial averages at a 5% confidence level (P= 0.05), and Microsoft Excel for computation and graph demonstration. Predicted model during optimization was assessed using the correlation coefficient R^2, the illustrated equation for response surface optimization was created based on experimental data, as shown in Equation 3.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} X_i X_j \quad \ldots(3)$$

Where; Y represents the objective function; β_0, β_i, β_{ii}, β_{ij} respectively denote the constant term, the linear coefficient, the squared coefficience and the interaction coefficience; X_i and X_j represent the survey variables.

Results and Discussion

Extraction with enzyme support is based on the catalytic abilities inherent to enzymes in aqueous solutions (Gardossi *et al.*, 2010). Cellulase enzymes are capable of breaking down cell walls, facilitating the efficient release and extraction of nutritional elements and bioactive compositions (Pinelo *et al.*, 2006; Puri *et al.*, 2012; Nguyen and Nguyen, 2021). The influence of cellulase concentration and pH on the total soluble solids (°Brix) and color (represented through L, a, b values) is presented in Table 1.

The results indicated that the total soluble solid (TSS) content of the extract rose from 2.30 to 2.33 °Brix as the concentration of the added cellulase enzyme increased from 1% to 2%. However, further increases in cellulase supplementation to 3% and 4% led to TSS values that increased but with no statistically significant differences. The results also showed that the pH of the solution was influenced by the TSS content of the extract. Specifically, the highest °Brix degree of the extract was achieved at pH 4.5 (2.35),

and it dropped as the pH of the solution continued to increase.

Table 1: *Effects of added enzyme concentration and pH on the Brix degree and color parameters (represented by L, a, b values) of the extracted solution from dried asparagus roots.*

Cellulase concentration (% v/w)	Brix	Color parameters		
		L	a	b
1	2.30*^{b}	38.83^{b}	-2.91^{a}	4.61^{a}
2	2.33^{a}	39.05^{ab}	-3.01^{ab}	5.13^{b}
3	2.33^{a}	39.07^{ab}	-3.02^{ab}	5.14^{b}
4	2.34^{a}	39.25^{a}	-3.05^{b}	5.16^{b}
Level of significance	**	ns	ns	**
pH				
4.5	2.35^{a}	39.33^{a}	-3.18^{c}	5.04^{b}
5.0	2.33^{b}	39.14^{ab}	-2.95^{b}	5.68^{a}
5.5	2.31^{b}	38.92^{b}	-2.54^{a}	4.25^{c}
6.0	2.31^{b}	38.81^{b}	-3.32^{d}	5.07^{b}
Level of significance	**	**	**	**
Significance level of interaction	**	**	**	**

**Average results of three repetitions. Distinct lowercase letters in each column differ statistically significantly, **difference at 1% significance level, and non-significant differences (ns) with no statistically significantly.*

When increasing the supplementary cellulase concentration, the changes in the L value and the b value tended to follow the same trend as the TSS content. However, the a value showed an opposite trend, decreasing with higher levels of supplementary cellulase. Additionally, the a and b values initially increased to an optimal point before gradually reducing as the pH of the solution rose. The a value reached its highest point at pH 5.5 (-2.54), and the b value reached its highest point at pH 5.0 (5.68). Meanwhile, the L value decreased with increasing pH, reaching its highest point (39.33) at pH 4.5.

The saccharose content increased when the cellulose concentration increased from 1 to 3% (from 1.23 to 1.51 g/100 g dry matter) (Figure 1A). However, when the cellulase concentration was further increased to 4%, the saccharose content increased but the difference was not statistically significant compared to the sample with 3% cellulase supplementation. The vitamin C content increased with the increasing cellulase supplementation and reached the highest level at 4% cellulase (1.27 g/100 g dry matter) (Figure 1B), which was statistically significant compared to the other samples. Both saccharose and vitamin C content decreased with the rising pH, with the highest levels observed at pH 4.5, which were 1.57 and 1.19 g/100 g dry matter, respectively.

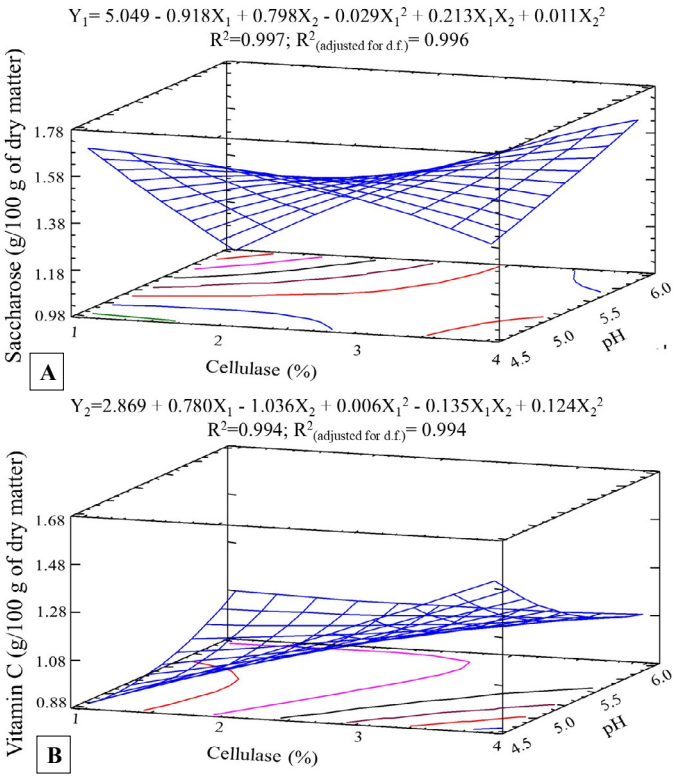

Figure 1: *The effect of added enzyme concentration and pH on the chemical compositions of the extracted solution from dried asparagus roots (A) Saccharose and (B) Vitamin C.*

When increasing the supplementary cellulase concentration, the phenolic and flavonoid content in the extracted solution exhibited a similar trend to vitamin C, Brix, and L value (Figure 2A, B). Specifically, the phenolic content increased and reached its highest level at 4% cellulase (0.61 g TAE/100 g dry matter). In contrast, the flavonoid content reached its highest point at 3% cellulase (0.14 g QE/100 g dry matter), which was not statistically significant when compared to the sample with 4% cellulase (0.14 g QE/100 g dry matter). Saponin content followed the same trend as the changes in the a value when increasing the supplementary cellulase concentration and reached its highest level at 1.39 g SE/100 g dry matter (Figure 2C). The results also showed that phenolic and saponin levels rose to a peak before gradually declining as the pH of solution increased. Saponin content reached its highest level at pH 5.0 (1.40 g SE/100 g dry matter), which was not statistically significant when compared to the sample at pH 5.5 (1.40 g SE/100 g dry matter). Similarly, phenolic content also reached its highest level at

pH 5.0 (0.60 g TAE/100 g dry matter). Meanwhile, flavonoid content increased with rising solvent pH. Specifically, phenolic content reached its highest point at pH 5.5 and with 3% cellulase supplementation, while flavonoid content reached its highest point with 1% cellulase supplementation at pH 5.5.

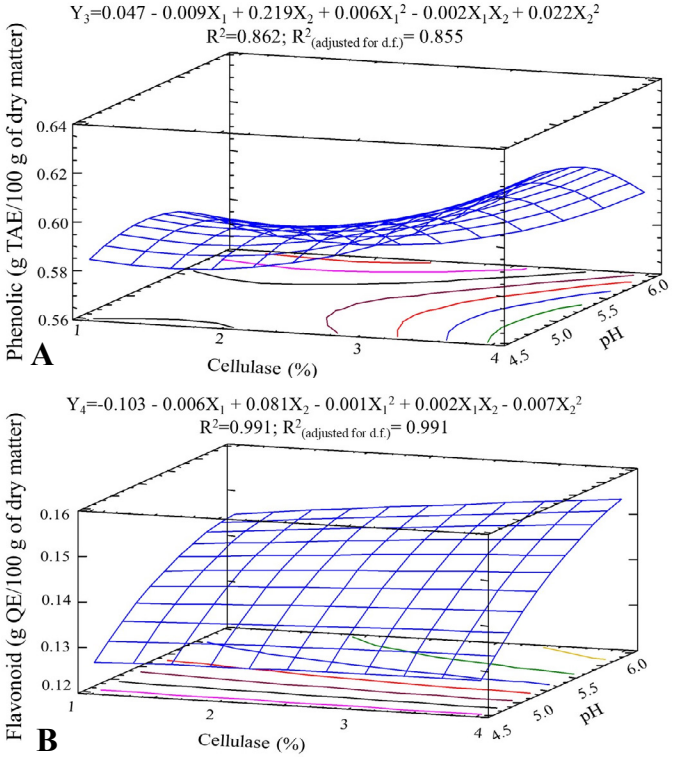

Figure 2: *The influence of the added enzyme concentration and pH in aqueous solvent extraction on the composition of bioactive compounds in tea extract from dried asparagus roots (A) Phenolic, (B) Flavonoid, and (C) Saponin.*

The antioxidant capability of the extract, as measured by its ability to scavenge DPPH radicals and its FRAP value, increased up to an optimal point and then decreased with rising solvent pH (Figure 3). However, FRAP value decreased with increasing supplementary enzyme concentration.

These changes can be explained due to the mechanism of cellulase during the extraction process. Cellulase is considered as commercial enzyme complexes typically contain various enzymes (Subhojit *et al.*, 2016). When adding a commercial enzyme complex to an oyster mushroom (*Pleurotus* spp.), the enzyme complex sequentially breaks down the structural components of the cell walls, such as cellulose and hemicellulose. This process disrupts the cell wall structure, facilitating the release of various compounds, including water and soluble substances (Ghandahari *et al.*, 2018; Nguyen and Nguyen, 2021). Enzyme activity directly catalyzes the cleavage of ether and ester bonds that link phenolic compounds to plant cell wall polymers (Heemann *et al.*, 2019). This breakdown promotes the release of soluble compounds, increasing the nutritional value of the extract and its antioxidant activity (Coniglio *et al.*, 2021). Moreover, under suitable substrate concentrations, the enzyme reaction rate is linearly proportional to enzyme concentration. However, when the enzyme concentration reaches a certain limit, the reaction rate does not increase further (Sridhar *et al.*, 2021) or may even decrease (Heemann *et al.*, 2019). Excessive cellulase enzymes can cause the solution to become viscous, hindering the enzymatic hydrolysis process (Yang *et al.*, 2019). Furthermore, the differences in the antioxidant capacity of the extract may be due to the inherent ability of enzymes to disrupt the plant cell wall structure, thus releasing oxidative compounds to different extents (Nath *et al.*, 2016).

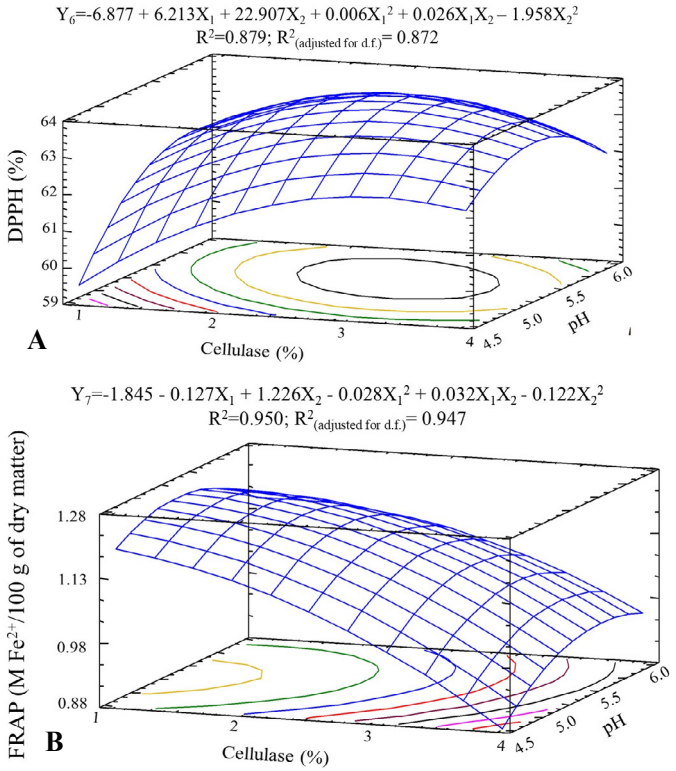

Figure 3: *The influence of the added enzyme concentration and pH in aqueous solvent extraction on the antioxidant activity of tea extracted from dried asparagus roots (a) DPPH, (b) FRAP.*

Phenolic compounds, flavonoids, and saponins are potent antioxidants and can be easily oxidized under alkaline conditions, while acidic conditions may inhibit the oxidation of these compounds, leading to higher yields. The changes in the bioactive compounds observed in this study indicated that a significant amount of these compounds was extracted in an acidic environment, which also supports the extraction of

other antioxidants, such as vitamin C (Ruenroengklin *et al*., 2008). Furthermore, enzymes are sensitive to the environmental pH, making it an important factor affecting enzyme reaction rates. The optimal pH for cellulase activity is between 4.5 and 6.0 (Lakmal *et al*., 2015). The pH optimum can maximize cellulase activity and cell wall disruption, but as pH values continue to rise, the hydrolysis of cellulase enzymes becomes less clear, and the hydrolysis of the cell wall becomes weaker (Li *et al*., 2020). Additionally, cellulase reaction conditions are altered and cellulase activity is reduced as the solvent pH increases (Wei *et al*., 2021).

The results from the sensory evaluation presented in Figure 4 show that the sample with 3% cellulase supplementation at pH 5.5 achieved the highest scores for aroma, bright yellow color, harmonious asparagus scent and the highest level of liking.

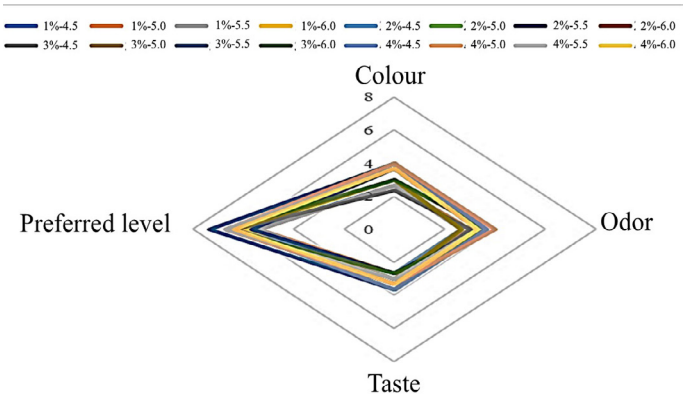

Figure 4: *The graph represents sensory evaluation for color, aroma, taste, and preference of dried asparagus roots extracted at different supplementary cellulase concentrations and pH levels.*

The analysis results showed that the determination coefficient R^2>0.86 of the predictive model was high, indicating that it can be used to predict the changes in sucrose content, vitamin C, bioactive compounds (phenolic, flavonoid, and saponin), and antioxidant capacity (DPPH and FRAP) of dried asparagus root's extract based on the supplementary cellulase concentration and pH (Figures 1-3). In the regression equation, if the influence coefficient of a variable has a positive value, it implies that an increase in the value of a variable with a positive influence coefficient will lead to an increase in the target function's value. Conversely, if the influence coefficient is negative, increasing the variable's value will result in a decrease in the target function's value (Nguyen *et al*., 2019). Moreover, when the influence coefficient of one variable is greater than the influence coefficient of the other variables, that variable has the most significant impact on the value of the target function (Le *et al*., 2017). The results showed that the sucrose content was the most affected by the cellulase concentration, while phenolic, flavonoid, saponin, and FRAP were influenced by the pH of the solvent.

Conclusions and Recommendations

The study selected a solvent pH of 5.5 and an enzyme concentration of 3% as suitable parameters for the subsequent experiments. Under these conditions, the extract obtained from dried asparagus roots retained optimal nutrients (including sucrose, vitamin C, phenolics, flavonoids, and saponins) along with antioxidant capability (measured by DPPH and FRAP assays). Simultaneously, it achieved the highest sensory liking. The results of this study contribute to diversifying value-added products, especially tea products from asparagus's by-products, and are consider as a reference for extraction processes assisted by cellulase.

Acknowledgment

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Novelty Statement

This study is among the first to focus on the valorization of green asparagus roots, an underutilized agricultural by-product in Vietnam, by optimizing enzyme-assisted extraction parameters to produce a high-quality tea extract. This research provides new insights into the systematic extraction process specifically aimed at maximizing both nutritional value and sensory quality and also addresses sustainability challenges by transforming agricultural waste into a valuable food product, thereby contributing to circular economy approaches in food processing and industrial innovation.

Author's Contribution

Nguyen Thi Ngoc Giang: Responsible for designing and conducting the experiment, analyzing the data, as well as writing, reviewing, and editing the manuscript. **Tran Van Khai:** Contributed to conducting the experiment and data analysis.

Conflict of interest

The authors have declared no conflict of interest.

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