

Article

Forage Potential of Faba Bean By-Products: A Comprehensive Analysis of Proximate Nutrients, Mineral Content, Bioactive Components, and Antioxidant Activities

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Abstract

The global feed gap, driven by seasonal shortages and climate change, highlights the need for novel forage resources. *Vicia faba* (Faba bean) produces substantial above-ground biomass as residue after fresh pod harvest, which remains underutilized. This study comprehensively evaluated the forage potential of faba bean leaves and stems across three growth stages: flowering (S1), pod development (S2), and ripening (S3). Dry matter content peaked at S2 in both tissues, while crude protein and fat content were highest at S1; carbohydrate levels increased progressively with maturation. Significant mineral concentrations, particularly K, Ca, and Mg, were detected, with leaves at S2 showing higher ash (i.e., mineral) content. Bioactive compounds (L-dopa, flavonols, total phenolics, and flavonoids) and antioxidant activities were most abundant at S1, with strong positive correlations between phenolics and antioxidant activities. Overall, faba bean residues offer proximate nutritional profiles comparable to traditional forages such as alfalfa and clover, while providing superior antioxidant potential. Their incorporation into animal feed systems before S3 could help mitigate seasonal forage shortages and enhance the nutritional quality of livestock diets.

Keywords: feed; *Vicia faba* L.; agricultural waste; growth stage; phenolics



Academic Editor: Jianbin Zhou

Received: 24 September 2025

Revised: 21 October 2025

Accepted: 23 October 2025

Published: 24 October 2025

Citation: Duan, S.; Kwon, S.-J.; Kim, J.W.; Kim, J.H.; Lee, J.W.; Kim, M.-S.; Baik, M.-Y.; Eom, S.H. Forage Potential of Faba Bean By-Products: A Comprehensive Analysis of Proximate Nutrients, Mineral Content, Bioactive Components, and Antioxidant Activities. *Agronomy* **2025**, *15*, 2473. <https://doi.org/10.3390/agronomy15112473>

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1. Introduction

Plant-based resources play vital roles not only in human diets but also in livestock feeding. The exploration of novel or underutilized plant sources has expanded dietary diversity and improved nutritional profiles for both humans and animals [1,2]. From a resource-utilization perspective, plant tissues unsuitable or inefficient for direct human consumption may provide substantial value in animal nutrition [3,4]. To meet the rising global demand for high-quality meat and dairy products, ensuring a diverse and stable supply of animal feed is increasingly important.

Among various categories of animal feed, forage crops constitute the nutritional foundation for herbivorous livestock. They are critical for maintaining optimal rumen

function and overall metabolic efficiency in ruminants such as cattle and sheep [4,5]. High-quality forage not only promotes livestock health and productivity but also improves the nutritional value of derived animal products, including meat and milk [2,4,6].

Globally, major forage crops include *Medicago sativa* (alfalfa), *Lolium* spp. (ryegrass), *Zea mays* (maize), *Oryza sativa* (rice), *Secale cereale* (rye), *Sorghum sudanense* (sudangrass), and *Phleum pratense* (timothy grass) [7–9]. Alfalfa alone accounts for approximately 39.2% of total global forage production [10]. However, many conventional forage species face seasonal growth limitations, contributing to the global “feed gap”, periodic shortages and quality decline in forage supply [7,11,12]. Climate change, through rising temperatures, prolonged droughts, and increasing pest and disease pressure, further threatens the yield stability of traditional forage systems [13]. In addition, shrinking availability of arable land suitable for forage cultivation compounds these challenges [14]. Consequently, the diversification of novel forage resources is an urgent priority, particularly in regions with low self-sufficiency or heavy dependence on imports.

Legumes of the Fabaceae family are widely valued as forage due to their high protein content, nitrogen-fixing ability, and broad adaptability [15]. Prominent forage legumes include alfalfa, clovers, and vetches. Faba bean (*Vicia faba* L.), also known as fava bean, field bean, horse bean, or broad bean, is cultivated across temperate and subtropical regions, including the Mediterranean, Near East, China, Egypt, Ethiopia, and Europe [16]. As of 2023, global faba bean cultivation covered approximately 2.8 million hectares, with total production reaching approximately 6.1 million tons [17].

Unlike crops cultivated solely for dry seeds, faba beans are often harvested at the immature pod or green bean stage for fresh consumption. However, after pod harvest, approximately 40–60% (*w/w*) of the above-ground biomass, primarily leaves and stems, remains in the field as agricultural residue [18]. Similarly to alfalfa and rye, *Vicia faba* exhibits strong winter hardiness and is typically sown in autumn and harvested in late spring or early summer. This growth cycle coincides with forage-deficient periods, and the strategic use of vegetative residues as forage may help bridge the seasonal feed gap and improve resource efficiency in integrated crop–livestock systems.

Forage quality is commonly assessed using both nutritional and quantitative criteria. Nutritional indicators include crude protein, crude fat, micronutrient content, and fiber composition [9,19], whereas quantitative indicators encompass seasonal yield and total biomass production [20]. Previous studies show that forage value varies by growth stage: delayed harvesting generally increases biomass yield but reduces nutritional quality due to secondary cell wall accumulation and declining intracellular soluble sugars and proteins [9]. Identifying the optimal harvest stage is therefore essential for balancing yield and feed quality. Despite this, comprehensive data on the nutritional indicators of faba bean leaves and stems remain limited, constraining the effective utilization of its biomass for forage. Beyond its basic nutritional profile, *Vicia faba* has been reported to contain significant amounts of L-dopa, a compound used in the treatment of Parkinson’s disease, along with various flavonoids [21,22]. Both exhibit antioxidant and health-promoting properties [21,22], and their incorporation into animal feed may improve livestock health, enhance product quality, and even extend the shelf life of meat and dairy products [23].

While previous faba bean research has predominantly focused on the qualitative and quantitative analysis of phytochemical constituents (e.g., L-dopa and flavonoids) in various tissues [24–26] or the forage potential of a single tissue (e.g., pod) at a specific stage [27], this study elucidates the dynamic and tissue-specific progression of nutritional and phytochemical profiles in both leaves and stems across three critical growth stages. Accordingly, the objective of this study was to evaluate the forage potential of these residues at different growth stages. Comprehensive analysis, including proximate composition, mineral

element profiles, bioactive compound content, and antioxidant activity, were conducted to determine the feasibility of utilizing *Vicia faba* residues as a novel, high-value forage resource. This integrated and temporal analysis provides novel insights into the optimal harvest time for maximizing the forage value of these underutilized by-products.

2. Materials and Methods

2.1. Plant Materials

Two faba bean cultivars were used: PI469181 (PI) and Won Jam 1 Ho (WJ). PI seeds were obtained from the USDA, whereas WJ is a cold-tolerant, high-yielding cultivar derived from PI through gamma irradiation breeding at the Korea Atomic Energy Research Institute (35.51° N, 126.83° E, Jeongeup, Republic of Korea) in 2023. Seeds were sown on 17 October 2024, at experimental fields of the Korea Atomic Energy Research Institute, where the soil was classified as silt loam with a pH of 6.4. All plants were grown under the same climatic conditions during the experimental period. The weather during the cultivation period (October 2024–June 2025) showed an average temperature of 10.2 °C and total precipitation of 88.9 mm (Korea Meteorological Administration). After overwintering, plants were sampled at three growth stages, S1, S2, and S3, between April and June 2025. Figure 1 shows the morphological changes in *Vicia faba* across these stages. Each treatment consisted of three biological replicates, and three individual plants included each treatment. Root tissues were removed, and aerial parts were rinsed thoroughly with distilled water to eliminate surface dust. Decayed or desiccated tissues were trimmed before separating leaves and stems. Samples were then dried in a hot-air dryer (Koencon Co., Ltd., Hanam, Republic of Korea) at <50 °C until constant weight was achieved (around 72 h), which was confirmed when successive weight measurements taken at 12 h intervals (using a precision balance with appropriate accuracy) showing negligible change. The dried material was ground with a mortar and passed through a 100-mesh sieve. The ground samples were stored in polyethylene bags at room temperature in the dark condition.



Figure 1. Morphology of two cultivars of faba beans at different growth stages.

2.2. Proximate Composition Analysis

Proximate composition was determined according to AOAC International official methods [28] of analysis with minor modifications. Moisture content was measured by oven-drying at 105 °C to constant weight, and ash content by heating at 550 °C. Crude protein was determined using the Kjeldahl method, with total nitrogen multiplied by 6.25. Crude fat was extracted with petroleum ether in a Soxhlet apparatus, while crude fiber was analyzed from the defatted residue after sequential digestion in H₂ SO₄ and NaOH.

2.3. Mineral Element Analysis

Mineral elements were analyzed following the method of Havlin and Soltanpour [29], using wet digestion. Inductively coupled plasma atomic emission spectroscopy (ICP-AES, OPTIMA 7300 DV, Perkin-Elmer, Waltham, MA, USA) was employed to quantify 11 mineral elements.

2.4. Bioactive Compounds Analysis

2.4.1. Sample Extraction

For extraction, 20 mg of sample was immersed in 1 mL 50% (*v/v*) aqueous methanol, sonicated at <40 °C for 60 min, and centrifuged at 12,000 rpm for 5 min. The supernatant was collected for subsequent analyses.

2.4.2. L-Dopa

The L-dopa analysis was conducted according to the method described by Duan et al. [30]. Filtered extracts (0.45 µm membrane) were analyzed by HPLC (Waters 2695 Alliance, Waters Inc., Milford, MA, USA) equipped with a Prontosil 120-5-C18-SH column (250 mm × 4.6 mm × 5 µm; Bischoff, Leonberg, Germany). The injection volume was 5 µL, with a flow rate of 0.8 mL/min. The mobile phases were (A) water with 0.3% formic acid and (B) acetonitrile with 0.3% formic acid, with a linear gradient: 98%A, 0–9 min; 98–20%A, 9–10 min; 20%A, 10–14 min; 20–98%A, 14–16 min; and 98%A, 16–20 min. L-dopa was monitored at 280 nm using a photodiode array detector (Waters Inc., Milford, MA, USA). Quantification was performed using a calibration curve of a L-dopa standard.

2.4.3. Flavonol

The flavonol analysis was conducted according to the method described by Duan et al. [31]. Filtered extracts (0.45 µm membrane) were analyzed using HPLC (Waters 2695 Alliance, Waters Inc., Milford, MA, USA) equipped with a Kinetex 100 Å column (150 mm × 4.6 mm × 5 µm; Phenomenex, Torrance, CA, USA). The injection volume was 5 µL, with a flow rate of 0.5 mL/min. The mobile phases were (A) water with 5% acetic acid and (B) acetonitrile, with a linear gradient: 95–86%A, 0–3 min; 86–84%A, 3–30 min; 84%A, 30–40 min; 84–10%A, 40–42 min; 10%A, 42–44 min; 10–95%A, 44–46 min; and 95%A, 46–49 min. Flavonols were detected at 370 nm with a photodiode array detector (Waters Inc., Milford, MA, USA). Quantification was achieved using calibration curves of kaempferitrin (for kaempferol glycosides) and rutin (for quercetin glycosides). The results are expressed as kaempferitrin equivalents (mg KE/g d.w.) and rutin equivalents (mg RE/g d.w.), respectively.

2.4.4. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC and TFC were determined according to the colorimetric methods [31]. For TPC, 50 µL of the extract was mixed with 650 µL of distilled water and 50 µL of Folin–Ciocalteu reagent. After 6 min of incubation, 500 µL of 7% sodium carbonate (Na₂CO₃) was added, and the mixture was incubated at room temperature for 90 min. Absorbance was measured at 750 nm, and TPC was expressed as milligrams of gallic acid equivalents (mg GAE/g d.w.) using a standard curve of gallic acid. For TFC, 100 µL of

extract was mixed with 640 μL of distilled water and 30 μL of 5% sodium nitrite (NaNO_2), followed by 5 min incubation. Then, 30 μL 10% aluminum chloride (AlCl_3) was added, and after 1 min, 200 μL of 1 mol/L sodium hydroxide (NaOH) was introduced. Absorbance was measured at 510 nm, and TFC was expressed as milligrams of catechin equivalents (mg CE/g d.w.) using a standard curve of catechin.

2.5. Antioxidant Activity

Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation assays [31]. For the DPPH assay, 20 μL of the sample was mixed with 980 μL of 0.1 mM DPPH solution and incubated in the dark for 30 min before absorbance was measured at 517 nm. For the ABTS assay, 20 μL of the sample was mixed with 980 μL of ABTS solution, incubated at room temperature for 10 min, before absorbance was measured at 734 nm. The ABTS solution was prepared by mixing 2.5 mM ABTS with 1.0 mM AAPH in $1\times$ phosphate-buffered saline, followed by incubation at 70 $^\circ\text{C}$ for 35 min with periodic shaking. After cooling to room temperature, the solution was filtered through a 0.45- μm syringe filter. In both assays, vitamin C served as the reference standard, and results were expressed as milligrams of vitamin C equivalents (VCE) per gram dry weight (mg VCE/g d.w.) using a standard curve.

2.6. Statistical Analysis

Values are presented as means of triplicate experiments. Significant differences among groups were determined using one-way ANOVA, followed by Fisher's least significant difference (LSD) test at $p < 0.05$, using SAS software (Enterprise Guide 7.1 version; SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Dry Matter and Proximate Composition

Table 1 summarizes the dry matter and proximate composition of leaves and stems of two *Vicia faba* cultivars across growth stages. Leaf and stem dry weights first increased, peaked at the S2 stage, and then declined. The sharp decrease in leaf dry weight at S3 was mainly due to senescence and abscission. Moisture content remained relatively stable in leaves but decreased significantly in stems, regardless of cultivar. Our previous research indicated water contents of 83.7–89.6% in leaves and 89.2–91.9% in stems at different maturity stages [30]. Such high moisture content contributes to juiciness, palatability, and texture, supporting their use as fresh forage in animal diets.

Table 1. Proximate composition in two cultivars of faba leaves and stems during development.

Cultivar		Leaf				Stem			
		S1	S2	S3	LSD	S1	S2	S3	LSD
Dry matter (g/plant)	PI	7.65 $\pm$ 1.05 c	44.62 $\pm$ 4.38 a	21.22 $\pm$ 1.52 b	9.50	8.14 $\pm$ 1.51 b	72.65 $\pm$ 9.72 a	61.72 $\pm$ 11.91 a	30.86
	WJ	8.78 $\pm$ 0.86 c	48.98 $\pm$ 2.68 a	19.75 $\pm$ 1.92 b	6.81	9.28 $\pm$ 1.40 b	80.34 $\pm$ 11.82 a	69.12 $\pm$ 8.62 a	29.36
Moisture (% d.w.)	PI	5.05 $\pm$ 0.11 b	5.13 $\pm$ 0.58 b	8.45 $\pm$ 0.37 a	1.39	5.62 $\pm$ 0.03 a	1.85 $\pm$ 0.40 b	1.47 $\pm$ 0.13 b	0.84
	WJ	5.70 $\pm$ 0.10 a	5.84 $\pm$ 0.10 a	5.72 $\pm$ 0.26 a	0.58	6.11 $\pm$ 0.16 a	2.39 $\pm$ 0.39 b	1.88 $\pm$ 0.23 b	0.96
Crude protein (g/100 g d.w.)	PI	28.66 $\pm$ 0.10 a	16.12 $\pm$ 0.54 b	11.44 $\pm$ 0.30 c	1.26	12.19 $\pm$ 0.46 a	5.00 $\pm$ 0.41 c	7.37 $\pm$ 0.89 b	2.15
	WJ	22.97 $\pm$ 0.10 a	19.47 $\pm$ 0.55 b	10.62 $\pm$ 1.31 c	2.85	7.48 $\pm$ 0.15 a	8.10 $\pm$ 0.76 a	4.75 $\pm$ 0.51 b	1.85
Crude fat (g/100 g d.w.)	PI	2.33 $\pm$ 0.15 a	1.89 $\pm$ 0.10 b	1.71 $\pm$ 0.10 b	0.41	1.97 $\pm$ 0.13 a	1.52 $\pm$ 0.11 b	1.47 $\pm$ 0.09 b	0.39
	WJ	2.24 $\pm$ 0.04 a	2.11 $\pm$ 0.22 a	1.80 $\pm$ 0.05 a	0.45	1.47 $\pm$ 0.14 ab	1.39 $\pm$ 0.15 b	1.81 $\pm$ 0.10 a	0.40
Carbohydrate (g/100 g d.w.)	PI	62.34 $\pm$ 0.14 c	74.39 $\pm$ 0.43 b	77.84 $\pm$ 0.44 a	1.26	78.84 $\pm$ 0.44 b	91.09 $\pm$ 0.78 a	89.07 $\pm$ 0.98 a	2.66
	WJ	67.94 $\pm$ 0.30 b	70.19 $\pm$ 0.71 b	80.69 $\pm$ 1.52 a	3.41	83.97 $\pm$ 0.34 c	87.43 $\pm$ 1.22 b	91.11 $\pm$ 0.22 a	2.56
Energetic value (kcal/100 g)	PI	385.01 $\pm$ 0.62 a	379.04 $\pm$ 2.46 a	372.46 $\pm$ 2.08 b	6.56	381.87 $\pm$ 0.92 b	398.06 $\pm$ 1.18 a	399.04 $\pm$ 1.23 a	3.87
	WJ	383.80 $\pm$ 0.50 a	377.65 $\pm$ 0.80 b	381.44 $\pm$ 0.94 a	2.66	379.04 $\pm$ 0.91 c	394.60 $\pm$ 1.78 b	399.73 $\pm$ 0.78 a	4.28
Ash (g/100 g d.w.)	PI	9.98 $\pm$ 0.43 b	14.23 $\pm$ 0.59 a	7.84 $\pm$ 0.25 c	1.55	11.03 $\pm$ 0.21 a	8.02 $\pm$ 0.46 b	7.68 $\pm$ 0.26 b	1.14
	WJ	9.34 $\pm$ 0.031 b	13.34 $\pm$ 0.11 a	10.25 $\pm$ 0.37 b	0.99	9.65 $\pm$ 0.29 a	8.13 $\pm$ 0.07 b	7.09 $\pm$ 0.34 c	0.96

Different lowercase letters indicate significant difference between stages at $p < 0.05$. PI and WJ indicate PI469181 and Won Jam 1 Ho, respectively.

For the three main macronutrients—crude protein, crude fat, and carbohydrates (Table 1)—similar variation patterns were observed in leaves and stems during development. Crude protein and crude fat content decreased gradually, while carbohydrates increased steadily, reflecting the shift from metabolically active tissues to mature organs enriched in structural carbohydrates. Despite these shifts, energy values remained similar in leaves and stems (PI: 372.46–399.04 kcal/100 g; WJ: 377.65–399.73 kcal/100 g; Table 1). These findings suggest similar energy potential of both tissues for animal feed. Onyeonagu and Eze [32] investigated crude protein and fat contents in five leguminous and five grass species, and reported a crude protein content of 7.87–23.58% in the legumes and 5.37–19.17% in the grasses, with fat contents of 0.15–0.65% and 0.15–0.55%, respectively. Hamacher et al. [33] evaluated the nutritive value of forage legumes and herbs (alfalfa, black medic, clover, yarrow, and chicory) and reported crude protein values of 9.3–23.0 g/100 g d.w. Mejri et al. [34] investigated the proximate composition of faba bean pods, and reported 18.93% carbohydrate, 23.81% protein, and <1% fat, yielding 139.24 kcal/100 g. Collectively, these findings reinforce the flexible utilization of faba bean macronutrients across growth stages and underscore its value as a fresh forage resource.

Ash content varied differently between leaves and stems, independent of cultivar. In leaves, levels increased initially and peaked at S2 (14.23 g/100 g d.w. in PI; 13.34 g/100 g d.w. in WJ) before declining, whereas stems showed a steady decrease (7.68–11.03 g/100 g d.w. in PI; 9.65–7.09 g/100 g d.w. in WJ). Under the same dry weight conditions, faba bean leaves consistently contained more ash than stems, making them a richer source of minerals. Hamacher et al. [33] reported ash contents of 9.1–14.2 g/100 g d.w. in 10 common forage crops, including ryegrass (7.9 g/100 g d.w.), alfalfa (9.7 g/100 g d.w.), and red clover (9.9 g/100 g d.w.). Ash, which reflects the total inorganic mineral content, plays essential roles in human and animal growth, bone formation, immune regulation, and production traits such as milk yield, egg laying, and fattening [35]. These findings demonstrate that both leaves and stems of faba bean are mineral-rich, with the leaves exhibiting higher concentrations, thereby offering greater potential for mineral supplementation in forage. To further clarify mineral composition, both macro- and microelement contents of leaves and stems were analyzed.

3.2. Mineral Element

Table 2 summarizes the mineral element composition of leaves and stems of two *Vicia faba* cultivars across developmental stages. Overall, mineral element variation mirrored total ash content. In leaves, both cultivars reached peak mineral concentrations at S2 (5.33% for PI; 5.94% for WJ), while in stems, levels declined steadily with development (5.02 to 4.16% for PI; 5.06 to 4.39% for WJ). Macro-elements accounted for >98% of the total mineral content in both tissues, following the descending order of $K > Ca > p > Mg > S$. In leaves, K, Ca, and Mg concentrations increased initially and peaked at S2 before declining, whereas *p* and S decreased consistently. In stems, Ca and Mg concentrations increased with growth, while that of other macro-elements declined significantly. Six microelements—Fe, B, Mn, Zn, Cu, and Mo—were detected in both tissues, with leaves generally containing higher concentrations than stems. Fe was the dominant microelement in both tissues, while the others were present only in small or trace amounts.

Juknevičius and Sabienė [36] studied the mineral elements in some grasses and legumes, and reported that K (13.0–28.3 g/kg d.w.) and Ca (3.08–17.0 g/kg d.w.) were key mineral elements in 13 forage crops, including alfalfa, white clover, quack grass, and smooth brome. Kappel et al. [37] investigated the mineral contents in some silages and forages, and reported that K (0.25–5.26%), Ca (0.21–1.15%), Mg (0.07–2.57%), and *p* (0.14–1.16%) contributed substantially to the mineral content of crops, including corn silage, sorghum silage, oats, and ryegrass. In the present study, we conducted the first

qualitative and quantitative analysis of mineral elements in faba bean leaves and stems. Results showed that both tissues are valuable sources of K (>1.44% or 14.4 g/kg d.w.), Ca (>0.37% or 3.7 g/kg d.w.), and Mg (>0.19% or 1.9 g/kg d.w.). Compared with the findings of Mejri et al. [34], who reported Ca (0.39 g/100 g), K (2.33 ppm), Mg (0.18 ppm), Na (0.39 ppm), Cu (0.13 ppm), Fe (0.74 ppm), and Zn (0.44 ppm) in faba bean pods, our results demonstrate that faba bean leaves and stems possess superior mineral profiles, especially in K, Ca, and Mg. While macro-elemental distribution in leaves was relatively uniform, stems displayed particularly high K concentrations (>2.24% or 22.4 g/kg d.w.), underscoring their potential as targeted K sources. These findings suggest that the aerial vegetative parts of faba bean, often regarded as by-products, hold substantial promise as mineral-rich feed ingredients or functional food resources. Furthermore, mineral element concentrations varied with cultivar and developmental stage. To maximize nutrient yield, harvesting during the vigorous growth phase, but before senescence, is recommended. In this section, the results establish a comprehensive mineral profile of faba bean leaves and stems across developmental stages, providing foundational data that were previously lacking. It serves as a critical reference for assessing the mineral supplementation value of these faba bean residues.

Table 2. Mineral element (%) in two cultivars of faba leaves and stems during development.

Cultivar			Leaf				Stem			
			S1	S2	S3	LSD	S1	S2	S3	LSD
Macro element	K	PI	1.95 ± 0.21 ab	2.61 ± 0.21 a	1.26 ± 0.29 b	0.82	3.60 ± 0.11 a	2.51 ± 0.26 b	2.24 ± 0.14 b	0.63
		WJ	2.39 ± 0.03 a	2.57 ± 0.12 a	1.44 ± 0.39 b	0.81	3.63 ± 0.30 a	2.74 ± 0.12 a	2.90 ± 0.44 a	1.09
	P	PI	0.61 ± 0.06 a	0.29 ± 0.02 b	0.32 ± 0.03 b	0.15	0.57 ± 0.02 a	0.38 ± 0.02 c	0.47 ± 0.02 b	0.07
		WJ	0.69 ± 0.00 a	0.45 ± 0.09 b	0.29 ± 0.01 b	0.18	0.60 ± 0.02 a	0.49 ± 0.13 a	0.39 ± 0.08 a	0.31
	S	PI	0.29 ± 0.03 a	0.16 ± 0.02 b	0.15 ± 0.03 b	0.09	0.24 ± 0.01 a	0.15 ± 0.01 b	0.18 ± 0.03 b	0.06
		WJ	0.28 ± 0.01 a	0.17 ± 0.02 b	0.18 ± 0.04 b	0.08	0.21 ± 0.01 a	0.12 ± 0.02 b	0.14 ± 0.04 ab	0.08
	Ca	PI	1.01 ± 0.02 b	1.79 ± 0.14 a	1.40 ± 0.21 ab	0.50	0.37 ± 0.04 b	0.85 ± 0.10 a	1.00 ± 0.07 a	0.25
		WJ	1.10 ± 0.05 b	2.23 ± 0.17 a	1.94 ± 0.29 a	0.68	0.41 ± 0.01 b	0.80 ± 0.02 a	1.09 ± 0.17 a	0.34
	Mg	PI	0.35 ± 0.01 ab	0.41 ± 0.04 a	0.27 ± 0.02 b	0.08	0.22 ± 0.02 a	0.26 ± 0.02 a	0.26 ± 0.02 a	0.06
		WJ	0.36 ± 0.01 a	0.48 ± 0.04 a	0.43 ± 0.05 a	0.14	0.19 ± 0.00 a	0.22 ± 0.02 a	0.27 ± 0.05 a	0.10
Micro element	Fe	PI	0.04 ± 0.00 a	0.05 ± 0.03 a	0.02 ± 0.00 a	0.06	0.02 ± 0.00 a	0.01 ± 0.00 b	0.01 ± 0.00 ab	0.01
		WJ	0.03 ± 0.00 a	0.03 ± 0.01 a	0.02 ± 0.00 a	0.02	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.00
	B	PI	Tr.	Tr.	Tr.	-	Tr.	Tr.	Tr.	-
		WJ	Tr.	Tr.	Tr.	-	Tr.	Tr.	Tr.	-
	Mn	PI	0.01 ± 0.00 b	0.01 ± 0.00 a	0.01 ± 0.00 a	0.00	Tr.	Tr.	Tr.	-
		WJ	0.01 ± 0.00 b	0.01 ± 0.00 b	0.02 ± 0.00 a	0.01	Tr.	Tr.	0.01 ± 0.00	-
	Zn	PI	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.00	Tr.	Tr.	Tr.	-
		WJ	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.00	Tr.	Tr.	Tr.	-
	Cu	PI	Tr.	Tr.	Tr.	-	Tr.	Tr.	Tr.	-
		WJ	Tr.	Tr.	Tr.	-	Tr.	Tr.	Tr.	-
	Mo	PI	Tr.	Tr.	Tr.	-	Tr.	Tr.	Tr.	-
		WJ	Tr.	Tr.	Tr.	-	Tr.	Tr.	Tr.	-
Total	PI	4.27 ± 0.31 b	5.33 ± 0.63 a	3.44 ± 0.11 b	0.96	5.02 ± 0.14 a	4.16 ± 0.25 b	4.17 ± 0.09 b	0.61	
	WJ	4.88 ± 0.04 ab	5.94 ± 0.22 a	4.34 ± 0.53 b	1.15	5.06 ± 0.30 a	4.39 ± 0.24 a	4.81 ± 0.44 a	1.17	

Different lowercase letters indicate significantly difference between stages at $p < 0.05$. Tr. indicates trace amount (<0.01%). PI and WJ indicate PI469181 and Won Jam 1 Ho, respectively.

3.3. Bioactive Compounds

3.3.1. L-Dopa and Flavonols

Figure 2 illustrates the changes in L-dopa in the leaves and stems of two faba bean cultivars across growth stages. Overall, L-dopa content declined from S1 to S2 and then remained relatively stable through S3 in both tissues, regardless of cultivar. Previous studies have shown that L-dopa content decreases with plant development and leaf maturity, following the trend: new leaves > young leaves > mature leaves [30]. Therefore, the sharp decline in L-dopa content observed from S1 to S2 in the present study may reflect shifts in leaf maturity composition, with a higher proportion of newly emerged leaves in S1 and more mature leaves in later stages. The leaves contained more than twice the L-dopa content in stems (e.g., WJ leaf S1: 23.17 mg/g d.w., WJ stem S1: 8.12 mg/g d.w.), highlighting leaves as a richer source. Furthermore, the L-dopa content in faba bean leaves documented

in our study is dramatically higher than the 0.51–1.26 mg/g d.w. range reported by Tesoro et al. [25] for faba bean seeds under different storage processes. Additionally, our methodological approach, focusing on separated leaves and stems, complements the work of Tesoro et al. [25], who developed a robust HPLC-UV method specifically for L-dopa quantification in faba beans, thereby reinforcing the reliability of such analyses for quality assessment in agricultural by-products.

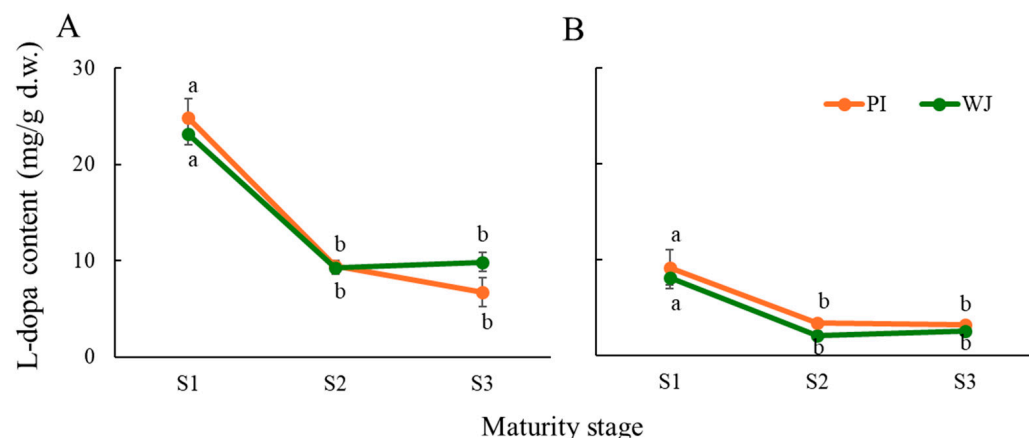


Figure 2. L-dopa content according to faba maturity stage in (A) leaf and (B) stem. Different lowercase letters indicate significant difference between stages at $p < 0.05$. PI and WJ indicate PI469181 and Won Jam 1 Ho, respectively.

Table 3 presents the flavonol composition of leaves and stems in both cultivars (PI and WJ) across three developmental stages. In all cases, total flavonol content was consistently higher in leaves than in stems. Most individual flavonols declined from S1 to S2 and then stabilized through S3. In PI, kaempferol-3-arabinoside-7-rhamnoside and kaempferol-glycoside predominated, whereas in WJ, kaempferol-3-arabinoside-7-rhamnoside and Kaempferol-3-acetyl-rhamnolactoside-7-rhamnoside were most abundant. Regarding flavonol composition, our results, which identified a diverse profile dominated by kaempferol glycosides, are in strong agreement with the foundational work of Neugart et al. [26]. A recent study by Vlasova et al. [27] evaluated the chemical composition and biological activity of faba bean pods, highlighting their potential as a feed additive for piglets. In their study, it was reported that L-dopa and flavonols were abundant. Compared with other major forage crops, faba bean leaves exhibited exceptionally high flavonol concentrations (>100 mg/g d.w.). For example, in alfalfa leaves, total flavonol content was 3.27 mg/g at the pre-flowering stage, dropping to 2.19 mg/g post-flowering [38]. Another study reported total flavone concentrations in alfalfa aerial parts ranging from 27 mg/g to 37 mg/g [39]. From a forage perspective, harvesting faba leaves at early stages (S1–S2) may provide substantial antioxidant benefits to livestock due to their high flavonol accumulation.

Table 3. Flavonol content in two cultivars of faba leaves and stems during development.

Flavonol (mg/g d.w.)	Cultivar	Leaf				Stem			
		S1	S2	S3	LSD	S1	S2	S3	LSD
Q-gly-1	PI	1.55 ± 0.08 a	0.48 ± 0.03 b	0.45 ± 0.05 b	0.20	0.27 ± 0.02 a	0.08 ± 0.01 b	0.10 ± 0.01 b	0.05
	WJ	0.84 ± 0.04 a	0.45 ± 0.04 b	0.46 ± 0.05 b	0.15	0.12 ± 0.00 a	0.05 ± 0.01 b	0.05 ± 0.01 b	0.01
Q-gly-2	PI	1.58 ± 0.04 a	0.54 ± 0.03 b	0.48 ± 0.06 b	0.16	0.36 ± 0.03 a	0.26 ± 0.02 b	0.27 ± 0.03 ab	0.09
	WJ	1.03 ± 0.04 a	0.53 ± 0.07 b	0.51 ± 0.04 b	0.18	0.21 ± 0.01 a	0.30 ± 0.01 a	0.30 ± 0.04 a	0.09
Q-3-rha-glc	PI	2.04 ± 0.17 a	0.72 ± 0.03 b	0.57 ± 0.12 b	0.41	0.34 ± 0.03 a	0.15 ± 0.02 b	0.14 ± 0.04 b	0.11
	WJ	2.44 ± 0.08 a	1.37 ± 0.14 b	1.26 ± 0.12 b	0.40	0.26 ± 0.01 a	0.16 ± 0.02 b	0.15 ± 0.02 b	0.06
Q-3-rha-gal (glu)-7-rha	PI	9.54 ± 0.50 a	2.71 ± 0.17 b	2.41 ± 0.55 b	1.51	1.41 ± 0.10 a	0.45 ± 0.07 b	0.47 ± 0.14 b	0.37
	WJ	9.43 ± 0.28 a	4.61 ± 0.50 b	4.78 ± 0.38 b	1.38	1.08 ± 0.03 a	0.58 ± 0.08 b	0.56 ± 0.04 b	0.19
Q-3-rha-ara- 7-rha	PI	0.46 ± 0.02 a	0.25 ± 0.01 b	0.24 ± 0.07 b	0.14	0.39 ± 0.04 a	0.34 ± 0.03 a	0.31 ± 0.02 a	0.12
	WJ	8.42 ± 0.26 a	3.25 ± 0.35 b	3.36 ± 0.30 b	1.06	1.02 ± 0.03 a	0.50 ± 0.09 b	0.48 ± 0.05 b	0.21

Table 3. Cont.

Flavonol (mg/g d.w.)	Cultivar	Leaf				Stem			
		S1	S2	S3	LSD	S1	S2	S3	LSD
Q-gly-3	PI	2.12 ± 0.04 a	0.61 ± 0.03 b	0.54 ± 0.06 b	0.16	0.41 ± 0.04 b	0.95 ± 0.15 a	0.81 ± 0.05 a	0.34
	WJ	0.60 ± 0.02 a	0.51 ± 0.01 ab	0.42 ± 0.06 b	0.13	0.08 ± 0.01 c	0.51 ± 0.03 a	0.22 ± 0.04 b	0.10
Total Q	PI	17.28 ± 0.79 a	5.31 ± 0.28 b	4.70 ± 0.89 b	2.45	3.17 ± 0.24 a	2.25 ± 0.29 b	2.11 ± 0.18 b	0.84
	WJ	22.76 ± 0.67 a	10.74 ± 1.10 b	10.79 ± 0.96 b	3.21	2.77 ± 0.07 a	2.10 ± 0.18 b	1.78 ± 0.19 b	0.54
K-3-rha	PI	0.58 ± 0.05 ab	0.67 ± 0.07 a	0.45 ± 0.05 b	0.20	0.36 ± 0.04 b	0.63 ± 0.06 a	0.65 ± 0.03 a	0.15
	WJ	0.59 ± 0.02 b	1.11 ± 0.05 a	0.87 ± 0.13 a	0.28	0.29 ± 0.01 c	0.62 ± 0.04 a	0.48 ± 0.04 b	0.11
K-3-rha-glc-7-rha-rha	PI	2.04 ± 0.08 a	1.39 ± 0.08 b	1.21 ± 0.06 b	0.26	0.44 ± 0.04 a	0.24 ± 0.03 b	0.26 ± 0.02 b	0.10
	WJ	0.66 ± 0.04 a	0.69 ± 0.07 a	0.65 ± 0.08 a	0.22	0.11 ± 0.00 a	0.10 ± 0.01 a	0.09 ± 0.01 a	0.02
K-3-rha-gal(glc)-7-rha	PI	2.49 ± 0.10 a	1.72 ± 0.11 b	1.48 ± 0.01 b	0.30	0.35 ± 0.04 a	0.25 ± 0.03 a	0.28 ± 0.01 a	0.11
	WJ	1.06 ± 0.02 a	1.04 ± 0.10 a	0.95 ± 0.10 a	0.28	0.14 ± 0.01 a	0.10 ± 0.01 b	0.09 ± 0.01 b	0.03
K-3-rha-ara-7-rha	PI	11.41 ± 0.62 a	9.14 ± 0.54 b	6.63 ± 0.78 c	2.26	1.30 ± 0.13 a	1.10 ± 0.11 ab	0.76 ± 0.17 b	0.47
	WJ	9.19 ± 0.50 a	9.9 ± 0.65 a	9.01 ± 0.60 a	2.03	1.29 ± 0.02 a	1.32 ± 0.12 a	1.34 ± 0.14 a	0.37
K-3-ara-7-rha	PI	36.46 ± 2.33 a	29.89 ± 2.37 ab	22.98 ± 1.63 b	7.40	5.50 ± 0.55 a	5.12 ± 0.48 a	4.40 ± 0.58 a	1.86
	WJ	29.81 ± 1.32 a	34.68 ± 2.65 a	31.40 ± 2.22 a	7.39	5.38 ± 0.05 a	5.29 ± 0.19 a	4.78 ± 0.30 a	0.71
K-3-acetyl-rha-gal-7-rha	PI	2.75 ± 0.07	Tr.	Tr.	0.13	0.93 ± 0.10	Tr.	Tr.	0.20
	WJ	44.05 ± 1.46 a	32.86 ± 3.03 b	30.04 ± 2.12 b	7.94	5.59 ± 0.13 a	4.08 ± 0.10 b	4.00 ± 0.37 b	0.81
K-gly	PI	24.98 ± 1.69 a	18.80 ± 1.40 b	12.20 ± 1.55 c	5.36	3.49 ± 0.57 a	3.90 ± 0.30 a	3.12 ± 0.69 a	1.89
	WJ	15.87 ± 0.59 b	22.04 ± 1.34 a	18.84 ± 1.88 ab	4.77	2.58 ± 0.11 a	2.96 ± 0.10 a	2.65 ± 0.17 a	0.45
K-3-acetyl-gal-7-rha	PI	13.63 ± 0.77 a	8.48 ± 0.64 b	5.92 ± 0.42 c	2.17	2.30 ± 0.36 a	2.23 ± 0.17 a	1.98 ± 0.35 a	1.06
	WJ	10.41 ± 0.31 a	11.6 ± 0.95 a	10.39 ± 0.75 a	2.49	1.63 ± 0.06 a	1.70 ± 0.04 a	1.51 ± 0.15 a	0.34
Total K	PI	94.34 ± 5.58 a	70.10 ± 5.21 b	50.88 ± 4.29 c	17.49	14.67 ± 1.77 a	13.47 ± 1.14 a	11.45 ± 1.76 a	5.49
	WJ	111.65 ± 3.97 a	113.92 ± 8.80 a	102.15 ± 7.84 a	24.85	17.01 ± 0.25 a	16.18 ± 0.44 a	14.93 ± 1.13 a	2.48
Total flavonol	PI	111.62 ± 6.36 a	75.41 ± 5.49 b	55.57 ± 4.99 c	19.52	17.84 ± 1.99 a	15.72 ± 1.36 a	13.57 ± 1.94 a	6.18
	WJ	134.40 ± 4.51 a	124.66 ± 9.87 a	112.95 ± 8.75 a	27.85	19.78 ± 0.18 a	18.28 ± 0.48 ab	16.71 ± 1.32 b	2.63

Different lowercase letters indicate significant difference between stages at $p < 0.05$. Abbreviations: K, kaempferol; Q, quercetin; rha, rhamnoside; rha-glc, rhamnoglucoside; gal, galactoside; rha-gal, rhamnogalactoside; rha-ara, rhamnoarabinoside; gly, glycoside.

3.3.2. TPC, TFC, and Antioxidant Activities

Figure 3A shows the TPC and Figure 3B TFC in faba bean leaves and stems across stages. Both tissues exhibited the highest TPC and TFC at S1, followed by a decline at S2, with stability thereafter. This pattern likely reflects the abundance of young leaves at S1, which typically show higher TPC and TFC than mature leaves [40], a difference linked to stronger enzyme activities and gene expression in phenolic biosynthesis pathways [41].

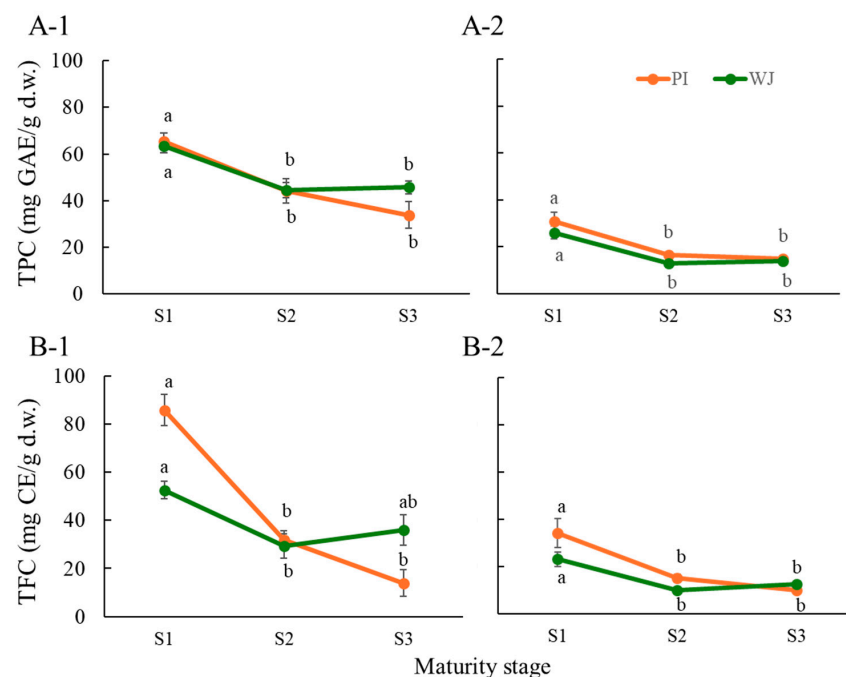


Figure 3. TPC (A) and TFC (B) of faba leaves and stems according to growth stage. The numeric values beside capital letters indicate leaves (1) and stems (2), respectively. Different lowercase letters indicate significant differences between stages at $p < 0.05$. PI and WJ indicate PI469181 and Won Jam 1 Ho, respectively.

Antioxidant activities, assessed using DPPH and ABTS radical scavenging assays, also varied with stage and tissue (Figure 4), closely paralleling TPC and TFC trends (Figure 3). In both cultivars, activity peaked at S1, dropped significantly at S2, and then remained stable or slightly decreased at S3. Leaves consistently demonstrated stronger radical scavenging capacity than stems across all stages. These results suggest that phenolic and flavonoid compounds are primary contributors to the antioxidant potential of faba bean tissues, consistent with findings by Duan et al. [40]. These results highlight the potential of faba bean leaves and stems as natural antioxidant sources for animal feed. Early developmental stages are especially valuable, with possible benefits for oxidative stability and livestock health. An original contribution of this section is the simultaneous quantification of L-dopa and the detailed profile of flavonol glycosides in both leaves and stems across growth stages. The results reveal that leaves are not only the primary repository for these bioactives but also that they accumulate these compounds at exceptionally high concentrations.

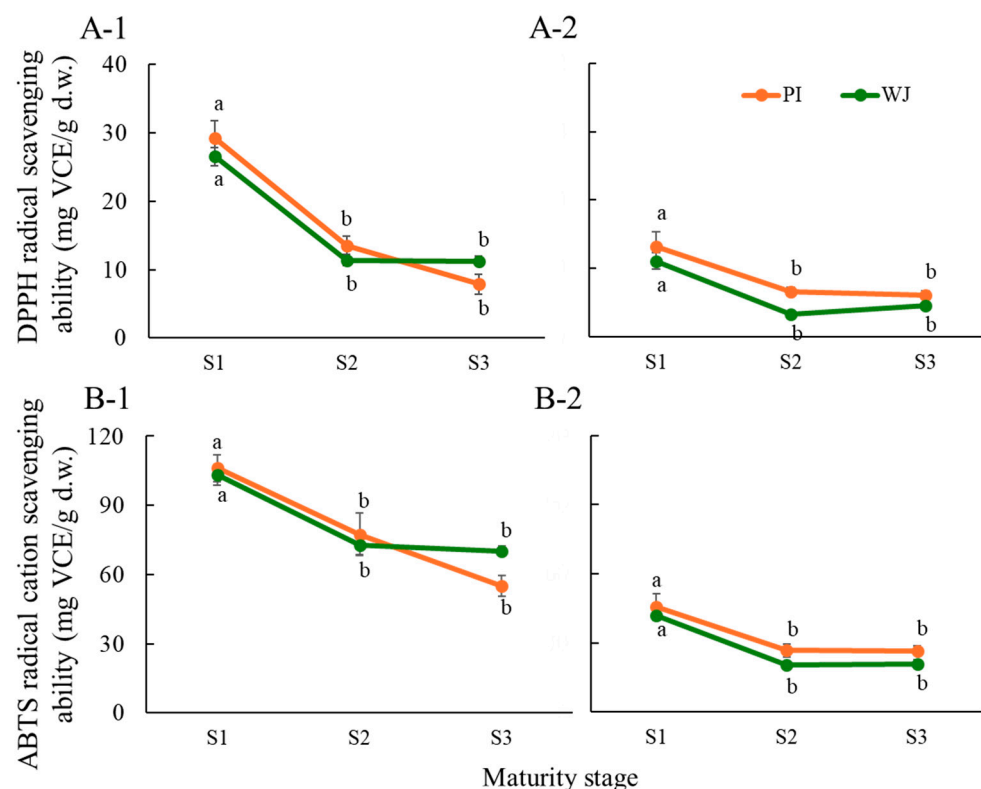


Figure 4. DPPH radical scavenging ability (A) and ABTS radical cation scavenging ability (B) of faba leaves and stems according to growth stage. The numeric values beside capital letters indicate leaves (1) and stems (2), respectively. Different lowercase letters indicate significant differences between stages at $p < 0.05$. PI and WJ indicate PI469181 and Won Jam 1 Ho, respectively.

4. Conclusions

This study demonstrates that faba bean by-products have strong potential as a high-quality forage resource. The leaves and stems showed favorable nutritional profiles, with substantial crude protein and fat contents at the flowering stage and increasing carbohydrate levels as plant matured. Mineral analysis revealed high concentrations of essential macro-elements, particularly K, Ca, and Mg, in leaves during pod development. In addition, faba bean by-products were rich in bioactive compounds, including L-dopa, flavonols, total phenolics, and total flavonoids, with peak levels at the flowering stage. These compounds were strongly correlated with antioxidant activities, indicating that early-harvested tissues may enhance oxidative stability and provide health benefits in animal diets. Compared with conventional forages such as alfalfa and clover, faba bean residues offer comparable

proximate nutritional value and superior antioxidant potential. Therefore, integrating faba bean leaves and stems into animal feed systems, particularly before the ripening stage, could help mitigate seasonal forage shortages, improve livestock diet quality, and promote sustainable agriculture by valorizing underutilized biomass.

Author Contributions: Conceptualization, M.-Y.B. and S.H.E.; methodology, S.D., J.W.K., J.H.K., J.W.L. and M.-S.K.; software, S.D. and J.W.K.; validation, S.D., S.-J.K., J.W.K., J.H.K., M.-Y.B. and S.H.E.; formal analysis, S.D.; investigation, S.D., J.W.K., J.H.K. and J.W.L., and M.-S.K.; resources, S.-J.K. and S.H.E.; data curation, S.D. and J.W.K.; writing—original draft preparation, S.D. and J.W.K.; writing—review and editing, M.-Y.B. and S.H.E.; visualization, S.D. and J.W.K.; supervision, M.-Y.B. and S.H.E.; project administration, S.H.E.; funding acquisition, S.-J.K. and S.H.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research Foundation of Korea (NRF; 2022R1A2C100769514) and by the research program of the Korea Atomic Energy Research Institute (Project No. 523420-25).

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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