

Centella asiatica (L.) Urb. Stem-Enriched Cookies: Reducing Glycemic Index and Improving Phenolic and Chlorophyll Release in the Intestine

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Asiatic pennywort (*Centella asiatica* (L.) Urb.) stems, although rich in dietary fiber and phenolic compounds, are usually discarded during processing. This study investigated the potential of Asiatic pennywort stem powder (APSP) as a partial substitute for soft wheat flour at varying levels (0–20%, w/w) in cookie production. Nutritional composition, physicochemical properties, glycemic response, release of phenolics and chlorophylls at the intestinal stage, and overall acceptability of the fortified cookies were analyzed and compared. The use of APSP markedly increased dietary fiber, total phenolic, total flavonoid, and chlorophyll contents of the fortified cookies. Consumer evaluation confirmed acceptance of cookies produced by substituting soft wheat flour with APSP up to 15% (w/w). The APSP fortification effectively reduced the glycemic index of cookies, placing them within the low-GI category of food products, and significantly improved the release of total phenolics (5.8–7.7 fold) and total flavonoids (4.7–6.5 fold) at the intestinal phase when compared to the control sample. Overall, the findings demonstrate that APSP is a promising sustainable ingredient for the development of health-oriented baked products, while supporting environmental sustainability and a circular economy.

Keywords: antioxidant capacity, Asiatic pennywort, consumer acceptability, dietary fiber, *in vitro* digestion

INTRODUCTION

Centella asiatica (L.) Urb., commonly known as Asiatic pennywort, a species classified within the *Apiaceae* family, is regarded as a medicinal plant that has traditionally been applied in Asia and South Africa for the treatment of wounds due to its antibacterial properties. In addition, it has been reported to exhibit a wide range of biological activities, including antibacterial, antiprotozoal, antitumor, antiviral, anxiolytic, anabolic, memory-enhancing, hepatoprotective, antiproliferative, and neuroprotective, as well as protective effects against gastric ulcers and diabetes [Das,

2011]. In addition to its therapeutic uses, Asiatic pennywort is eaten as a fresh vegetable and is commonly processed into beverages in several countries. Its stems are often discarded or used for animal feeding and their application in food formulations has not yet been documented. However, this residue could retain substantial amounts of dietary fiber as well as phenolic compounds, including especially flavonoids [Das, 2011]. Beyond their nutritional advantages, incorporating Asiatic pennywort stems into food formulations can also help mitigate environmental problems and support a circular economy.

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Cookies represent an important category of snack items due to their extended shelf life and the wide range of flavors available to consumers. However, their typically high levels of sugar and fat, combined with a low fiber content, render them nutritionally unbalanced [Yoo & Kim, 2009]. The rise in unhealthy dietary patterns, frequent intake of calorie-rich foods, and increasingly sedentary lifestyles have played a major role in the significant surge of obesity and diabetes, conditions that have now escalated to an epidemic level [Hariharan *et al.*, 2022]. Growing evidence linking diet to chronic diseases in the scientific literature has further increased public awareness of the importance of healthy eating [Nickel *et al.*, 2024]. As a result, considerable research efforts have been focused on reformulating cookies by reducing sugar and fat levels and enriching them with dietary fiber and bioactive compounds [Alqahtani *et al.*, 2024; Udeh *et al.*, 2024; Weldeyohannis Gebremariam *et al.*, 2024]. The utilization of tea leaves [Nguyen *et al.*, 2022] or *Asparagus officinalis* L. hard stems [Tran *et al.*, 2024] in cookie/cracker making has recently been reported to improve dietary fiber and phenolic levels, leading to additional health benefits. Nevertheless, the stems of Asiatic pennywort have not yet been incorporated into cookie formulations in the literature.

The objective of this research was, therefore, to characterize Asiatic pennywort stem powder (APSP) and evaluate its suitability for partial substitution of soft wheat flour (SWF) in cookie production. This study examined how APSP incorporation influences nutritional composition, physicochemical properties, glycemic response, release of phenolics and chlorophylls at the intestinal phase, and consumer preferences. This practical innovation contributes to sustainable development by reducing agricultural and food processing waste and promoting the utilization of by-products, in line with global sustainability initiatives.

MATERIALS AND METHODS

■ Raw materials and chemicals

Asiatic pennywort stems, which were manually separated from the whole plant, were obtained from local fields in Dong Thap Province (Vietnam). Upon arrival at the laboratory, the stems were thoroughly washed to eliminate contaminants by tap water and then were subsequently dehydrated in a laboratory convection oven at 50°C for 4 h until the moisture content decreased below 12 g/100 g. The dried stems were pulverized and passed through a 70-mesh sieve (210 µm aperture size). The resulting Asiatic pennywort stem powder (APSP) was sealed in polyethylene packaging and stored under refrigerated (4°C), light-protected conditions until use. Soft wheat flour (SWF) was supplied by Dai Phong Flour Joint Stock Company (Can Tho City, Vietnam). Additional ingredients, including lecithin, monocalcium phosphate, baking powder, table salt, saccharose, and soybean cooking oil were obtained from a local retail market.

Enzyme preparations used for dietary fibre analysis included Termamyl SC DS (α -amylase), Dextrozyme GA (glucoamylase), and Alcalase 2.5 L (protease), all obtained from Novozymes (Bagsværd, Denmark). For the *in vitro* digestion experiments,

salivary α -amylase, pepsin derived from porcine gastric mucosa, pancreatin from porcine pancreas (8×USP specifications), and bile salts were supplied by Merck KGaA (Darmstadt, Germany). Analytical-grade reagents, including Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, gallic acid, Trolox, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and 3,5-dinitrosalicylic acid (DNS), were also purchased from Merck KGaA.

■ Cookie preparation

Five formulations with different levels of SWF replacement with APSP (0, 5, 10, 15, and 20%; w/w) were used to produce cookies designated as C0, C5, C10, C15, and C20, respectively. The total weight of flours (SWF and APSP) in the formulation was 125 g. The remaining ingredients and the cookie preparation process followed the procedure described by Mai *et al.* [2022]. In addition to flours, the formulation also contained butter (58.33 g), one whole chicken egg, isomalt (38.83 g), water (10.83 mL), baking soda (1.33 g), table salt (0.55 g), vanilla (0.5 g), and acesulfame potassium (0.15 g). A solution of isomalt, table salt, and acesulfame potassium in water was added to the egg (beaten for 4 min), and the mixture was blended in a KitchenAid mixer (KSMC895OB, Whirlpool Corporation, Benton Harbor, MI, USA) for 4 min. Butter, vanilla, and baking soda were subsequently incorporated, and the mixture was agitated at 100 rpm for 4 min. Wheat flour and the corresponding ASPs were then added, followed by kneading at 100 rpm for 2 min. The resulting dough was manually rolled into a 4-mm-thick sheet and shaped into circular pieces using a 33-mm-diameter cookie cutter. The shaped dough pieces were baked at 175°C for 15 min in an HO-K60B oven (Häfele SE & Co KG, Nagold, Germany). After baking, cookies were packaged in aluminum zip-lock stand-up pouches and stored at 25°C for 24 h prior to subsequent analyses.

■ Nutritional composition analysis

Moisture content was determined using an MB23 moisture analyzer (Ohaus, Parsippany, NJ, USA) by drying the samples at 105°C until a constant weight. Total dietary fiber (TDF) (991.43 AOAC), soluble dietary fiber (SDF) (993.19 AOAC), insoluble dietary fiber (IDF) (991.42 AOAC), total starch (996.11 AOAC), ash (942.05 AOAC), protein (979.09 AOAC), and lipid (948.22 AOAC) contents were determined following relevant standard protocols of the Association of Official Analytical Chemists (AOAC) [AOAC, 2000]. Total carbohydrate content was estimated by difference, subtracting determined ash, lipid, and protein contents from the total dry matter. Contents of these components in APSP, SWF, and cookies were expressed as g/100 g dry matter (dm). Energy value of cookies (kcal/100 g) was calculated based on an Equation (1), considering dietary fiber as a non-caloric component in human nutrition [Ozgoren *et al.*, 2019]:

$$\text{Energy value} = (\text{Carbohydrates} \times 4) + (\text{Proteins} \times 4) + (\text{Lipids} \times 9) \quad (1)$$

■ Determination of chlorophyll content, total flavonoid content, total phenolic content, and antioxidant capacity

For chlorophyll content (CC) determination, 100 mg of APSP, SWF or cookies were homogenized in a mortar with 4 mL of 80% (v/v) aqueous acetone [Roshanak *et al.*, 2016]. The resulting homogenate was quantitatively transferred into a Falcon tube using additional 4 mL of 80% (v/v) aqueous acetone. All tubes were protected from light and maintained on ice throughout the procedure. The samples were centrifuged at 1,500×g for 10 min at 4°C using a 3K30 centrifuge (Sigma Zentrifugen Ltd., Osterode am Harz, Germany). Following centrifugation, the supernatant volume was adjusted to 10 mL using the same solvent, and absorbance was recorded at 663.2 nm ($A_{663.2}$) and 646.8 nm ($A_{646.8}$) using the UV5Bio spectrophotometer (Mettler Toledo, Switzerland) with a 1-cm optical path cuvette. Chlorophyll concentration in the extract (C_{total} , mg/L) was calculated as the sum of chlorophyll *a* concentration (C_a) and chlorophyll *b* concentration (C_b), which were obtained according to Equation (2) and Equation (3), respectively:

$$C_a = 12.25 A_{663.2} - 2.79 A_{646.8} \quad (2)$$

$$C_b = 21.50 A_{646.8} - 5.10 A_{663.2} \quad (3)$$

The chlorophyll content of APSP, SWF or cookies was subsequently expressed on a dry matter basis (mg/100 g dm).

For total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity determinations, APSP, SWF and cookies were extracted with acidified aqueous methanol (methanol/HCl/water, 80/1/20, v/v/v) at 30°C for 1 h; the solid to solvent ratio was 1/15 (w/v) [Tran *et al.*, 2024].

TPC was determined using the colorimetric assay with the Folin-Ciocalteu reagent, following the procedure previously employed by Ayele *et al.* [2022] with slight modification. Briefly, 500 µL of the diluted extract (prepared by diluting 500 µL of the primary extract to 50 mL with 80% (v/v) methanol) were mixed with 2.5 mL of the Folin-Ciocalteu reagent and 2 mL of a 7.5% sodium carbonate solution. The reaction mixture was incubated at 45°C for 15 min, after which absorbance was measured at 765 nm using 80% (v/v) methanol as the blank. Results were expressed as mg of gallic acid equivalents (GAE) per kg of dm of APSP, SWF, or cookies.

TFC was determined according to the method described by Manye *et al.* [2023]. An aliquot of extract (0.5 mL) was combined with 2 mL of distilled water and 0.15 mL of a 5% sodium nitrite solution. After 6 min of incubation, 0.15 mL of a 10% aluminum chloride solution was added, and the mixture was allowed to react for an additional 6 min. Subsequently, 2 mL of a 4% sodium hydroxide solution were introduced, and distilled water was added to obtain a final volume of 5 mL. The mixture was thoroughly vortexed and left to stand for 15 min at room temperature. The absorbance of the resulting pink-colored complex was measured at 510 nm. Quercetin was used as the standard,

and TFC was calculated from the quercetin calibration curve and expressed as mg quercetin equivalents (QE) per kg of dm.

Antioxidant capacity was evaluated using ferric reducing antioxidant power (FRAP) [Benzie & Devaki, 2018] and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays [Gulcin & Alwasel, 2023]. For the DPPH assay, 0.1 mL of the extract (ASPS extract was diluted in solvent at a 1 to 3 ratio (v/v); other extracts were not diluted) was combined with 3.9 mL of a 60 µM DPPH radical solution prepared in methanol. The mixture was incubated at room temperature in the absence of light. Absorbance was recorded at 515 nm immediately after mixing and again after 30 min of incubation, with methanol serving as the blank. For the FRAP assay, 0.2 mL of the extract (ASPS extract was diluted in solvent at a 1 to 15 ratio (v/v); other extracts were not diluted) was mixed with 3.8 mL of freshly prepared FRAP reagent consisting of 25 mL of acetate buffer (0.3 M, pH 3.6), 2.5 mL of a TPTZ solution (10 mM in 40 mM HCl), and 2.5 mL of an $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ solution (20 mM). The reaction mixture was incubated in the dark at 37°C for 5 min prior to absorbance measurement at 593 nm. Acidified methanol was used as the blank. For both assays, antioxidant capacity was quantified using a Trolox standard curve and expressed as µmol of Trolox equivalents (TE) per kg of APSP, SWF, or cookies dry matter.

■ Determination of physical properties

Water and oil holding capacities of SWF and APSP were determined based on previously established methods [Jose *et al.*, 2022]. Briefly, 1 g of powder was dispersed in 10 mL of distilled water/soybean cooking oil and maintained at room temperature for 30 min. The suspension was subsequently centrifuged at 2,000×g for 30 min at 5°C. The water/oil holding capacities were expressed as g of water/oil retained per g of sample on a dry matter basis.

Cookie diameter (mm) and thickness (mm) were recorded using a digital vernier caliper, and the spread ratio was computed as the quotient of diameter to thickness [Tran *et al.*, 2024].

Texture profile of cookies was assessed with a TA-XT plusC textural analyzer (Stable Micro Systems Co., Godalming, United Kingdom). The hardness (N) of the final cookies was quantified through a three-point bending test [Mudgil *et al.*, 2017]. A trigger force of 25 g and a 50-kg load cell were employed. Texture measurements were performed at a pre-test speed of 1.5 mm/s, test speed of 2.0 mm/s, and post-test speed of 10 mm/s, with a test distance of 10 mm. The distance between the two supporting points was set at 50 mm. The maximum fracture force recorded at the point of cookie breakage into two major pieces was taken as the hardness value.

Color attributes (L^* , a^* , and b^*) were measured instrumentally using a CM-3700A colorimeter (Konica Minolta, Osaka, Japan). The L^* value represents lightness, whereas a^* and b^* values correspond to the red–green and yellow–blue chromatic dimensions, respectively, with positive and negative values indicating the respective color directions. The total color difference (ΔE) of cookies prepared with APSP compared to the control cookies

without APSP was calculated following Equation (4) reported by Weldeyohanis Gebremariam *et al.* [2024].

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (4)$$

where: parameters without a subscript refer to the test samples, while those with the subscript "0" refer to the control samples.

■ Sensory evaluation

Overall consumer acceptability of the cookies was assessed employing a nine-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) [Long *et al.*, 2025]. Sixty panelists (30 men and 30 women, nonsmokers and untrained), aged 18–35, were recruited from students and personnel of the Vietnam National University (Ho Chi Minh City, Vietnam). Before participation, panelists were screened for food allergies and intolerances, and individuals with known allergies or intolerances to wheat/gluten or any ingredients used in the cookie formulations were excluded. Panelists were not informed of the specific formulation or ingredient composition of the samples prior to evaluation. Eligibility required participants to consume cookies at least once *per week*. Samples were labelled with random three-digit numbers and exhibited in a randomized sequence. Purified water was supplied for palate cleansing during evaluations.

The study protocol was approved the Ethics Review Board at the University of Social Sciences and Humanities, Vietnam National University – Ho Chi Minh City (VNU-HCM); approval number 01/GCN-XHNV-HDDNC from November 21st, 2022. Panelists were instructed to read an information sheet and sign a consent form. They provided their voluntary consent and were assured that their responses would remain confidential. They were also informed they could withdraw at any point without any consequences.

■ Glycemic index determination

In vitro glycemic index (GI) for cookie samples was determined in accordance with a protocol outlined by Naknaen *et al.* [2016]. Briefly, 0.5 g of each ground sample (dry matter basis) was suspended in 10 mL of HCl–KCl buffer (pH 1.5) and agitated for 10 min. Subsequently, pepsin (1 mg dissolved in 10 mL of HCl–KCl buffer, pH 1.5) was added, and the mixture was incubated in a shaking water bath at 40°C for 40 min. The resulting suspension was diluted to 25 mL using 0.1 M phosphate buffer (pH 6.9), and the pH was verified with a pH meter. Pancreatic amylase (5 mL, 2.6 U/5 mL) was then introduced, followed by incubation in a shaking water bath at 37°C. At predetermined intervals (0, 15, 30, 60, 90, 120, 150, and 180 min), 1-mL aliquots were withdrawn and immediately heated in boiling water for 5 min before being cooled on ice. The aliquots were centrifuged at 7,800×g for 10 min. An aliquot of the resulting supernatant (1 mL) was combined with 1 mL of 0.1 M sodium acetate buffer (pH 4.5), and the pH was subsequently verified. Amyloglucosidase (30 mL) was then added, and the mixture was incubated at 60°C in a shaking water bath for 45 min. The amount of glucose

released from the samples was quantified using a Glucose Liq-uicolor assay kit (Human, Wiesbaden, Germany) and expressed as mg/g dry sample.

The hydrolysis index (HI) was determined from the starch hydrolysis curve obtained over 180 min. It was expressed as the percentage ratio between the area under the hydrolysis curve of the sample and that of white bread analyzed under identical conditions. The glycemic index (GI) was subsequently calculated according to Equation (5):

$$GI = 39.71 + 0.549 \times HI \quad (5)$$

■ Determination of phenolic and chlorophyll release during the *in vitro* digestion

In vitro digestion of the cookie samples was performed in accordance with a protocol outlined by Brodkorb *et al.* [2019]. The digestion process consisted of three sequential phases: oral, gastric, and intestinal.

For the oral phase, 2.5 g of a crushed cookie sample were mixed with 15 mL of distilled water, and pH was adjusted to 6.5. After incubation for 2 min, the mixture was subjected to the gastric digestion phase. During the gastric phase, 15 mL of distilled water were added into mixture and acidified to pH 2.2 using 6 M HCl. Subsequently, 2.5 mL of a pepsin solution (12.5 mg/mL prepared in 0.1 M HCl) were added. The mixture was incubated for 1 h.

For intestinal digestion, another 15 mL of distilled water were added into mixture, and pH was adjusted to 7.5 with 1 M NaOH. A pancreatin solution (2.5 mL, 10 mg/mL in 1 M NaOH) was then added, followed by incubation for 4 h. All digestion phases were conducted at 37°C in an orbital shaking incubator (Solaris SK2000 R, Thermo Fisher, Waltham, MA, USA) operating at 120 rpm. The mixtures after intestinal digestion were centrifuged at 4°C and 4,924×g for 15 min, and collected supernatants were analyzed for TPC, TFC, and CC as described above.

■ Statistical analysis

Every cookie formulation was produced in three independent batches. Data were shown as mean and standard deviation. Statistical processing was done using Statgraphics Centurion XVII software (v.18.1.12, Statgraphics Technologies, Inc., The Plains, VA, USA). Differences among treatments were identified using Tukey's honestly significant difference test at a significance level of $p < 0.05$. Correlation was calculated from the covariance between two variables, standardized by their respective standard deviations.

RESULTS AND DISCUSSION

■ Characteristics of Asiatic pennywort stem powder and soft wheat flour

The nutritional quality, antioxidant capacity, and functional characteristics of APSP and SWF are summarized in **Table 1**. APSP exhibited higher contents of protein, lipid, and ash compared to SWF. Besides, it contained substantially less total carbohydrate

Table 1. Characteristics of Asiatic pennywort stem powder and soft wheat flour used in the study.

Parameter	Asiatic pennywort powder	Soft wheat flour
Protein (g/100 g dm)	18.24±0.16 ^a	11.86±0.45 ^b
Ash (g/100 g dm)	11.94±0.05 ^a	0.41±0.01 ^b
Lipid (g/100 g dm)	5.53±0.29 ^a	2.75±0.05 ^b
Carbohydrate (g/100 g dm)	64.02±0.24 ^b	84.96±0.42 ^a
Total starch (g/100 g dm)	11.31±0.30 ^b	76.88±0.06 ^a
Total dietary fiber (g/100 g dm)	50.03±0.44 ^a	4.34±0.10 ^b
Insoluble dietary fiber (g/100 g dm)	39.57±0.58 ^a	2.77±0.14 ^b
Soluble dietary fiber (g/100 g dm)	10.46±0.48 ^a	1.57±0.05 ^b
Total phenolics (mg GAE/kg dm)	20,296±978 ^a	1,013±17 ^b
Total flavonoids (mg QE/kg dm)	15,979±800 ^a	764±36 ^b
Chlorophyll (mg/100 g dm)	430.5±5.2 ^a	0.0±0.0 ^b
DPPH radical scavenging activity (μmol TE/kg dm)	4,574±135 ^a	414±10 ^b
Ferric reducing antioxidant power (μmol TE/kg dm)	10,799±188 ^a	211±4 ^b
<i>L</i> *	57.52±0.33 ^b	92.79±0.57 ^a
<i>a</i> *	−12.03±0.24 ^b	0.23±0.01 ^a
<i>b</i> *	54.77±0.11 ^a	6.26±0.17 ^b
Water holding capacity (g/g dm)	6.00±0.23 ^a	1.56±0.06 ^b
Oil holding capacity (g/g dm)	2.28±0.03 ^a	1.28±0.06 ^b

Results are shown as mean ± standard deviation. Means with various superscripts (a–b) in the same row differ significantly ($p < 0.05$). GAE, gallic acid equivalent; QE, quercetin equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TE, Trolox equivalent; dm, dry matter.

than SWF, with starch content being approximately 7 times lower. Notably, TDF accounted for approximately 78% of the total carbohydrates in APSP. The levels of TDF, IDF, and SDF in APSP were 12-, 14-, and 7-fold higher, respectively, than those of SWF, with IDF representing the dominant fiber fraction. Both SDF and IDF are well recognized for their distinct physiological benefits [He *et al.*, 2022]. SDF is known for its positive effects on cholesterol reduction and blood glucose regulation while IDF contributes to digestive health by increasing fecal bulk and facilitating intestinal transit, which may help reduce the risk of constipation [He *et al.*, 2022].

TPC and TFC of APSP were 20 and 21 times, respectively, higher than those of SWF (Table 1). APSP contained chlorophylls, *i.e.*, compounds with various bioactive properties, including anti-inflammatory, antioxidant, and detoxifying, as well as the ability to improve digestion [Pareek *et al.*, 2017].

Correspondingly, in FRAP and DPPH assays, APSP showed higher antioxidant capacity compared to SWF (Table 1). Moreover, it displayed darker, greener, and yellower color than SWF, as indicated by *L**, *a** and *b** values, respectively. APSP exhibited significantly ($p < 0.05$) higher water-holding capacity, which can be attributed to the abundant hydroxyl groups present in both SDF and IDF [Boulos *et al.*, 2000]. Its oil-holding capacity was also significantly ($p < 0.05$) higher than that of SWF. The difference in functional properties between APSP and SWF indicates that APSP may affect the textural and other physical attributes of the fortified cookies.

■ Nutritional quality, bioactive compound content, and antioxidant capacity of cookies prepared with Asiatic pennywort stem powder

As summarized in Table 2, increasing the substitution level of SWF with APSP from 0% to 20% (w/w) resulted in a slight increase in lipid content and a 17% rise in protein content. The cookies prepared with 20% (w/w) APSP exhibited a 2.2-fold increase in ash content, while their total starch content decreased by approximately 36% compared with the control counterpart. Progressive enrichment with APSP significantly ($p < 0.05$) enhanced dietary fiber content in the cookies. Specifically, contents of TDF, SDF, and IDF increased approximately threefold, twofold, and threefold, respectively, when the APSP reached 20% of the total flour in cookie recipe. According to the guidelines for use of nutrition and health claims [Codex Alimentarius, 1997], cookies containing 5% (w/w) APSP can be qualified as high-fiber products, as their TDF content exceeded 6 g/100 g dry matter. Concurrently, the caloric value of cookies declined by about 9% as the APSP ratio in flour increased from 0% to 20% (w/w).

The SWF substitution with APSP significantly increased TPC, TFC, and CC levels in the cookies (Table 1). As a consequence, the cookies prepared with 20% (w/w) APSP exhibited enhancements in antioxidant capacity, with DPPH radical scavenging activity and FRAP increasing 68-fold and 32-fold, respectively, compared to the control. Strong positive correlations were observed between TPC and TFC with antioxidant capacity measured by both DPPH ($r = 0.970$ and 0.989 , respectively) and FRAP ($r = 0.940$ and 0.974 , respectively) assays. A positive correlation was also noted between the DPPH radical scavenging activity and FRAP ($r = 0.992$). Tran *et al.* [2024] revealed that the incorporation of asparagus hard stems into crackers also increased their total phenolic content and antioxidant activity; moreover, the TPC showed a strong positive correlation with the results of FRAP and DPPH assays.

■ Physical characteristics and sensory scores of cookies prepared with Asiatic pennywort stem powder

The dimensions, color parameters, and hardness of cookies prepared without and with APSP are shown in Table 3. Increasing SWF substitution with APSP from 0% to 20% (w/w) led to a 9% reduction in cookie diameter and a 51% decrease in thickness. Cookie thickness is closely related to air retention within

Table 2. Nutritional quality, bioactive compound content, and antioxidant capacity of cookies prepared by substituting 0, 5, 10, 15, and 25% (w/w) of wheat flour with Asiatic pennywort stem powder (C0, C5, C10, C15, and C20, respectively).

Parameter	C0	C5	C10	C15	C20
Protein (g/100 g dm)	10.52±0.25 ^c	11.24±0.25 ^{bc}	11.48±0.27 ^{bc}	11.79±0.51 ^{ab}	12.29±0.41 ^a
Ash (g/100 g dm)	1.26±0.03 ^c	1.69±0.06 ^{bc}	2.04±0.09 ^{bc}	2.42±0.11 ^{ab}	2.89±0.10 ^a
Lipid (g/100 g dm)	10.33±0.01 ^e	10.43±0.02 ^d	10.47±0.01 ^c	10.55±0.03 ^b	10.65±0.03 ^a
Total starch (g/100 g dm)	74.17±1.05 ^a	69.0±0.72 ^b	62.82±1.02 ^c	55.05±1.21 ^d	47.78±1.14 ^e
Total dietary fiber (g/100 g dm)	4.26±0.02 ^e	6.42±0.20 ^d	9.16±0.41 ^c	10.82±0.12 ^b	13.11±0.10 ^a
Soluble dietary fiber (g/100 g dm)	1.40±0.04 ^d	1.76±0.04 ^c	2.63±0.10 ^b	2.48±0.12 ^b	3.07±0.11 ^a
Insoluble dietary fiber (g/100 g dm)	2.86±0.02 ^e	4.66±0.23 ^d	6.54±0.32 ^c	8.34±0.04 ^b	10.04±0.21 ^a
IDF/SDF index	2.04±0.07 ^c	2.65±0.19 ^b	2.49±0.04 ^b	3.37±0.17 ^a	3.27±0.19 ^a
Energy value (kcal/100 g)	429.53±0.07 ^a	419.71±0.94 ^b	407.55±1.97 ^c	399.81±0.31 ^d	389.27±0.15 ^e
Total phenolics (mg GAE/kg dm)	88.2±1.1 ^e	133.3±1.5 ^d	195.9±8.8 ^c	250.1±6.6 ^b	283.5±6.9 ^a
Total flavonoids (mg QE/kg dm)	69.7±3.3 ^e	125.0±5.1 ^d	176.7±2.7 ^c	203.1±6.6 ^b	227.7±1.3 ^a
Chlorophyll (mg/100 g dm)	n.d.	12.8±0.6 ^d	25.0±1.2 ^c	41.2±1.9 ^b	52.4±1.5 ^a
DPPH radical scavenging activity (μmol TE/kg dm)	20.2±1.8 ^e	233.2±7.5 ^d	532.0±18.0 ^c	856.6±24.9 ^b	1,380.3±17.2 ^a
Ferric reducing antioxidant power (μmol TE/kg dm)	45.4±1.1 ^e	352.5±4.8 ^d	739.3±3.6 ^c	1,011.2±9.4 ^b	1,462.4±13.3 ^a

Results are shown as mean ± standard deviation. Means with various superscripts (a–e) in the same row differ significantly ($p < 0.05$). IDF, insoluble dietary fiber; SDF, soluble dietary fiber; GAE, gallic acid equivalent; QE, quercetin equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TE, Trolox equivalent; n.d., not detected; dm, dry matter.

Table 3. Physical properties of cookies prepared by substituting 0, 5, 10, 15, and 25% (w/w) of wheat flour with Asiatic pennywort stem powder (C0, C5, C10, C15, and C20, respectively).

Parameter	C0	C5	C10	C15	C20
Diameter (mm)	45.45±0.11 ^a	44.56±0.6 ^b	43.67±0.29 ^{bc}	42.63±0.25 ^{cd}	41.35±0.51 ^d
Thickness (mm)	11.57±0.31 ^a	9.50±0.50 ^b	7.80±0.30 ^c	6.69±0.18 ^d	5.68±0.29 ^e
Spread ratio	3.93±0.11 ^e	4.69±0.22 ^d	5.60±0.23 ^c	6.37±0.14 ^b	7.29±0.32 ^a
Hardness (N)	4,133±112 ^e	4,593±227 ^d	4,895±100 ^c	5,284±106 ^b	5,718±137 ^a
L^*	71.64±0.20 ^a	53.78±0.14 ^b	48.87±0.08 ^c	45.82±0.12 ^d	37.45±0.35 ^e
a^*	0.81±0.03 ^a	−12.96±0.70 ^b	−15.12±0.1 ^c	−20.20±0.17 ^d	−22.54±0.38 ^e
b^*	43.87±0.52 ^d	51.47±0.46 ^a	48.10±0.05 ^b	46.11±0.15 ^c	41.52±0.57 ^e
ΔE	0.00±0.00 ^e	23.81±0.48 ^d	28.11±0.07 ^c	33.36±0.05 ^b	41.48±0.26 ^a

Results are shown as mean ± standard deviation. Means with various superscripts (a–e) in the same row differ significantly ($p < 0.05$). L^* value represents lightness; a^* value represents green–red color; b^* value represents yellow–blue color; ΔE , total color difference.

the dough matrix [Nammakuna *et al.*, 2016], which in turn depends on the foaming stability of the ingredients [Sun *et al.*, 2023]. In our study, the formulations containing lower APSP levels, and therefore having a higher gluten content, were more capable of entrapping air during baking, which resulted in their greater vertical expansion. Conversely, higher APSP

incorporation significantly increased the spread ratio, which is an important indicator of cookie quality, with a higher value generally associated with more desirable cookie characteristics [Mudgil *et al.*, 2017].

Cookie hardness increased by approximately 1.4 times as the APSP ratio in the total flour rose to 20% (w/w) (Table 3),

The underlying mechanisms of this phenomenon warrant further investigation, particularly with respect to gluten network development, gluten-starch-fiber interactions, and the interactions of other ASPS components under the fixed water conditions used in this study. Grzelczyk *et al.* [2025] also noted that the hardness and breaking energy of cookies made with bamboo flour were higher than those of the control counterpart in the 3-point test.

The substitution of SWF with APSP also reduced lightness (L^*) while intensifying greenness (a^*) and yellowness (b^*) of the cookies (Table 3). These color changes were mainly attributed to the presence of natural pigments in Asiatic pennywort (chlorophylls). The total color difference for all cookies produced with ASPS compared to the control (without ASPS) was greater than 5, making them visually discernible.

Overall acceptability scores (Figure 1) indicated that the cookies prepared with up to 15% (w/w) APSP in the total flour were generally acceptable (overall acceptability score >5); however, APSP incorporation should preferably be limited to 10% (w/w) to achieve greater consumer acceptance and slight preference for the product (overall acceptability score >6). During the short interview, most participants indicated that the reduced acceptability at higher APSP levels was mainly due to these substantial reductions in cookie size, combined with increased hardness. The reduced expansion of the cookies is attributed to the fiber of APSP, which interferes with the development of the gluten network and gluten–starch matrix, contributing to gas retention [Long *et al.*, 2025; Sun *et al.*, 2023]. In addition, the strong WHC of fiber limits water availability for starch hydration, swelling, and gelatinization [Ibrahim *et al.*, 2023]. Consequently, the fiber–starch–protein matrix becomes more compactly structured, resulting in a harder cookie texture.

■ Glycemic index and release of phenolics and chlorophylls from cookies with Asiatic pennywort stem powder at the intestinal phase

The GI of cookies prepared with different APSP ratios in the total flour is shown in Figure 2. The GI is a key parameter used to determine how quickly carbohydrate-rich foods elevate postprandial blood glucose levels. Maintaining a diet consisting of low-GI foods has been associated with reduced risks of obesity, diabetes, cardiovascular diseases, certain cancers, and several chronic health conditions [Kaur *et al.*, 2022]. The control cookie showed a GI value of 56.8 when glucose was used as the reference. Because of replacing sucrose with isomalt and acesulfame potassium, the control sample fell into the medium-GI category (GI=56–69) [Atkinson *et al.*, 2021], while several commercial cookies have been classified as high-GI products [Trinidad *et al.*, 2010]. All cookies prepared with 5–20% APSP in the total flour (w/w) were categorized as low-GI foods (GI<56). In particular, the cookies from the formulation containing 20% (w/w) APSP had a GI value approximately 10% lower than that of the control counterpart, likely owing to the decreased starch proportion in the fortified cookies. Additionally, some types of phenolics

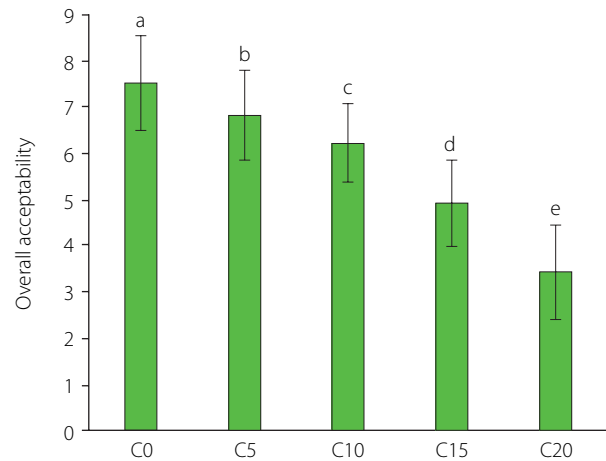


Figure 1. Overall acceptability of cookies prepared by substituting 0, 5, 10, 15, and 25% (w/w) of wheat flour with Asiatic pennywort stem powder (C0, C5, C10, C15, and C20, respectively). Means designated by various lowercase letters (a–e) differ significantly ($p < 0.05$).

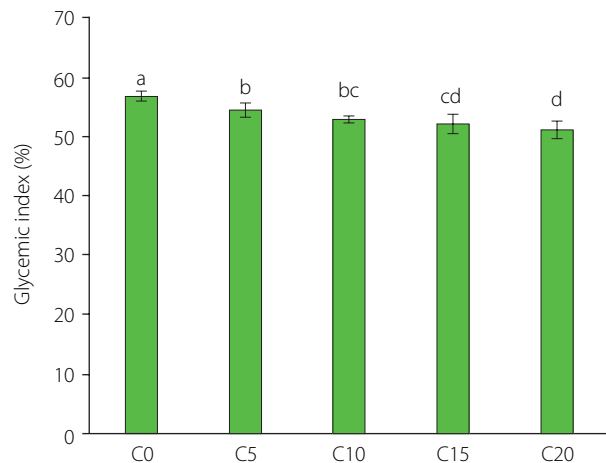


Figure 2. Glycemic index of cookies prepared by substituting 0, 5, 10, 15, and 25% (w/w) of wheat flour with Asiatic pennywort stem powder (C0, C5, C10, C15, and C20, respectively). Means designated by various lowercase letters (a–d) differ significantly ($p < 0.05$).

may form hydrogen bonds with starch molecules, limiting its hydrolysis into glucose during digestion [Chi *et al.*, 2017; Zhu, 2015]. Besides, the phenolic compounds may inhibit the activities of α -amylase and α -glucosidase, thereby lowering the GI [Sun & Miao, 2020].

Contents of total phenolics, total flavonoids, and chlorophylls released at the intestinal digestion phase are shown in Figure 3. The total phenolic content released from the fortified cookies was 5.8–7.7 times higher than that of control cookies, and the released TPC showed strong positive correlations with antioxidant capacity determined by the DPPH assay ($r=0.997$) and the FRAP assay ($r=0.992$). Moreover, at the intestinal phase of digestion, the total content of released flavonoids was 4.7–6.5 times higher than that in the cookies prepared without APSP. The increased total phenolic and total flavonoid contents of the *in vitro* digested samples when compared to respective untreated samples may be attributed to the release of a large proportion of these

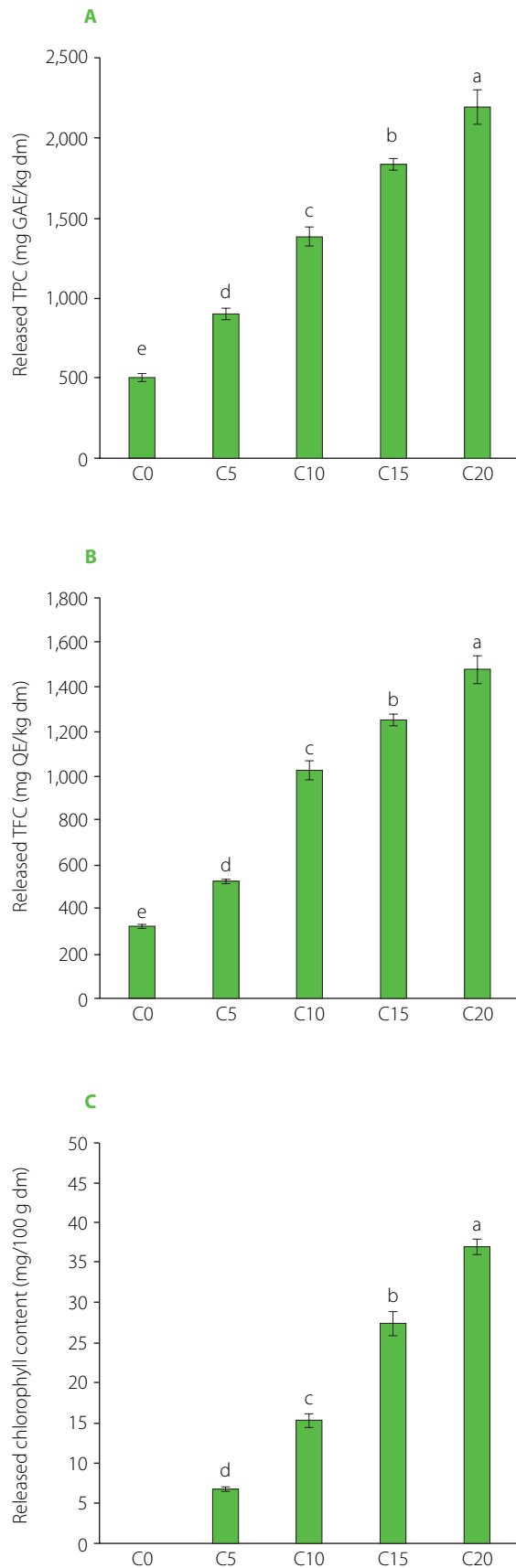


Figure 3. Contents of total phenolics, TPC (A), total flavonoids, TFC (B), and chlorophyll (C) released from cookies prepared by substituting 0, 5, 10, 15, and 25% (*w/w*) of wheat flour with Asiatic pennywort stem powder (C0, C5, C10, C15, and C20, respectively) at the intestinal digestion phase. Means designated by various lowercase letters (a–e) differ significantly ($p < 0.05$). GAE, gallic acid equivalent; QE, quercetin equivalent; dm, dry matter.

compounds from their previously bound forms within the fiber–protein–starch–lipid matrices, facilitated by digestive enzymes during *in vitro* simulation [Lucas-González *et al.*, 2021]. Pinto *et al.* [2023] observed that, after the intestinal phase, the TPC did not differ significantly from that of the sample not subjected to the enzymatic treatment, whereas the TFC and antioxidant capacity, as determined by the DPPH and FRAP assays, increased significantly. Additionally, after digestion in the intestinal phase, the chlorophyll content increased when the APSP proportion in the cookie recipe increased from 0 to 20% (*w/w*) (Figure 3C), most likely due to the chlorophyll naturally present in APSP. On the other hand, compared with the corresponding undigested sample, the chlorophyll content was lower after digestion. This finding aligns with observations made by Viera *et al.* [2022] who have reported that chlorophyll is extensively degraded under acidic digestive conditions.

CONCLUSIONS

Overall, APSP fortification significantly enhanced the nutritional composition and functional properties of the cookies, suggesting its potential application in the development of health-promoting food products. The substitution of wheat flour with APSP up to 20% (*w/w*) resulted in significant enhancements in the dietary fiber, total phenolic, total flavonoid, and chlorophyll contents of the cookies. Moreover, antioxidant capacity of the cookies prepared with APSP increased substantially in FRAP and DPPH assays. Although the use of APSP undesirably modified several physicochemical parameters of the cookies (hardness and color), consumers still accepted those prepared with 15% (*w/w*) APSP. However, based on the hedonic scores, APSP supplementation should be limited to 10% (*w/w*) to make them acceptable by consumers (score ≥ 6). APSP fortification also decreased the GI of cookies, placing them within the low-GI category foods, while promoting the release of phenolics at the intestinal phase when compared to the control sample. Generally, these results demonstrate that APSP could be used as a functional and sustainable ingredient of cookies. Further studies should examine how APSP affects cookie quality during storage.

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CONFLICT OF INTERESTS

All authors declare that we have no conflicts of interest.

ADDITIONAL INFORMATION

The study protocol was approved the Ethics Review Board at the University of Social Sciences and Humanities, Vietnam National University – Ho Chi Minh City (VNU-HCM); approval number 01/GCN-XHNV-HDDDNC from November 21st, 2022.

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