Phenolic composition, antioxidant capacity and inhibitory effects on α-glucosidase and lipase of immature faba bean seeds

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Abstract

Immature faba bean seeds of five varieties were investigated for changes in phenolic composition, antioxidant capacity and inhibitory effects on α-glucosidase and lipase at three edible stages (S1, S2 and S3). Phenolics and antioxidant capacity in seed coats were significantly higher than those in cotyledons, and the highest was found of FD18 at stage S3. While phenolic content in cotyledons decreased steadily with the harvest time delayed. Eight phenolic compounds were confirmed with epicatechin and catechin prominent and their levels showed a great variability depending on varieties, maturation stages and plant parts of faba bean seeds. Unexpectedly, the inhibitory effects of purified phenolics on α-glucosidase were even stronger than that of the positive control acarbose, and the inhibitory effects on lipase in the cotyledons declined gradually from stage S1 to S3. Significant positive correlations between phenolics from seed coats and antioxidant capacity, and phenolics from cotyledons and the enzyme inhibitory effects were observed. Overall, the immature faba bean seed, especially its seed coat, has great potential as a functional food.

Introduction

Oxidative stress has been repetitively shown to be a hallmark of many diseases linked with metabolic or vascular disorders including diabetes and hypertension.[1] Natural phenolic compounds endogenous to food of plant origin have been well studied as antioxidants, which can delay or inhibit oxidative damage, thus preventing the onset of oxidative stress-related diseases in the human body.[2] In addition to antioxidant activity, phenolic compounds also play an important role in the inhibition of digestive enzymes, including α-glucosidase, lipase, and α-amylase.[3,4] The inhibition of α-glucosidase and lipase is considered as one of the more effective strategies for managing type 2 diabetes[5] and obesity.[6] It has been reported that phenolic compounds from some legumes have a strong inhibitory effect on α-glucosidase and lipase activities.[7] It is worth mentioning that condensed tannins, complex flavonoid polymers naturally present in cereals, legume seeds, and fruits, are important in human health, which participate in the prevention of cancers and cardiovascular diseases.[8]

Faba bean (Vicia faba L.) has been gaining increasing attention for health benefits as human diet. It is an important protein source with 22.4–36.0% proportion.[9] It contains significant amounts of complex carbohydrates, vitamins, minerals, and various bioactive compounds such as phenolic compounds, tocopherols, triterpenic acids,[10,11] trypsin inhibitors,[12] hemagglutinin[13], L-dopa[14], which were
reported to have potential health benefits like antioxidant activities\textsuperscript{[11,15]}, anti-inflammatory activity\textsuperscript{[16]}, hepatoprotective effect\textsuperscript{[17]}, anticancer\textsuperscript{[12,13]}, antiobesity\textsuperscript{[18]}, lowering cholesterol levels\textsuperscript{[19]}, and the treatment of Parkinson’s disease\textsuperscript{[14]}

Many studies focus on mature or dry faba bean seeds; however, the information about fresh or immature faba bean seeds which are generally consumed in many areas like Chile\textsuperscript{[20]} and China is few. Boukhanouf et al.\textsuperscript{[21]} reported that the immature faba bean seeds had significantly higher phytochemical contents and displayed a better antioxidant activity than those of mature ones. Additionally, compared with mature faba bean seeds, immature faba bean seeds possess much more levels of glucose, fructose, and sucrose, as well as fewer contents of oligosaccharides (raffinose, stachyose, averbascose), which are antinutritional factors causing flatulence.\textsuperscript{[22]} In the present study, five varieties of immature faba bean seeds at three edible stages were investigated for changes in phenolics, antioxidant capacity, and inhibitory effects on α-glucosidase and lipase. It is expected to provide useful information for developing immature faba bean seeds-based functional foods with improved health benefits and suggests a potential role of immature faba bean consumption in managing weight and control of blood glucose.

\textbf{Materials and methods}

\textbf{Faba bean materials}

Five varieties of faba bean, namely ‘Fengdou6’ (FD6), ‘Fengdou13’ (FD13), ‘Fengdou15’ (FD15), ‘Fengdou17’ (FD17) and ’Fengdou18’ (FD18), were selected for this study. These five varieties (“Fengdou” series) were bred by Dali academy of Agricultural Sciences, and popularized to the entire Yunnan Province for planting because of their high yield, good quality, strong resistance, and wide adaptability.\textsuperscript{[23,24]} All cultivars of faba bean were planted on October 19, 2016 at Research Bases for Faba Bean in Dali Academy of Agricultural Sciences (Yunnan, China; latitude, 100° 24’ N; longitude, 25° 45’ E; altitude, 2090 m). Immature faba bean pods were picked by hand at three edible stages S1 (138 DAS), S2 (156 DAS) and S3 (173 DAS) when the plants were fresh and green, where DAS refers to days after seeding, because fresh faba bean seeds are usually consumed between these three edible stages in Yunnan. At each stage, fresh weight of 100 seeds was recorded, and moisture percentage of freshly harvested faba bean seeds was determined using an oven-dry method according to the Chinese standard GB 5009. 3–2016\textsuperscript{[25]} All faba bean seeds were vacuum freeze-dried (Scientz-ND, Ningbo Xinzhi Biotechnoloy Co, Ltd) and separated in three parts: the whole seeds, the seed coats and the cotyledons. The plant materials were ground, sieved, and then stored until analysed.

\textbf{Reagents}

Acetonitrile (HPLC-grade) was purchased from Fisher Scientific Co. (USA). Ferulic acid, p-coumaric acid, caffeic acid, protocatechuic acid, catechin, epicatechin, p-hydroxybenzoic acid, quercetin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu phenol reagent, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), diammonium salt (ABTS), 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) 2,2-diphenyl-1-picrylhydrazyl (DPPH), α-glucosidase (G5003, ≥10 units/mg protein), pancreatic lipase (L0382, ≥20,000 units/mg protein), 4-nitrophenyl-α-D-glucopyranoside (pNPG), and 4-methylumbellifery oleate (4-MUO) were purchased from Sigma Chemical Co. (USA). All other chemicals were of analytical grade.
Analysis of phenolics

Extraction and purification of phenolics

The phenolics were extracted based on a procedure as described previously \cite{26} with some modifications. Twenty grams of dried faba bean seed coat and cotyledon flour were mixed with 400 mL of 80% methanol-water under an ultrasonic wave for 10 min at room temperature for three times. After centrifugation, the combined supernatants were concentrated to about 20 mL using a rotary vacuum evaporator. The aqueous suspension was adjusted at pH 2.0 using 6 M hydrochloric acid and extracted six times with ethyl acetate (30 mL each). The ethyl acetate extract was reduced until dried under vacuum at 35°C, and the resulting residue was dissolved in 60 mL of pure water. The phenolics were purified by X-5 macroporous resin. Briefly, 10 g of the pretreated resin was added to the phenolic solution and continually shaken using a water-bath shaker at 120 r/min and 25°C for 24 h. The resin was then first washed twice with ultrapure water and then desorbed with 50 mL of 70% ethanol at 120 r/min and 25°C for 24 h. Desorption solution was evaporated at 35°C under vacuum, and the resulting residue was freeze-dried to obtain dry extracts.

Analysis of total phenolic content (TPC), total flavonoids content (TFC) and condensed tannins content (CTC)

The TPC was determined according to the Folin-Ciocalteu colorimetric method described by Gao et al. \cite{26} and the results were expressed as mg gallic acid equivalent (GAE) per g DW of sample. The TFC and CTC were determined according to the method described by Zhang et al. \cite{3} with slight modifications and the results were expressed as mg catechin equivalent (CAE) per g DW of the sample.

HPLC analysis of phenolic compounds

The analysis of phenolic composition was carried out by Baginsky et al. \cite{20} with some modifications. An Agilent1200 HPLC chromatograph system (Agilent, California, USA) was used for quantification of individual phenolic compounds. Aliquots of 10 μL of the final solutions were subjected to reversed-phase chromatographic separation at 37°C on a C18 column (Agilent, ZORBAX SB-C18, 5 μm, 4.6 mm × 250 mm). Two mobile phases were used, which are as follows: A, 100% acetonitrile, and B, acetic acid/H₂O (1:99, v/v). The gradient profile was 0–7 min, 0–5% A; 7–9 min, 5% A; 9–38 min, 5–40% A; 38–40 min, 40–42% A; 40–45 min, 42–0% A, with a flow rate of 1 mL/min. Phenolics were detected at a wavelength of 280 nm. The content of the phenolic compounds was quantified with the curve and expressed as mg per g DW of the sample.

Antioxidant capacity

The DPPH radical scavenging capacity was assessed as described by Turkoglu et al. \cite{27} The reducing ability was determined using ferric reducing antioxidant power (FRAP) assay according to the method as described by Netzel et al. \cite{28} Trolox equivalent antioxidant capacity (TEAC) were performed according to the method of Re et al. \cite{29} The DPPH radical scavenging capacity and TEAC were calculated as μmol Trolox equivalent (TE) per g DW of the sample. The FRAP values were expressed as μmol Fe(II) per gram DW of the sample.

Enzyme inhibition assay

α-Glucosidase inhibition assay

The α-glucosidase inhibitory activity was measured as described by Ma et al. \cite{30} with slight modifications. Briefly, 0.1 mL of phenolic solutions or acarbose solutions with different concentrations were added to 0.1 mL of 1.25 U/mL α-glucosidase solution. Then, 0.1 mL of 1.5 mmol/L pNPG solution was added to each tube, and the mixture was incubated at 37 °C for 30 min. The reaction was then terminated by the addition of 3 mL of 1 mol/L Na₂CO₃. The absorbance at 400 nm was
then measured. The control contained 0.1 mL buffer solution in place of the α-glucosidase solution, and the background had 0.1 mL buffer solution instead of the pNPG solution. The inhibitory activity was calculated using the equation: inhibitory activity (%) = \[1-\frac{A_{sample}-A_{background}}{A_{control}}\]. The α-glucosidase inhibitory activity was expressed as IC$_{50}$ which was calculated from the percent inhibition of the serial dilutions. IC$_{50}$ is defined as the concentration of extract required to inhibit 50% of the enzyme activity, and expressed as milligram phenolic extract per millilitre.

**Lipase inhibition assay**

The inhibition of lipase activity was determined as described by Zhang, et al. [3] with minor modifications. Pancreatic lipase and 4-MUO served as the reaction enzyme and fluorogenic substrate, respectively. In brief, the mixture of 0.2 mL of substrate (0.1 mM) and 0.1 mL sample solution or orlistat with different concentrations were incubated at 37°C for 15 min, followed by addition of 0.05 mL of enzyme solution (0.55 mg/mL) in Tris-HCl buffer (0.1 M, pH 8.0) and 2.65 mL of ultrapure water in each tube. After reaction at 37°C for 1 h, the fluorescence was measured at excitation wavelength of 365 nm and emission wavelength of 460 nm with a Fluorescence Spectrophotometer. The control contained 0.1 mL ultrapure water in place of the sample solution. The inhibitory activity was calculated using the equation: inhibitory activity (%) = \[1-\frac{A_{sample}}{A_{control}}\]. The lipase inhibitory activity was also expressed in IC$_{50}$.

**Statistical analysis**

All experiments were conducted three times independently, and the results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by the Tukey’s multiple range test was used to compare the means among different groups by SPSS (version 17.0). Differences were considered significant at $p < 0.05$. Correlation analysis was performed by SPSS (version 17.0) Pearson correlation program.

**Results and discussion**

**Physical parameters at different edible stages**

The physical characteristics of faba bean seeds at different edible stages are tabulated in Table 1. During the three edible stages, the colour of seed coats and cotyledons changed varying from yellow green to white and deep green to sandy white, respectively. The changes of the colour can be attributed to the changes in colouring matter profiles synthesized in faba bean seeds such as chlorophyll, carotenoids, and anthocyanins, as well as the changes in moisture content, seed size, and density during the development of faba bean seeds. Weight of the 100 fresh seeds increased significantly, whereas moisture declined rapidly. As a result, the 100 seeds dry weight and the rate of dry matter accumulation of faba bean seeds increased significantly with the harvest time delayed. These results are in consistent with legume seed ontogenesis, when seed moisture typically ceases to increase during the accumulation of storage materials (such as starch) within seeds. In addition, the 100 seeds weight of different faba bean cultivars was different at the same edible stage and FD6 was lower than other varieties.

**Phenolic compounds at different edible stages**

**Total phenolic content, total flavonoids content, and condensed tannins content**

The levels of total phenolics, total flavonoids, and condensed tannins in phenolic extracts from faba bean seed coats and cotyledons are tabulated in Table 2. The lowest and highest TPC, TFC and CTC in seed coats were found for FD6 at S2 stage and FD18 at S3 stage, respectively. Whereas, in cotyledons the contents of TPC, TFC, and CTC decreased steadily with the harvest time delayed regardless of the varieties, suggesting that with the faba bean seed maturation, the content of phenolic compounds decreased gradually, which is consistent with the result of Boukhanouf et al.[21]
Table 1. Physical parameters of faba bean seeds at different edible stages.

| Variety | Edible stage | Seed coat color | Cotyledon color | 100-Seed fresh weight (g) | Moisture (%) | 100-Seed dry weight (g) | % Dry matter accumulation |
|---------|--------------|----------------|----------------|--------------------------|--------------|------------------------|--------------------------|
| FD6     | S1           | Yellow green   | Deep green     | 154.38 ± 0.5 aA          | 77.86 ± 1.5 cBC | 34.17 ± 0.1 aB         | 22.14 ± 1.3 aAB          |
|         | S2           | Light yellow   | Yellow green   | 212.26 ± 1.7 bA          | 69.75 ± 2.6 bAB | 64.21 ± 0.5 bA         | 30.25 ± 2.1 bAB          |
|         | S3           | White          | Sandy white    | 238.21 ± 1.2 cA          | 62.56 ± 0.8 aA | 89.18 ± 0.4 cA         | 37.44 ± 0.6 cA           |
| FD13    | S1           | Yellow green   | Deep green     | 238.24 ± 3.1 aC          | 74.19 ± 0.3 cA | 61.49 ± 0.8 aD         | 25.81 ± 0.3 aC           |
|         | S2           | Light yellow   | Yellow green   | 280.36 ± 5.3 bCD         | 68.15 ± 0.4 bA | 89.30 ± 1.7 bC         | 31.85 ± 0.5 bB           |
|         | S3           | White          | Sandy white    | 307.11 ± 3.1 cC          | 58.87 ± 0.9 aA | 126.31 ± 1.3 cC        | 41.43 ± 0.8 cA           |
| FD15    | S1           | Yellow green   | Deep green     | 160.22 ± 4.2 aA          | 79.86 ± 1.0 cC | 32.26 ± 0.9 aA         | 20.14 ± 1.0 aA           |
|         | S2           | Light yellow   | Yellow green   | 229.30 ± 2.8 bB          | 73.18 ± 1.1 bC | 61.50 ± 0.8 bA         | 26.82 ± 1.3 bA           |
|         | S3           | White          | Sandy white    | 276.73 ± 0.8 cB          | 61.98 ± 1.6 aA | 105.21 ± 0.3 cB        | 38.02 ± 1.9 cA           |
| FD17    | S1           | Yellow green   | Deep green     | 212.62 ± 2.2 aB          | 75.39 ± 2.5 cAB | 52.32 ± 0.5 aC         | 24.61 ± 2.2 aBC          |
|         | S2           | Light yellow   | Yellow green   | 270.85 ± 2.4 bC          | 70.86 ± 1.2 bAB | 78.93 ± 0.7 bB         | 29.14 ± 2.0 bAB          |
|         | S3           | White          | Sandy white    | 320.46 ± 2.4 cD          | 61.38 ± 1.3 aA | 123.76 ± 0.9 cC        | 38.62 ± 1.4 cA           |
| FD18    | S1           | Yellow green   | Deep green     | 275.05 ± 1.3 aD          | 74.32 ± 1.0 bA | 70.63 ± 0.3 aE         | 25.68 ± 1.6 aC           |
|         | S2           | Light yellow   | Yellow green   | 284.75 ± 5.6 aD          | 69.74 ± 1.0 bAB | 86.16 ± 1.7 bC         | 30.26 ± 0.9 bAB          |
|         | S3           | White          | Sandy white    | 310.80 ± 6.6 bC          | 60.41 ± 3.1 aA | 123.05 ± 2.6 cC        | 39.59 ± 2.1 cA           |

S1-S3 refer to the different edible stages. Data are expressed as mean values of three independent replicates ± SD. Values within a column with different lowercase letters for the same faba bean variety at different edible stages are statistically different at $p < 0.05$. Values within a column with different uppercase letters for different faba bean varieties at same edible stage are statistically different at $p < 0.05$. 
In general, the changing patterns of TPC, TFC and CTC were similar, and significant correlations were observed among them (Table 6).

Additionally, the data obtained indicated that the TPC, TFC, and CTC in seed coats were higher than those in cotyledons, which were in consonance with the findings obtained by other studies.\[16,19]\n
It is believed that since flavonoids are often accumulated in specialized cells and according to their roles as ultraviolet screens and as antioxidants, these compounds are very well-placed in the epidermal layers or in the cuticle of leaves and fruits.\[16]\n
The results obtained in this study are in consistent with such placement of flavonoids. In general, the contents of total phenolics, total flavonoids and condensed tannins in faba bean seeds are closely relation to cultivars, parts and development stages of plants.

**Phenolic composition of phenolic extract**

The representative HPLC chromatogram of the phenolic extracts was shown in supplementary Fig.1 and phenolic profiles in faba bean seed coats and cotyledons at different edible stages are tabulated in Table 3. In seed coats, a total of 7, 7, 8, 8 and 8 individual phenolic compounds were detected in the phenolic extracts for FD6, FD13, FD15, FD17, and FD18 during the three edible stages, respectively. Furthermore, epicatechin and catechin were the most abundant phenolic compounds in all cultivars at the three edible stages with the content in the range of 134.79–353.78 and 181.64–420.57 μg/g DW, respectively. In cotyledons, catechin, epicatechin, quercein and ferulic acid were identified from all varieties at the three edible stages with the levels in the range of 8.55–65.93, 10.72–45.50, 7.14–59.95, and 4.10–6.88 μg/g DW, respectively. It was interesting to note that p-hydroxybenzoic acid was detected only at stage S3, which was probably due to that the synthesis of p-hydroxybenzoic acid occurs as the maturation stage of the seed approaches.

In the current study, the results obtained showed that epicatechin and catechin were the predominant phenolics, along with phenolic acids as minor compounds. Our results were generally consistent with the conclusion conducted by Baginsky et al.\[20]\ that the epicatechin and catechin were the major phenolic compounds in immature seeds of ten faba bean varieties from Chile. Whereas, for the mature faba bean seeds, Amarowicz et al.\[14,31]\ reported that catechin gallate, digallate procyanidin dimer, and gallate procyanidin dimer were the major phenolics present in the

| Variety | Edible stage | Seed coats | Cotyledons |
|---------|--------------|------------|------------|
|         | TPC (mg GAE/g DW) | TFC (mg CAE/g DW) | CTC (mg CAE/g DW) | TPC (mg GAE/g DW) | TFC (mg CAE/g DW) | CTC (mg CAE/g DW) |
| FD6     | S1           | 2.92 ± 0.1 cB | 8.61 ± 0.0 bAB | 4.30 ± 0.3 cB | 0.25 ± 0.0 cA | 0.59 ± 0.0 cAB | 0.26 ± 0.0 bA |
|         | S2           | 1.62 ± 0.0 aA | 6.04 ± 0.1 aA  | 2.19 ± 0.1 aA | 0.11 ± 0.0 bB | 0.33 ± 0.1 bC | 0.20 ± 0.0 bA |
|         | S3           | 1.91 ± 0.0 aA | 6.38 ± 0.4 aA  | 3.18 ± 0.0 bB | 0.06 ± 0.0 aA | 0.19 ± 0.0 aA | 0.10 ± 0.0 aA |
| FD13    | S1           | 2.11 ± 0.2 aA | 6.93 ± 0.1 aA  | 3.32 ± 0.1 aA | 0.19 ± 0.0 bA | 0.49 ± 0.0 bAB | 0.28 ± 0.0 bA |
|         | S2           | 2.77 ± 0.0 aBC | 7.16 ± 1.0 aA  | 4.03 ± 0.2 abcC | 0.10 ± 0.0 abB | 0.32 ± 0.0 abB | 0.19 ± 0.0 abA |
|         | S3           | 2.51 ± 0.0 aB | 8.34 ± 0.0 bB  | 4.71 ± 0.1 bcC | 0.08 ± 0.0 abB | 0.17 ± 0.0 aA | 0.12 ± 0.0 aA |
| FD15    | S1           | 2.81 ± 0.0 bB | 9.20 ± 1.3 bAB | 3.61 ± 0.2 bAB | 0.27 ± 0.0 bB | 0.63 ± 0.0 bcC | 0.34 ± 0.0 bA |
|         | S2           | 2.38 ± 0.2 bB | 6.19 ± 0.1 aA  | 2.81 ± 0.0 abB | 0.12 ± 0.0 abB | 0.37 ± 0.0 bcC | 0.22 ± 0.0 abA |
|         | S3           | 1.78 ± 0.1 aA | 6.17 ± 0.8 aA  | 2.66 ± 0.1 aA | 0.03 ± 0.0 aA | 0.12 ± 0.5 aA | 0.12 ± 0.0 aA |
| FD17    | S1           | 2.95 ± 0.8 bB | 7.82 ± 0.3 abA | 3.32 ± 0.2 aA | 0.15 ± 0.0 bA | 0.41 ± 0.0 bA | 0.33 ± 0.0 bA |
|         | S2           | 3.24 ± 0.1 aB | 8.02 ± 1.1 bA  | 4.70 ± 0.1 bdC | 0.08 ± 0.0 abB | 0.20 ± 0.0 abB | 0.20 ± 0.0 abA |
|         | S3           | 1.86 ± 0.2 aA | 5.16 ± 0.1 aA  | 2.96 ± 0.2 aAB | 0.06 ± 0.0 aA | 0.14 ± 0.0 aA | 0.16 ± 0.0 aA |
| FD18    | S1           | 3.71 ± 0.0 bC | 10.97 ± 0.1 bB | 5.24 ± 0.1 bcC | 0.15 ± 0.0 abB | 0.46 ± 0.0 bAB | 0.25 ± 0.0 bA |
|         | S2           | 2.79 ± 0.1 abc | 8.07 ± 0.1 aA  | 4.04 ± 0.1 acC | 0.06 ± 0.0 aA | 0.16 ± 0.0 aA | 0.17 ± 0.0 abA |
|         | S3           | 4.71 ± 0.1 cC | 13.25 ± 0.3 ccC | 7.91 ± 0.1 cdC | 0.03 ± 0.0 aA | 0.15 ± 0.0 aA | 0.13 ± 0.0 aA |

S1-S3 refer to the different edible stages. Data are expressed as mean values of three independent replicates ± SD. Values within a column with different lowercase letters for the same faba bean variety at different edible stages are statistically different at \( p < 0.05 \). Values within a column with different uppercase letters for different faba bean varieties at same edible stage are statistically different at \( p < 0.05 \).
Table 3. Phenolic compound content (μg/g DW) in phenolic extracts from faba bean seeds at different edible stages.

| Variety | Edible stage | Protocatechuic acid | p-Hydroxybenzoic acid | Catechin | Caffeic acid | Epicatechin | p-Coumaric acid | Ferulic acid | Quercetin |
|---------|--------------|----------------------|-----------------------|----------|-------------|-------------|----------------|--------------|-----------|
| **Seed coats** | | | | | | | | | |
| FD6 | S1 | nd | 56.92 ± 4.2 bB | 353.78 ± 16.3 cC | 22.06 ± 1.5 aA | 420.57 ± 25.5 bD | 42.00 ± 2.0 bB | 14.08 ± 0.9 aA | nd |
| S2 | nd | 25.46 ± 1.8 aA | 234.78 ± 14.9 bC | 23.16 ± 1.4 aB | 343.53 ± 22.7 bB | 13.10 ± 0.7 aA | 16.03 ± 0.7 aA | 21.49 ± 1.9 bB |
| S3 | nd | 34.41 ± 1.3 aB | 134.79 ± 4.9 aA | 22.99 ± 1.7 aA | 212.62 ± 14.8 aA | 11.77 ± 0.9 aAB | 10.89 ± 0.7 aA | 12.34 ± 0.8 aA |
| FD13 | S1 | nd | 155.95 ± 6.1 aA | nd | 191.02 ± 10.7 aA | nd | 36.90 ± 1.1 bB | 30.05 ± 1.5 bA |
| S2 | nd | 39.32 ± 2.0 aB | 198.81 ± 12.9 bABC | nd | 355.16 ± 19.7 cB | 19.52 ± 1.5 bB | 13.65 ± 0.6 aA | 28.77 ± 1.4 bC |
| S3 | nd | 41.63 ± 2.5 aC | 159.76 ± 7.0 aAB | 19.79 ± 0.9 A | 283.29 ± 12.7 bB | 13.36 ± 0.7 aB | 10.89 ± 0.7 aA | 12.45 ± 0.6 aA |
| FD15 | S1 | 26.92 ± 1.5 bB | 50.31 ± 3.1 bC | 185.10 ± 6.1 aAB | nd | 244.81 ± 12.5 bA | nd | 22.21 ± 1.5 aB |
| S2 | nd | 71.83 ± 3.1 aC | 221.80 ± 10.8 aC | 18.66 ± 1.6 A | 181.64 ± 6.8 aA | 12.90 ± 0.6 aA | nd | 18.49 ± 0.8 bB |
| S3 | 12.52 ± 0.8 aB | 28.81 ± 1.4 aA | 24.06 ± 1.6 aA | 22.73 ± 1.2 bB | 240.60 ± 10.6 aAB | 17.37 ± 0.9 bA | 13.92 ± 0.8 aA | 26.50 ± 1.4 bA |
| FD17 | S1 | 26.87 ± 1.6 bB | 24.84 ± 2.0 aA | 180.44 ± 9.5 aA | 16.56 ± 0.8 aA | 221.71 ± 14.2 aA | 10.71 ± 0.8 aA | 11.56 ± 0.9 aA | 12.76 ± 0.8 aA |
| S2 | 15.84 ± 0.8 aA | 26.86 ± 2.0 aA | 173.07 ± 9.1 aB | 17.43 ± 1.5 aB | 217.70 ± 15.3 aB | 10.32 ± 0.7 aA | nd | 13.03 ± 0.7 aA |
| S3 | nd | 19.96 ± 1.6 aA | 312.92 ± 19.2 bABC | nd | 191.02 ± 10.7 aA | nd | 22.21 ± 1.5 aB |
| FD18 | S1 | 18.85 ± 1.2 bB | 47.98 ± 3.0 bB | 232.19 ± 12.8 bABC | nd | 358.23 ± 19.8 bC | 28.40 ± 1.9 bB | 14.43 ± 1.4 aA | nd |
| S2 | 13.14 ± 1.2 aA | 36.47 ± 1.8 aA | 224.92 ± 7.4 aB | 18.38 ± 1.4 aAB | 255.98 ± 18.7 aA | 11.14 ± 0.7 aA | 11.21 ± 0.9 aA | 14.12 ± 0.4 aA |
| S3 | 16.85 ± 1.1 abA | 61.24 ± 3.3 cD | 233.38 ± 8.7 aC | 35.43 ± 2.4 bB | 272.80 ± 11.1 aB | 24.69 ± 0.4 bC | nd | 17.55 ± 0.7 aB |
| **Cotyledons** | | | | | | | | | |
| FD6 | S1 | nd | 16.50 ± 1.0 aA | nd | 45.50 ± 2.0 cC | nd | 11.22 ± 0.8 cB | 59.95 ± 2.4 cB |
| S2 | nd | nd | 51.28 ± 4.4 cC | nd | 27.39 ± 0.4 bC | nd | 7.43 ± 0.4 bB | 24.36 ± 1.8 bA |
| S3 | nd | 6.21 ± 1.7 A | 33.20 ± 1.2 bB | nd | 18.87 ± 0.1 aA | nd | 4.95 ± 0.3 aA | 10.87 ± 0.5 aA |
| FD13 | S1 | nd | 65.93 ± 2.2 cD | nd | 34.32 ± 2.1 bA | nd | 6.22 ± 0.3 aA | 34.39 ± 1.8 cA |
| S2 | nd | nd | 34.00 ± 2.6 bB | nd | 25.51 ± 1.4 bC | nd | 6.46 ± 0.2 aB | 24.49 ± 1.3 bA |
| S3 | nd | 8.36 ± 0.5 A | 8.55 ± 0.4 aA | nd | 16.94 ± 0.4 aAB | nd | 5.08 ± 0.2 aAB | 7.14 ± 0.7 aA |
| FD15 | S1 | nd | 28.67 ± 2.0 bB | nd | 16.3 ± 0.6 aA | nd | 5.12 ± 0.3 aA | 47.85 ± 1.9 cB |
| S2 | nd | nd | 52.97 ± 5.6 aC | nd | 15.90 ± 1.0 aB | nd | 6.88 ± 0.3 aB | 27.40 ± 1.6 aA |
| S3 | nd | 6.88 ± 0.6 A | 13.67 ± 1.2 aA | nd | 19.32 ± 0.2 bB | nd | 6.02 ± 0.3 aB | 10.49 ± 0.6 aB |
| FD17 | S1 | nd | 53.49 ± 3.0 bC | nd | 38.08 ± 2.6 bB | nd | 10.18 ± 0.8 bB | 37.53 ± 2.6 cA |
| S2 | nd | nd | 23.49 ± 2.4 aB | nd | 10.72 ± 0.8 aA | nd | 4.94 ± 0.2 aA | 26.91 ± 1.6 aB |
| S3 | nd | 6.07 ± 0.5 A | 30.35 ± 3.4 aB | nd | 14.74 ± 0.2 aA | nd | 5.26 ± 0.4 aAB | 9.97 ± 0.5 aAB |
| FD18 | S1 | nd | 60.37 ± 4.2 bCD | nd | 14.04 ± 1.3 aA | nd | 6.67 ± 0.5 bA | 36.27 ± 1.4 cA |
| S2 | nd | nd | 19.36 ± 1.8 aA | nd | 10.89 ± 0.7 aA | nd | 4.19 ± 0.3 aA | 25.73 ± 1.2 bB |
| S3 | nd | 6.71 ± 0.8 A | 12.27 ± 1.1 aA | nd | 18.94 ± 0.8 bB | nd | 4.67 ± 0.2 aA | 10.76 ± 0.7 aB |

S1-S3 refer to the different edible stages. nd, not detected. Data are expressed as mean values of three independent replicates ± SD. Values within a column with different lowercase letters for the same faba bean variety at different edible stages are statistically different at \( p < 0.05 \). Values within a column with different uppercase letters for different faba bean varieties at same edible stage are statistically different at \( p < 0.05 \).
extract from Albus cultivar, and gallate procyanidin dimer, gallate procyanidins and acetylated kaempferol hexose were the abundant phenolics present in the extract from Polis cultivar. The presence of prodelphidins and procyanidins in broad bean was also confirmed by Abureidah et al.\[32\].

Bekkara et al.\[33\] found that seed coats of Alfred cultivar were dominated by catechin derivatives, condensed tannins and flavones, whereas cotyledon consisted mainly of phenolic acids. In contrast, the seed coats of Blandine cultivar contained flavones, flavonols, dihydroflavonols and phenolic acids. These results above proved that differences in phenolic composition in relation to varieties, maturation stages, and plant parts of faba bean seeds.

**Antioxidant capacity at different edible stages**

DPPH radical scavenging capacity, TEAC and FRAP assays were employed to test the antioxidant capacity of phenolics extracted from faba bean seed coats and cotyledons and the results are shown in Table 4. The data obtained indicated that antioxidant capacity of seed coats was significantly higher than that of cotyledons due to higher total phenolics, total flavonoids and condensed tannins contents, and the strongest antioxidant capacity was found of FD18 at stage S3 with the values of DPPH, TEAC and FRAP 24.27 μmol TE/g DW, 73.96 μmol TE/g DW and 98.49 μmol Fe(II)/g DW, respectively.

The correlations between the antioxidant capacity and TPC, TFC and CTC were established, and correlation coefficients (r) are tabulated in Table 6. Significant correlations were found between TPC, TFC and CTC, and DPPH radical scavenging capacity, FRAP and TEAC in seed coats, whereas no significant correlation was observed in cotyledons. It has been reported that the phenolics, flavonoids and tannin contents of ethanol extract from faba bean seeds significantly correlated to TEAC, and phenolic content of acetone extract positively to FRAP and inversely related to DPPH radical scavenging capacity.\[16\]. Additionally, significant correlations were reported between total phenolics and tannins, and antioxidant activity, while there was no significant correlation between flavonols and antioxidant activity in seeds of low-tannin faba bean genotypes.\[34\]. These differences in correlation between phenolic profiles and antioxidant capacity could be attributed to the divergence of plant materials, evaluation systems, extraction methods, and complicated extracts containing two or more antioxidant substances and so on. On the other hand, the antioxidant activity in extracts is associated with not only phenolic content but also the phenolic compositions.

### Table 4. Antioxidant capacity of faba bean seeds at different edible stages.

| Variety | Edible stage | DPPH value (μmol TE/g DW) | TEAC value (μmol TE/g DW) | FRAP value (μmol Fe(II)/g DW) | DPPH value (μmol TE/g DW) | TEAC value (μmol TE/g DW) | FRAP value (μmol Fe(II)/g DW) |
|---------|--------------|---------------------------|---------------------------|-------------------------------|---------------------------|---------------------------|-------------------------------|
| FD6     | S1           | 14.46 ± 1.1 aA            | 43.76 ± 1.1 aA            | 49.46 ± 1.1 bA                | 0.61 ± 0.0 bD             | 1.05 ± 0.0 cD             | 4.74 ± 0.2 cC               |
|         | S2           | 12.17 ± 1.1 aA            | 39.71 ± 0.9 aAB           | 32.64 ± 0.6 aA                | 0.29 ± 0.0 aB             | 0.59 ± 0.0 bA             | 1.08 ± 0.1 bB               |
|         | S3           | 13.25 ± 1.0 aA            | 40.96 ± 1.2 aA            | 43.56 ± 3.0 bA                | 0.26 ± 0.0 aA             | 0.36 ± 0.0 aA             | 0.58 ± 0.0 aA               |
| FD13    | S1           | 14.82 ± 0.3 aA            | 42.08 ± 0.5 aA            | 43.20 ± 1.0 aA                | 0.22 ± 0.0 aA             | 0.37 ± 0.0 aA             | 0.30 ± 0.0 aA               |
|         | S2           | 14.55 ± 0.4 aAB           | 49.09 ± 4.8 aB            | 52.42 ± 3.2 aBC               | 0.37 ± 0.0 bC             | 0.71 ± 0.0 bB             | 1.46 ± 0.0 bC               |
|         | S3           | 15.59 ± 1.3 aA            | 48.48 ± 0.7 aBC           | 55.18 ± 4.5 aA                | 0.34 ± 0.0 bB             | 0.64 ± 0.0 bC             | 1.63 ± 0.0 bC               |
| FD15    | S1           | 14.56 ± 0.5 aA            | 41.87 ± 0.7 bA            | 44.74 ± 1.8 aA                | 0.30 ± 0.0 aB             | 0.54 ± 0.0 bBC            | 0.46 ± 0.0 aA               |
|         | S2           | 12.71 ± 1.0 aA            | 37.72 ± 0.9 aA            | 37.89 ± 0.3 aAB               | 0.36 ± 0.0 bC             | 0.69 ± 0.0 cB             | 0.51 ± 0.0 aA               |
|         | S3           | 11.73 ± 1.6 aA            | 36.30 ± 0.9 aA            | 36.16 ± 3.0 aA                | 0.27 ± 0.0 aA             | 0.30 ± 0.0 aA             | 0.50 ± 0.0 aA               |
| FD17    | S1           | 15.24 ± 0.7 aAB           | 41.35 ± 1.0 aA            | 49.74 ± 2.9 aA                | 0.58 ± 0.0 cD             | 0.65 ± 0.0 aC             | 0.90 ± 0.0 aB               |
|         | S2           | 16.53 ± 0.3 bB            | 51.23 ± 2.5 bC            | 59.24 ± 4.8 aC                | 0.29 ± 0.0 aB             | 0.68 ± 0.0 aB             | 1.15 ± 0.1 bB               |
|         | S3           | 11.80 ± 1.4 aA            | 39.18 ± 1.3 aA            | 37.81 ± 0.7 aA                | 0.36 ± 0.0 bB             | 0.68 ± 0.0 aC             | 1.89 ± 0.1 cD               |
| FD18    | S1           | 17.87 ± 0.9 aB            | 55.49 ± 0.1 aB            | 65.02 ± 2.9 aA                | 0.40 ± 0.0 cC             | 0.43 ± 0.0 aAB            | 0.44 ± 0.0 aA               |
|         | S2           | 13.42 ± 0.9 aAB           | 48.45 ± 2.1 aBC           | 52.35 ± 3.0 aBC               | 0.18 ± 0.0 aA             | 0.54 ± 0.1 aA             | 1.09 ± 0.1 bB               |
|         | S3           | 24.27 ± 1.3 bB            | 73.96 ± 3.6 bC            | 98.49 ± 3.4 bB                | 0.31 ± 0.0 bAB            | 0.52 ± 0.0 aB             | 1.41 ± 0.0 cB               |

S1-S3 refer to the different edible stages. Data are expressed as mean values of three independent replicates ± SD. Values within a column with different lowercase letters for the same faba bean variety at different edible stages are statistically different at \( p < 0.05 \). Values within a column with different uppercase letters for different faba bean varieties at same edible stage are statistically different at \( p < 0.05 \).
The correlation coefficient was also determined between different antioxidant capacity assays. Significant correlations were found among DPPH radical scavenging capacity, FRAP and TEAC assays (Table 6). The significant correlations between the antioxidant capacities are expected because these assays share the same principle of electron transfer reaction.

**Inhibitory effects on \( \alpha \)-glucosidase and lipase at different edible stages**

The IC\(_{50}\) values of the inhibitory effects of the phenolic extracts from faba bean seed coats and cotyledons on \( \alpha \)-glucosidase ranged from 0.05 to 0.14 and 0.57 to 4.74 mg/mL, respectively (Table 5). Acarbose, a \( \alpha \)-glucosidase inhibitor, was used as positive control in many types of research to evaluate the inhibitory effect of bioactive compounds on \( \alpha \)-glucosidase.\[^{3,30}\] Surprisingly, in the present study, the IC\(_{50}\) values of the inhibitory effects of the phenolic extracts were even significant lower than that of the acarbose. Additionally, the inhibitory activities on \( \alpha \)-glucosidase found in this study were markedly higher than those of reported for other beans.\[^{35}\] For most faba bean varieties, no significant difference of IC\(_{50}\) values was observed in seed coats, while it increased gradually with the harvest time delayed in cotyledons. The strongest inhibitory activity on \( \alpha \)-glucosidase was found in seed coats of cultivars FD13 and FD18. Furthermore, the inhibitory effect on \( \alpha \)-glucosidase of the phenolics from seed coats was much stronger than that of cotyledon, which could be due to the divergence of phenolic content and composition. The IC\(_{50}\) values for lipase inhibition from seed coats and cotyledons ranged from 3.84 to 6.10 and 2.97–6.89 mg/mL, respectively (Table 5). The IC\(_{50}\) values of inhibitory effects on lipase increased steadily with the harvest time delayed in cotyledons, whereas it first decreased then increased in seed coats for most varieties, that is, the strongest inhibitory effects were found at stage S2. The strongest inhibitory activity on lipase was found of FD6 in cotyledons at edible stage S1.

Correlation analysis (Table 6) showed that in cotyledons, significant correlations were observed between IC\(_{50}\) values for \( \alpha \)-glucosidase and TPC and TFC. Similarly, significant correlations were found between IC\(_{50}\) values for lipase and TPC, TFC and CTC. In contrast, in seed coats, TPC, TFC and CTC weakly correlated to the IC\(_{50}\) values of inhibitory effects on \( \alpha \)-glucosidase and lipase, suggesting that other non-phenolic compounds in the extract may also contribute to the inhibitory activities.

**Table 5.** Enzyme inhibitory effects of the faba bean seeds at different edible stages.

| Variety | Edible stage | \( \alpha \)-Glucosidase inhibitory effect IC\(_{50}\) value (mg/mL) | Lipase inhibitory effect IC\(_{50}\) value (mg/mL) |
|---------|--------------|------------------------------------------------|----------------------------------|
|         | Seed coats   | Cotyledons                                       | Seed coats | Cotyledons |
| FD6     | S1           | 0.13 ± 0.0 aC | 0.82 ± 0.0 bB | 5.03 ± 0.1 bBC | 2.97 ± 0.1 aA |
|         | S2           | 0.14 ± 0.0 aB | 1.79 ± 0.0 cA | 3.93 ± 0.0 aA | 3.62 ± 0.1 aA |
|         | S3           | 0.14 ± 0.0 aC | 0.63 ± 0.0 aA | 5.53 ± 0.1 cCD | 4.78 ± 0.3 bA |
| FD13    | S1           | 0.05 ± 0.0 aA | 1.96 ± 0.0 aC | 4.62 ± 0.0 aAB | 3.37 ± 0.0 aB |
|         | S2           | 0.05 ± 0.0 aA | 2.51 ± 0.0 bB | 6.10 ± 0.1 bC | 4.11 ± 0.1 bB |
|         | S3           | 0.06 ± 0.0aA | 4.45 ± 0.1 cC | 5.74 ± 0.1 bD | 4.93 ± 0.2 cA |
| FD15    | S1           | 0.12 ± 0.0 aC | 0.57 ± 0.0 aA | 4.58 ± 0.2 ba | 3.63 ± 0.0 aB |
|         | S2           | 0.13 ± 0.0 aAB | 4.24 ± 0.0 cD | 3.84 ± 0.1 aA | 5.65 ± 0.1 bC |
|         | S3           | 0.13 ± 0.0 aC | 3.97 ± 0.0 bB | 4.30 ± 0.2 abA | 6.89 ± 0.2 cB |
| FD17    | S1           | 0.09 ± 0.0 aB | 2.54 ± 0.0 aB | 4.47 ± 0.1 aA | 3.49 ± 0.0 aB |
|         | S2           | 0.13 ± 0.0 bAB | 2.59 ± 0.0 aB | 4.69 ± 0.1 abB | 3.73 ± 0.0 bB |
|         | S3           | 0.09 ± 0.0 aB | 4.10 ± 0.0 bB | 4.86 ± 0.1 bB | 5.68 ± 0.1 cA |
| FD18    | S1           | 0.07 ± 0.0 aA | 3.17 ± 0.0 aE | 5.14 ± 0.0 aC | 3.38 ± 0.2 aB |
|         | S2           | 0.06 ± 0.0 aA | 3.54 ± 0.0 bC | 4.98 ± 0.1 aB | 4.07 ± 0.1 aB |
|         | S3           | 0.07 ± 0.0 aAB | 4.74 ± 0.0 cD | 5.22 ± 0.0 aBC | 5.46 ± 0.3 bA |
| Acarbose|              | 6.26 ± 0.0     |                  | 0.73 ± 0.0     |

S1–S3 refer to the different edible stages. Data are expressed as mean values of three independent replicates ± SD. Values within a column with different lowercase letters for the same faba bean variety at different edible stages are statistically different at \( p < 0.05 \). Values within a column with different uppercase letters for different faba bean varieties at same edible stage are statistically different at \( p < 0.05 \).
Data from the present study showed that the phenolic extract from immature faba bean seed coats and cotyledons were the excellent source of α-glucosidase and lipase inhibitors. Therefore, fresh faba bean seed could be used as a useful dietary adjunct for the management of blood glucose and obesity.

### Conclusion

In summary, phenolic profiles, antioxidant capacity as well as the inhibitory effects of phenolics on α-glucosidase and lipase were investigated in immature faba bean seeds of five varieties. The results showed that immature faba bean seeds were rich in phenolic compounds with different changing patterns depending on cultivars, edible stages, and morphological parts being tested. Therefore, the choice of the varieties and edible stages are the important factors that should be considered while consuming immature faba bean seed. Furthermore, phenolic extracts from both seed coats and cotyledons possess the strong inhibitory effects on α-glucosidase and lipase, indicating that immature faba bean seed is a potential source of functional food in management of diabetes and obesity. Additionally, the TPC, TFC, CTC, antioxidant activity, and inhibitory effect on α-glucosidase of seed coats were significantly higher than those of cotyledons, suggesting that great attention should be paid on the functionality and edibility of seed coats because they are traditionally discarded or used as animal feed when consuming.

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