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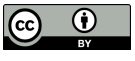
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Original article

Enzymatically hydrolysed asparagus (*Asparagus officinalis* L.) hard-stem exhibits the ability to inhibit angiotensin-converting enzyme (ACE)

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Abstract Asparagus (*Asparagus officinalis* L.) hard-stem, woody bottom of the asparagus stalk, is a rich source of bioactive compounds and is discarded as an eco-friendly agricultural waste. Therefore, we intended to explore the antioxidant, amino acid composition, and antihypertensive potentials of enzymatically hydrolysed asparagus hard-stem. The treated samples exhibited higher total free amino acids (11437 mg 100 g^{-1}), total phenolics (1749 mg 100 g^{-1}), and ability to inhibit (78.38%) angiotensin-converting enzyme (ACE) compared to control (ACE inhibition of 46.88%). Hydrophobic amino acids (39%) and gallic acid (48%) were the highly eluted bioactive compounds. The ability to inhibit ACE had been positively correlated with hydrophobic amino acids (r = 0.959–0.987) and gallic acid (r = 0.966), indicating the role of amino acids and phenolics in controlling ACE reactions. Thus, asparagus hard-stem can be a potential source to develop natural health supplements for the management of hypertension and related health risks.

Keywords angiotensin-converting enzyme (ACE), asparagus hard-stem, free amino acids, phenolic compounds, waste valorisation.

Introduction

Hypertension, high blood pressure, is a chronic medical condition that occurs due to rise in systolic or diastolic blood pressure, possibly leading to severe health complications and significantly increases the risk of heart, cerebrovascular, and chronic kidney diseases (Zhou *et al*., 2021). Globally, hypertension is one of the major causes of morbidity & mortality and has been diagnosed in 1.28 billion adults aged 30–79 years, in which 46% of adults are unaware of this condition (WHO, 2021). Modifiable risk factors, including unhealthy diets, physical inactivity, being obese, and regular consumption of tobacco and alcohol, are the major risk factors for hypertension (WHO, 2021). Thus, it is likely that the consumption of heart-unhealthy processed foods and lack of exercise develop hypertension that can increase the risk of several health complications.

Over the years, hypertension has been managed by the angiotensin-converting enzyme (ACE) inhibitors, which effectively lower the blood pressure both in systolic and diastolic conditions and decrease the oxygen demand from the heart (Herman *et al*., 2021). Since then, many artificial ACE inhibitor drugs, such as sulphydryl-containing ACE inhibitors (e.g. captopril), dicarboxylic-containing ACE inhibitors (e.g. enalapril), and phosphorus-containing ACE inhibitors (e.g. fosinopril), are used for the control of hypertension. However, the use of artificial ACE inhibitors showed adverse side effects on human health including first-dose hypotension, acute kidney injury, hyperkalaemia, and contraindications during pregnancy. In addition, other complications, such as cough, angioedema, and anaphylactoid reactions, have been attributed to ACE inhibitor therapy (Townsend, 2016). This represented the need of an alternative, natural, plant-based green approach as a first-line of treatment to control hypertension. This further directed scientific communities to focus on plant-based ethnopharmacological remedies with hypotensive and antihypertensive therapeutic values (Ahmad *et al*., 2015; Chukwuma *et al*., 2019; Ajeigbe *et al*., 2021). Although plant-based natural products have historically proven their therapeutic potential, particular attention is given to environmental sustainability for the recovery of active pharmaceutical ingredients and the development of novel bioactive compounds from plant by-products. This further revitalised the

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valorisation of plant-based by-products to meet the United Nations Sustainable Development Goals (SDGs).

Asparagus (*Asparagus officinalis* L.), a perennial herb and stem vegetable native to the eastern Mediterranean and Asia Minor, is now globally cultivated in more than 60 countries (Guo *et al*., 2020). The edible part of the asparagus, commonly called as stalks, corresponds to approximately 50% of its total and other 50% discarded as by-products in the form of hard-stems, roots, and leaves during processing (Sriyab *et al*., 2021). For this reason, many national and international organisations are enforcing sustainable utilisation of waste generated from fruits and vegetables through technological developments. In recent years, asparagus hard-stem has attracted the scientific attention due to the presence of similar bioactive compounds as in the main products, such as phenolic compounds, saponins, and dietary fibre (Chitrakar *et al*., 2019; Romani *et al*., 2021; Sriyab *et al*., 2021). Fresh asparagus hard-stems contain more than 91% water, and the remaining content is composed of protein (15.90%), crude fat (1%), crude fibre (1.80%), total sugars (5.70%), and reducing sugars (5.50%) (Guan *et al*., 2015). A review demonstrated that the asparagus hard-stem extracts showed health benefits against numerous cancers, including colon cancer and human breast cancer, through certain inhibitory effects (Guo *et al*., 2020). Recently, studies showed the potential use of asparagus hard-stem as a possible co-adjuvant agent on tumour growth, metastasis of breast, colon, breast cancer, and pancreatic cancer cell lines (Mfengwana & Mashele, 2019; Romani *et al*., 2021). However, to date, there have been no studies that utilised the asparagus hard-stem as a potential source to develop health supplements for the management of cardiovascular diseases, such as hypertension and other related health risks.

Thus, we hypothesise that the bioactive compounds extracted from asparagus hard-stem can be a potential source featuring antioxidant and antihypertensive activity. Currently, antihypertensive plant-based botanical products are of interest worldwide to avoid fewer side effects of hypertension-controlling synthetic drugs and ACE inhibitors. Therefore, we intended to explore the antioxidant, amino acid composition, and antihypertensive potentials of enzymatically hydrolysed asparagus hard-stem.

Materials and methods

2.1. Sample procurement and preparation

The fresh asparagus (1 kg; *Asparagus officinalis* L.) samples were purchased from the local market in Pingtung County, Taiwan. The superior quality samples were packed in plastic crates, covered with perforated film, and transported to the laboratory within 20 min. The samples were washed briefly under tap water and then softly patted on bibulous paper to remove the surface water. Then, the samples were lined up on a cutting board (40 × 25 cm) and cut off the hard-stem with a chef's knife. The hard-stem was identified by visual observation where stalks' green colour starts to fade to white and/or stringy stems appear to start towards the ends. The hard-stem samples (length: 5 cm with a thickness of 1–1.50 cm) were cut into small pieces and dried in an oven (Kingtech Scientific Co., Ltd., Taipei, Taiwan) at 50 °C until no change in weight was observed. The dried samples were ground to a powder (Rong Tsong Precision Technology Co., Taichung City, Taiwan) and stored in enclosable Ziplock bags (17 × 25 cm) at −20 °C until further use.

2.2. Enzymatic hydrolysis

The enzymatic hydrolysis of the samples was carried out with enzyme bromelain. Briefly, the enzyme bromelain was added to sample (0.50 g) in different concentrations (1, 3, 5, 7, and 10 U mL^{-1}) and then hydrolysis was carried out at different hydrolysis temperatures of 30, 40, 50, 60, and 70 °C. Then, the enzyme activity was terminated by heating at 100 °C for 10 min and then cooled in ice for 5 min. The reaction mixture was centrifuged (Hitachi CF15RXII, Tokyo, Japan) at 10,000 r.p.m. for 10 min and filtered with Whatman® filter paper No. 3 (Whatman International Ltd., Maidstone, England, UK). The extracts thus obtained were stored in amber-coloured glass vials (28 × 57 mm) at 4 °C and re-dissolved in water for further experimental analysis.

2.3. Total free amino acids

Total free amino acid content was quantified according to Chen *et al*. (2017) with minor modifications. Using an aspartic acid calibration curve, the content of total free amino acids was determined and reported as mg aspartic acid equivalents/100 g dried weight of the sample (mg/100 g DW). The detailed methodology is described in Supplementary.

2.4. Total phenolic content

The total phenolic content (TPC) of the sample was determined according to Yeo & Shahidi (2017). The TPC was calculated using a gallic acid calibration curve and represented in milligrams of gallic acid equivalents per 100 grams of dried weight of the

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sample (mg GAE/g DW). The detailed methodology is described in Supplementary.

2.5. Antioxidant activities

2.5.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging activity of the sample was determined following Sun *et al.* (2019) with modifications. The DPPH radical scavenging activity was expressed as % inhibition using the following equation (1). The detailed methodology is described in Supplementary.

$$\%inhibition = \left[\left(\frac{A_{control} - A_{sample}}{A_{Control}}\right) \times 100\right] \quad (1)$$

where $A_{control}$ = absorbance of control and A_{sample} = absorbance of the sample.

2.5.2. FRAP (ferric reducing antioxidant power) activity

The FRAP ability of samples was determined according to the Sun *et al.* (2019) with modifications. The FRAP activity was calculated in relation to the reducing power of $FeSO_4$ standard solution and expressed as μM $FeSO_4$ equivalent per litre of dried weight of the sample (μM/L DW). The detailed methodology is described in Supplementary.

2.6. ACE (angiotensin-converting enzyme) inhibition

ACE inhibition activity of samples was determined according to He (2017). In brief, a control sample or test sample was prepared by mixing 500 mU ACE (5 μL) with sodium-borate buffer (45 μL) and incubated at 37 °C for 30 min. Then, N-hippuryl-L-histidyl-L-leucine (5 mM, 12.50 μL) was added to the reaction mixture and incubated at 37 °C for 10 min with continuous agitation at 450 rpm. The reaction was terminated by the addition of 1 M HCl (75 μL), filtered through 0.45-μm PVDF filter, and injected (20 μL) in the HPLC system using a Mightysil RP-18 GP column (250 × 4.60 mm, 5 μm) at room temperature. The analysis was performed through two different mobile phases: solvent A (0.10% trifluoroacetic acid in water) and solvent B (methanol) with a linear gradient of 0 min (85%, B)–15 min (60%, B)–20 min (10%, B). The constant flow rate of 1 mL min^{-1} was maintained throughout the analysis. Single-wavelength UV absorption of 280 nm was used for the detection of compounds. The ACE inhibition was determined using equation (2) based on a comparison of the concentration of an inhibitor with or without.

$$\%inhibition = \left[1 - \left(\frac{S_{inhibitor} - S_{inhibitor}}{S_{control} - S_{blank}}\right) \times 100\right] \quad (2)$$

2.7. Amino acid composition

The amino acid composition of samples was determined according to Kazan *et al.* (2019). Briefly, sample or standard (100 μL) was dissolved in 0.10 M HCl to the final concentration of 10^{-3} M L^{-1} and then filtered through 0.45-μm PVDF filters for further experimental analysis using HPLC. Chromatography was performed using a Mightysil RP-18 GP column (250 × 4.60 mm, 5 μm) at a temperature of 40 °C with 20 mM acetate buffer (pH 7.60) containing 3% tetrahydrofuran (v/v) (mobile phase A), while mobile phase B was a mixture of 100 mM acetate buffer:acetonitrile:methanol (1:2:2). The system was run with the linear gradient elution programme with different time intervals at a constant flow rate of 1 mL min^{-1} and the injection volume was 20 μL. The amino acid derivatisation reagents were prepared freshly and mixed with samples before injecting into a column. The compounds were detected at an excitation wavelength of 340 nm with an emission wavelength at 450 nm. The composition of amino acids was determined based on the comparison between the retention time of the standards and samples. The total amounts of each compound were expressed as mg/g protein.

2.8. Determination of individual phenolic compounds

The individual phenolic compounds were determined using an HPLC system (L-7100, Hitachi, Tokyo, Japan). The separation was performed on C_{18} column (250 × 4.60 mm; 5 μm; Kanto Chemical Co., Inc., Tokyo, Japan) at room temperature. The mobile phase consisted of 0.30% formic acid in ultrapure water (solvent A) and 100% methanol (solvent B) (Lu *et al.*, 2021). The elution of solvents in a gradient was programmed as follows: 0–20 min, 80% A; 30–45 min, 60% A; 70–75 min, 40% A. The mobile phase flow rate was kept constant at 1 mL min^{-1}. Sample extracts (20 μL) and mobile phase solvents were filtered through a 0.45-μm nylon filter membrane. The detection wavelength was set at 280 nm based on maximum ultraviolet (UV) absorption of individual phenolic compounds. The individual phenolic compounds were identified and were represented in milligrams per 100 g of the sample's dried weight (mg/100 g DW).

2.9. Statistical analysis

All experimental analyses were performed in triplicate and the results were expressed as mean ± standard deviation. Mean differences were analysed by one-way analysis of variance (ANOVA) and Duncan's multiple comparison test using SPSS 20.0 software (IBM, Armonk, NY, USA) at the significance levels of $p < 0.01$. The same software was used to run Pearson's correlation analysis at the significance levels of $p < 0.01$ and 0.05.

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Results and discussion

3.1. Enzyme concentration and hydrolysis temperature

The increase in enzyme concentration exhibited the increase in total free amino acids, which was found to be 1.04-fold significantly ($p < 0.05$) higher at the final enzyme concentration of 10 U/mL^{-1} compared to the initial concentration of 1 U mL^{-1} (Fig. 1). Similarly, ACE inhibition was also significantly ($p < 0.05$) varied and showed a higher ACE inhibition (78.40%) at the enzyme concentration of 7 U mL^{-1}, but decreased to 61.60% after 7 U mL^{-1}. For hydrolysis temperature, 50 °C showed a positive effect on total free amino acids and ACE inhibition, which significantly ($p < 0.05$) increased to 11357 mg 100 g^{-1} and 78.40%, respectively, compared with that of control and other hydrolysis temperatures (Fig. 1). It was obvious that the total free amino acids and ACE inhibition activity may decrease with increase in temperature due to increased enzyme deactivation at higher temperatures. Moreover, several physical and chemical factors, such as temperature and enzyme concentration, may influence the relationship between enzyme activity and enzyme concentration. The moderate increase in temperature can improve the effectiveness of the enzyme–substrate interaction due to an exposure of H_2 bonds to proteases, as a result, the extraction is more efficient (Sandoval-Gallardo *et al.*, 2020). Based on these findings, the hydrolysis temperature of 50 °C and enzyme concentration of 7 U mL^{-1} resulted in the increased yield of total free amino acids and ACE inhibition. Thus, these optimal conditions were selected for further analysis.

3.2. Total free amino acids, total phenolic content, antioxidant, and ACE inhibition activities

The total free amino acids, total phenolic, antioxidant (DPPH radical and FRAP), and ACE inhibition

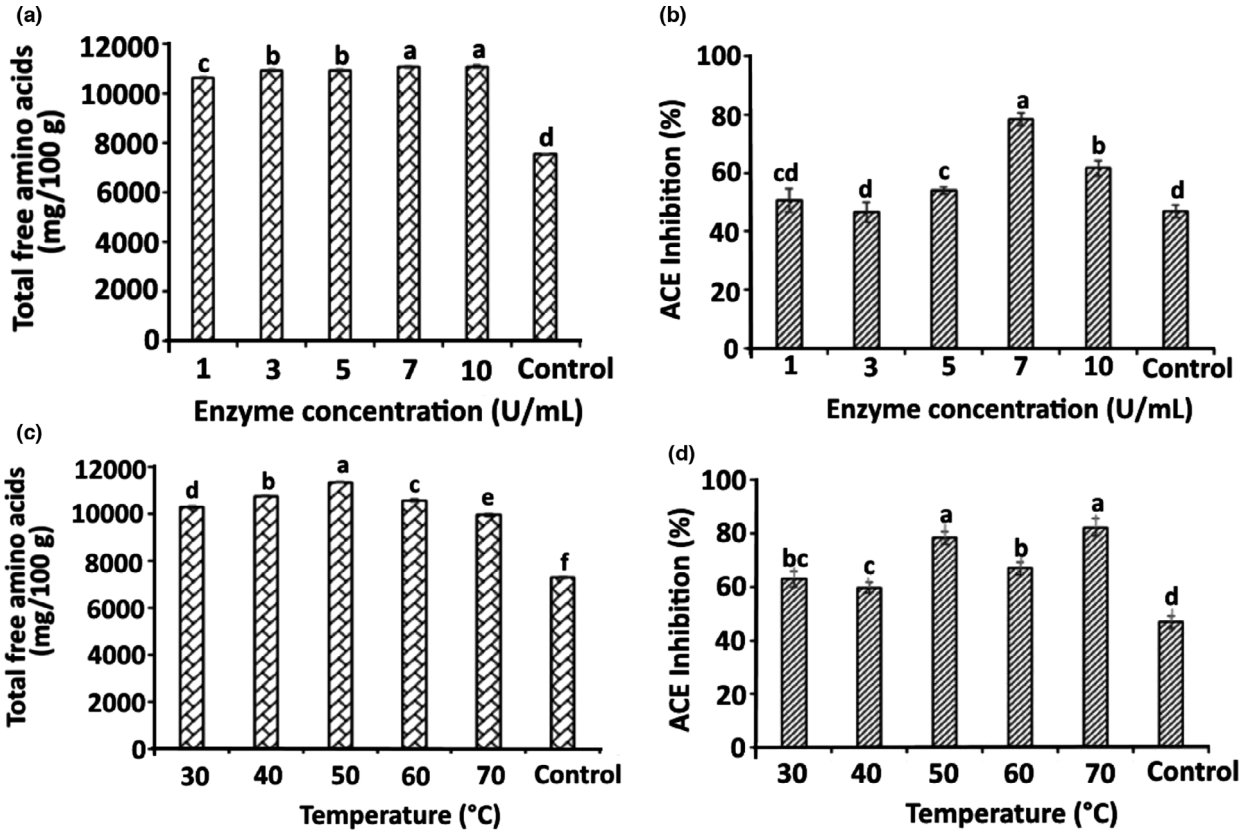


Figure 1 Effect of enzyme concentration and hydrolysis temperature on asparagus hard-stem. Effect of enzyme concentration on total free amino acids (a) and ACE inhibition (b) and effect of hydrolysis temperature on total free amino acids (c) and ACE inhibition (d). Error bar represents the standard deviation of the mean determined from three independent determinations. Error bars along with lower-case alphabets (a to f) show the significant difference ($p < 0.05$) among the samples treated with different enzyme concentrations and hydrolysis temperature based on the analysis of variance (ANOVA) and Duncan's multiple range tests. ACE, angiotensin-converting enzyme.

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activities of asparagus hard-stem treated with or without enzymatic hydrolysis are shown in Table 1. The total free amino acid and total phenolic content increased from 7448 to 11437 mg 100 g^{-1} and 1160 to 1749 mg 100 g^{-1}, respectively, which represents the 50% increase in samples treated with enzymatic hydrolysis compared to control. For antioxidant activity, samples treated with enzymatic hydrolysis exhibited significantly ($p < 0.05$) higher DPPH free radical inhibition (60%) than the control (56%), indicating the improved DPPH free radical scavenging activity with the enzymatic hydrolysis. However, FRAP activity demonstrated a significantly ($p < 0.05$) lower reducing activity (1072 μM L^{-1}) for samples treated with enzymatic hydrolysis than the control (1322 μM L^{-1}). For ACE inhibition, samples treated with enzymatic hydrolysis exhibited 1.67-fold higher inhibition than the control (46.88%), demonstrating a similar trend as total free amino acids, total phenolics, and DPPH scavenging activity. These findings broadly support the results of earlier study (Chen *et al*., 2021), which showed the increased total phenolic content and DPPH free radical scavenging activity for djulis hull, leaf, and seedling treated with enzymatic hydrolysis. Thus, the increased bioactive potential could be related to the hydrolytic reactions and a high percentage act of the enzyme on asparagus cell wall components, which resulted in the release of bioactive compounds during extraction.

The inhibition activity of samples using DPPH and FRAP assays showed differences, which could be related to different reagents used in each method or differences in stereochemical structures of reagents. The variations also occur due to solubility of extracts in different solvent systems and different units used to determine the antioxidant capacity using DPPH and FRAP methods. Thus, antioxidant activities using DPPH and FRAP methods showed discrepancies in the antioxidant potential of the sample extracts. Nevertheless, DPPH assay is a well-accepted assay for the screening of antioxidant activity of a compound, plant extract, and/or other biological materials (Kedare & Singh, 2011). Overall, enzymatic hydrolysis improved the release of bioactive compounds and thus favoured the antioxidant capacities to prevent the formation of free radicals.

Table 1 Total free amino acids, phenolic, antioxidant, and ACE inhibitory activities of asparagus hard-stem treated with or without enzymatic hydrolysis[1]

Content	Control	Enzymatic hydrolysis
Total free amino acids (mg/100 g)	7448 ± 27.71[a]	11437.33 ± 24.44[b]
Total phenolic content (mg GAE/100 g)	1160.43 ± 13.39[a]	1749.13 ± 5.22[b]
DPPH (%)	56.61 ± 10.52[a]	60.71 ± 11.98[b]
FRAP (μM/L)	1322 ± 20[b]	1072 ± 17.32[a]
ACE inhibition (%)	46.88 ± 2.24[a]	78.38 ± 2.23[b]

[1]Values were presented as mean ± standard deviation of three independent replicates. The mean values followed by lower-case alphabets (a and b) show the significant difference ($p < 0.05$) between the samples treated with or without enzymatic hydrolysis based on the analysis of variance (ANOVA) and Duncan's multiple range tests. GAE, gallic acid equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ACE, angiotensin-converting enzyme.

3.3. Amino acid composition

Enzymatically hydrolysed samples had a significantly ($p < 0.05$) high content of amino acids (6426 mg 100 g^{-1}), which was 1.22-fold higher than the control (5252 mg 100 g^{-1}). Interestingly, the release of amino acids, such as aspartic acid, asparagine, glutamine, and alanine in enzymatically hydrolysed samples, showed significantly ($p < 0.05$) lower value than the control (Table 2). On the other hand, other amino acids, such as histidine, valine, threonine, arginine, tyrosine, γ-aminobutyric acid, tryptophan, phenylalanine, isoleucine, leucine, and lysine, significantly ($p < 0.05$) showed 1.02 to 5.10-times higher content after enzymatic hydrolysis compared to control. Additionally, hydrophobic

Table 2 Amino acid composition of asparagus hard-stem treated with or without enzymatic hydrolysis[1]

Amino acid composition (mg/100 g)	Control	Enzymatic hydrolysis
Aspartic acid	248.14 ± 15.20[a]	213.46 ± 3.18[b]
Glutamic acid	169.83 ± 5.32[a]	161.47 ± 4.93[b]
Asparagine	1327.87 ± 25.25[a]	1250.73 ± 41.25[b]
Serine	381.01 ± 32.46[a]	363.30 ± 11.04[b]
Glutamine	776.06 ± 38.76[a]	708.34 ± 8.71[b]
Histidine	187.85 ± 21.26[a]	191.21 ± 9.03[b]
Threonine	135.04 ± 13.63[b]	376.29 ± 6.08[a]
Arginine	240.14 ± 15.31[b]	259.81 ± 2.47[a]
Alanine	417.50 ± 5.53[a]	320.97 ± 28.29[b]
Tyrosine	77.76 ± 1.12[b]	214.65 ± 7.62[a]
γ-Aminobutyric acid	634.08 ± 110.46[b]	925.84 ± 140.77[a]
Valine	140.73 ± 11.14[a]	152.01 ± 4.08[b]
Methionine	102.50 ± 35.86[a]	92.72 ± 21.04[b]
Tryptophan	68.07 ± 35.45[a]	105.17 ± 9.81[b]
Phenylalanine	65.16 ± 5.56[b]	194 ± 1.87[a]
Isoleucine	80.40 ± 4.92[b]	112.70 ± 2.82[a]
Leucine	101.65 ± 5.41[b]	277.94 ± 2.67[a]
Lysine	99.16 ± 19.41[b]	505.99 ± 59.14[a]
Total	5252.97 ± 2.28[b]	6426.60 ± 5.67[a]

[1]Values were presented as mean ± standard deviation of three independent replicates. The mean values followed by lower-case alphabets (a and b) show the significant difference ($p < 0.05$) between the samples treated with or without enzymatic hydrolysis based on the analysis of variance (ANOVA) and Duncan's multiple range tests.

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amino acids (alanine, valine, leucine, isoleucine, phenylalanine, methionine, and tryptophan) exhibited an increase of 39% after enzymatic hydrolysis, indicating that most of the amino acids were hydrophobic with long carbon chains. These variations in amino acid composition could be attributable to the deamination and decarboxylation of the amino acid structure (Shen & Sergi, 2021). There are, however, other possible facts including consequences of hydrolysis that enhanced the polymerisation of amino acids with each other or together to form different types of peptides and proteins (Kiewiet *et al*., 2018). Due to an extensive hydrolysis, the peptide bonds in proteins may decompose and promote the elution of hydrophobic amino acids. Generally, protein degradation and amino acid metabolism in oxidising conditions could enhance the production of amino acids. Another study by Liu *et al*. (2021) concluded the ACE inhibitory potential of antioxidant amino acids, such as valine, alanine, leucine, tyrosine, and phenylalanine, extracted from wheat gluten oligopeptides. Taken together, the enzymatic hydrolysis enhanced the release of amino acids from asparagus hard-stem, especially hydrophobic and aromatic amino acids, which may play a vital role in the inhibition of ACE and also have the ability to chelate zinc ions in the ACE active site, thereby blocking the catalytic activity of ACE (Al Shukor *et al*., 2013).

3.4. Individual phenolic compounds

A total of 8 phenolic compounds were detected in the enzymatically hydrolysed sample with a total quantity of 638 mg 100 g^{-1}, while 11 compounds were identified in the control sample with a total quantity of 543 mg 100 g^{-1}, which was 1.17-fold lower content than the total individual phenolic content of treated samples (Table 3). Among the identified phenolic compounds, gallic acid and syringic acid showed a significant increase in their quantity after enzymatic hydrolysis, which was found to be 2-fold higher than the control. However, the quantity of other phenolic compounds, such as catechin, chlorogenic acid, caffeic acid, ferulic acid, and cinnamic acid, was found to be significantly ($p < 0.05$) decreased in samples treated with enzymatic hydrolysis than those in control. Catechin, epicatechin, and *p*-coumaric acid present in control found to be not detected after the enzymatic treatment, indicating the transformation zmodification of phenolic compounds during enzymatic hydrolysis. Rutin showed insignificant difference ($p > 0.05$) between the samples treated with or without enzymatic hydrolysis. The increase in gallic acid content could be ascribed to the formation of natural plant cell wall that enhanced the hardness of the outer cell wall (Tsai *et al*., 2010). Thus, enzymatic hydrolysis facilitated the cleavage of bonds in molecules present in the outer cell wall of asparagus hard-stem and favoured the release of gallic acid. A similar behaviour was observed by Tsai *et al*. (2010), Wallis & Chen (2012), and Pinelo *et al*. (2006), in which authors concluded the role of gallic acid, ferulic acid, and other phenolic compounds in stiffening the structure of plant cell walls by binding among polysaccharides and carboxyl group of phenolic compounds. A study evaluated the interdependence of phenolic compounds, such as gallic acid and vanillic acid, extracted from the leaves of *Elsholtzia communis* which showed the ability to inhibit ACE and control the occurrence of high blood pressure (Nath *et al*., 2020). Based on the aforementioned studies and our findings, it was observed that the generation of phenolic compounds, such as gallic acid, could be used for the prevention of elevated blood pressure and improve heart health.

Table 3 Individual phenolic compounds of asparagus hard-stem treated with or without enzymatic hydrolysis[1]

Phenolic compounds (mg/100 g)	Control	Enzymatic hydrolysis
Gallic acid	205.37 ± 31.37[b]	547.68 ± 26.97[a]
3,4-Dihydroxybenzoic acid	44.77 ± 1.39[a]	6.54 ± 0.19[b]
Esculetin	ND[†]	ND
Catechin	148.93 ± 4.64	ND
Chlorogenic acid	85.53 ± 0.96[a]	29.62 ± 4.91[b]
Vanilic acid	ND	ND
Caffic acid	3.65 ± 0.13[a]	1.24 ± 0.02[b]
Syringic acid	0.70 ± 0.02[b]	1.37 ± 0.21[a]
Epicatechin	4.66 ± 0.23	ND
p-Coumaric acid	3.22 ± 1.73	ND
Ferulic acid	4.40 ± 0.89[a]	2.21 ± 0.85[b]
Sinapic acid	ND	ND
Ethyl 3,4-dihydroxybenzoate	ND	ND
Rutin	45.53 ± 1.97[a]	43.46 ± 1.53[a]
Ellagic acid	ND	ND
Morin	ND	ND
Cinnamic acid	1.19 ± 0.04[a]	1 ± 0.03[b]
Quercetin	ND	ND
Kaempferol	ND	ND
Total	543.55 ± 10.28[b]	638.33 ± 5.24[a]

[1]Values were presented as mean ± standard deviation of three independent replicates. The mean values followed by lower-case alphabets (a and b) show the significant difference ($p < 0.05$) between the samples treated with or without enzymatic hydrolysis based on the analysis of variance (ANOVA) and Duncan's multiple range tests.
[†]ND = not detected.

3.5. Correlation

The correlation among amino acids, phenolic compounds, total free amino acids, total phenolic content, and ACE inhibition is shown in Table 4. The correlation (r) between amino acids and total free amino acids ranged from 0.640 to 0.999 ($p < 0.05$), revealing

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Table 4 The correlation coefficient among total free amino acids, total phenolic content, and ACE inhibition[1]

	TFAA	TPC	ACE inhibition
Amino acids			
Threonine	0.997**	0.996**	0.980**
Arginine	0.740	0.730	0.690
Tyrosine	0.998**	0.997**	0.987**
γ-Aminobutyric acid	0.817*	0.812*	0.760
Valine	0.640	0.620	0.590
Tryptophan	0.660	0.650	0.590
Phenylalanine	0.999**	0.997**	0.982**
Isoleucine	0.980**	0.976**	0.959**
Leucine	0.999**	0.998**	0.982**
Lysine	0.985**	0.984**	0.960**
Phenolic compounds			
Gallic acid	0.991**	0.989**	0.966**
Syringic acid	0.998**	0.997**	0.980**
p-Coumaric acid	0.570	0.560	0.540

[1]Values were presented as mean of three independent replicates. The mean values followed superscript ** and * represents significance difference at $p < 0.01$ and 0.05, respectively, based on Pearson's correlation analysis. TFAA, total free amino acids; TPC, total phenolic content; ACE, angiotensin-converting enzyme.

a strong positive relationship. This striking interdependence could be contributed to the amino acids, such as threonine, tyrosine, *r*-aminobutyric acid, phenylalanine, isoleucine, leucine, and lysine ($r = 0.817$, $p < 0.05$ to 0.999, $p < 0.01$). Similarly, total phenolic content significantly had a positive correlation with amino acids, such as threonine, tyrosine, *r*-aminobutyric acid, phenylalanine, isoleucine, leucine, and lysine ($r = 0.812$, $p < 0.05$ to 0.998, $p < 0.01$). A strong positive correlation was found between ACE inhibition and amino acids, such as threonine ($r = 0.980$, $p < 0.01$), arginine ($r = 0.690$), tyrosine ($r = 0.987$, $p < 0.01$), *r*-aminobutyric acid ($r = 0.760$), valine ($r = 0.590$), tryptophan ($r = 0.590$), phenylalanine ($r = 0.982$, $p < 0.01$), isoleucine ($r = 0.959$, $p < 0.01$), leucine ($r = 0.982$, $p < 0.01$), and lysine ($r = 0.960$, $p < 0.01$). Phenolic compounds, such as gallic acid and syringic acid, were highly correlated with ACE inhibition ($r = 0.966$ and 0.980, respectively, at $p < 0.01$). Previous study by Zieliński *et al.* (2020) reported a significantly ($p < 0.05$) strong correlation between the total phenolic content and ACE inhibition. Another study by Paiva *et al.* (2017) concluded the strong correlations between ACE inhibition and antioxidant activities in relation to amino acids and phenolic compounds during enzymatic hydrolysis, suggesting that the ACE inhibition activity might be more related to the amino acid composition and phenolic compounds of asparagus hard-stem. Thus, the correlation analysis proved that the presence of phenolic compounds and amino acids in asparagus hard-stem is the main basis to exert their ACE inhibition activity.

Conclusions

The results of this study showed that the asparagus hard-stem is a rich source of amino acids, phenolic compounds, and has ACE inhibitory activity. Hydrophobic amino acids and gallic acid are the major bioactive compounds and the release of these was enhanced by the enzymatic hydrolysis of asparagus hard-stem. The increase in amino acids and phenolic compounds, especially gallic acid, played a vital role in inhibiting ACE. Based on these findings, enzymatic hydrolysis can promote the release of bioactive compounds, which can act as natural ACE inhibitors. Hence, this study expanded the potential use of asparagus hard-stem exerting antioxidant and ACE inhibitory activities as a promising natural alternative for the development of novel functional supplements rich in health-promoting compounds for controlling elevated blood pressure and related health risks.

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Conflict of interest

The authors declare no conflict of interests.

Author contribution

Yu-Chun Huang: Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead). **Kandi Sridhar:** Resources (equal); Validation (supporting); Visualization (supporting); Writing – original draft (lead); Writing – review & editing (supporting). **Pi-Jen Tsai:** Conceptualization (lead); Funding acquisition (lead); Project administration (lead); Supervision (lead); Writing – review & editing (lead).

Ethical statement

Ethics approval was not required for this research.

Peer review

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Data availability statement

The research data are available within the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary Material

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