

# Chemical Profile and In Vitro Bioactivity of *Vicia faba* Beans and Pods <sup>†</sup>

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**Abstract:** *Vicia faba* L. (faba bean) (Fabaceae) is cultivated worldwide as a crop for human consumption. In this study, beans and pods were investigated for their phytochemical content and their potential nutraceutical properties as a strategy to counteract metabolic syndrome (MetS). Pods represent a faba bean industrial processing by-product. Pod ethanol extract showed the highest total phenol and flavonoid content. High Performance Liquid Chromatography (HPLC) analysis revealed that in both pods and bean, (+)-catechin and (–)-epicatechin were the two most abundant compounds. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Ferric Reducing Antioxidant Power (FRAP), β-carotene bleaching were used to test *V. faba* antioxidant activity. The inhibition of alpha-amylase, alpha-glucosidase, and lipase was studied. Pod extract showed an ABTS radical scavenging ability (IC<sub>50</sub> value of 1.5 mg/mL) comparable to ascorbic acid (IC<sub>50</sub> value of 1.7 mg/mL) used as a positive control, whereas bean extract was the most active in protecting lipid peroxidation. A promising alpha-glucosidase inhibitory activity was also observed with the edible portion of faba beans (IC<sub>50</sub> value of 38.31 mg/mL). Collectively, our results demonstrated the potential health properties of *V. faba* edible and inedible portions.

**Keywords:** *Vicia faba*; beans; pods; by-products; carbohydrate hydrolyzing enzyme; lipase; antioxidant

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## 1. Introduction

Pulse crops have recently received attention from consumers and researchers for the high content in dietary fibers, micronutrients, and bioactive phytochemicals. Their low glycemic indexes and fats, in addition to their health promoting properties, including anti-diabetic and anti-obesity, make them one of the most important components of the human diet [1]. Among pulse crops, *Vicia faba* L. (Fabaceae) has received particular attention due to its nutraceutical, functional, and economic importance. This species represents a potential source of affordable alternative proteins in developed and underdeveloped countries. Moreover, it is rich in phenolic compounds that are recognized as healthy phytochemicals [2].

In this study, beans and pods were investigated for their phytochemical content and nutraceutical properties as a strategy to counteract metabolic syndrome (MetS), a group of risk factors, including insulin resistance and consequently impaired glucose tolerance, dyslipidemia, and obesity. In this context, the oxidative stress plays a key role [3]. The efficacy of phytochemicals from foods is a topic of great interest not only to cure but also

to prevent MetS onset. Moreover, pods represent a faba bean industrial processing by-product. The possible reuse of by-products can contribute to innovation and growth in the functional food and nutraceutical industry due to their high bioactive phytochemical content [4]. For this purpose, the following analyses were done: i. Evaluation of total phenols (TPC), flavonoids (TFC) and carotenoids (TCC) content; ii. Quantification of selected markers; iii. Evaluation of antioxidant activity through different *in vitro* assays (2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), Ferric Reducing Antioxidant Power (FRAP), and  $\beta$ -carotene bleaching test); iv. Inhibition of  $\alpha$ -amylase,  $\alpha$ -glucosidase, and lipase enzymes.

## 2. Materials and Methods

### 2.1. Sample and Extraction Procedure

Commercial faba beans were bought in the market in Cosenza, Calabria (Italy). Beans and pods were manually separated. Samples (50 g) were exhaustively softened by an ultrasound-assisted maceration process with ethanol (48 h  $\times$  3 times). The resultant solutions were dried under reduced temperature and pressure using a rotary evaporator to give an extraction yield of 4.2 and 5.2% (weight/weight) (w/w) for pods and beans, respectively.

### 2.2. Total Phenols, Flavonoids, and Carotenoids Content

The total phenol content (TPC) was evaluated using the Folin–Ciocalteu method as previously reported [5]. The sample at a concentration of 1.5 mg/mL was mixed with a solution of Folin–Ciocalteu reagent and water. Then, 1.5 mL of 20% sodium carbonate was added, and the mixture was incubated at 25 °C. The absorbance was measured at 765 nm using a UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy). The total phenol content was expressed as milligrams of chlorogenic acid equivalents (CAE)/g of dried weight (DW). Total flavonoid content (TFC) was determined spectrophotometrically using a method based on the formation of a flavonoid–aluminum complex [6]. The sample was mixed with aluminum chloride solution (2%) in a 1:1 ratio and incubated at room temperature for 15 min. The absorbance was measured at 510 nm. TFC was expressed as mg quercetin equivalents (QE)/g DW. The total carotenoid content (TCC) was determined as previously described [7]. Briefly, the extract was added to NaCl 5% solution, vortexed for 30 s, and centrifuged at 4500 rpm for 10 min. The supernatant (100  $\mu$ L) was diluted with 0.9 mL of *n*-hexane and measured at 460 nm. TCC was expressed as mg  $\beta$ -carotene equivalents/g Fresh Weight (FW).

### 2.3. HPLC–DAD Phenolic Profile

The high performance liquid chromatography (HPLC) analysis was performed using a Knauer instrument (Asi Advanced Scientific Instruments, Berlin, Germany) and a UV-Vis diode array detector (DAD) following the procedure previously described [8]. Selected polyphenols were quantified by comparing their retention time with those of standards and confirmed with characteristic spectra using a photodiode array detector and literature data. Calibration curves, detection limits (LOD), and quantification limits (LOQ) of analytical methods for the determination of phytochemicals in samples are reported in Table 1.

**Table 1.** Quantification of *Vicia faba* beans and pods selected markers.

Markers mg/Kg	Pods	Beans	Sign.
(−) Epicatechin	698.15 ± 4.71 <sup>a</sup>	378.23 ± 1.76 <sup>b</sup>	**
(+) Catechin	498.63 ± 3.52 <sup>a</sup>	378.63 ± 1.77 <sup>b</sup>	**
Syringic acid	76.24 ± 0.57 <sup>a</sup>	55.32 ± 0.42 <sup>b</sup>	**
Rutin	33.68 ± 0.28 <sup>a</sup>	25.77 ± 0.20 <sup>b</sup>	**
Quercetin-3- <i>O</i> -glucoside	28.64 ± 0.25 <sup>a</sup>	15.64 ± 0.13 <sup>b</sup>	**
Myricetin	1.81 ± 0.02 <sup>a</sup>	2.58 ± 0.03 <sup>a</sup>	**

Data represent means ± SD (standard deviation) (*n* = 3). Differences were evaluated by a one-way analysis of variance (ANOVA) test completed with a multicomparison Tukey’s test. \*\**p* < 0.05. Means in the same column with different small letters differ significantly (\*\**p* < 0.05).

#### 2.4. In Vitro Antioxidant Activity

The in vitro antioxidant activities of samples were evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), Ferric Reducing Antioxidant Power (FRAP), and β-carotene bleaching assays.

The ABTS assay was applied using the methodology described by Leporini et al. [7]. A solution of ABTS radical cation was prepared. After 12 h, the solution was diluted with ethanol to an absorbance of 0.70 at 734 nm using a UV-Vis Jenway 6003 spectrophotometer. A dilution of extracts in ethanol was added to 2 mL of diluted ABTS<sup>+</sup> solution to test the following concentrations from 400 to 1 µg/mL. After 6 min, the absorbance was read at 734 nm.

DPPH radical scavenging activity was determined according to the technique reported by Leporini et al. [7]. An aliquot of 1.5 mL of 0.25 mM DPPH radical (DPPH·) in ethanol was mixed with 12 µL of samples to test concentrations ranging from 1000 to 1 µg/mL. The absorbance was determined at 517 nm with a UV-Vis Jenway 6003 spectrophotometer. Ascorbic acid was used as a positive control in both radical scavenging activity assays.

In the β-carotene bleaching test, a mixture of linoleic acid, Tween 20, and β-carotene was prepared as previously described [8]. β-Carotene was added to linoleic acid 100% Tween 20. After evaporation of the solvent and dilution with water, the emulsion was added to a 96-well microplate containing samples in ethanol concentrations ranging from 100 to 2.5 µg/mL. The plate was left to incubate at 45 °C for 30 and 60 min. The absorbance was measured at 470 nm. Propyl gallate was used as a positive control.

For the preparation of the FRAP reagent, a mixture of 10 mM tripyridyltriazine (TPTZ) solution, 40 mM HCl, 20 mM FeCl<sub>3</sub>, and 0.3 M acetate buffer (pH 3.6) was prepared [8]. A sample at a concentration of 2.5 mg/mL in ethanol was mixed with FRAP reagent and water; the absorption of the reaction mixture was measured at 595 nm after 30 min of incubation at 25 °C. Ethanol solutions of known Fe (II) concentration, in the range of 50 to 500 µM (FeSO<sub>4</sub>), were used for obtaining the calibration curve. The FRAP value was expressed as µM Fe(II)/g. Butylated hydroxytoluene (BHT) was used as a positive control.

#### 2.5. Hypoglycemic and Hypolipidemic Effects

In the α-amylase inhibitory assay, the enzyme solution was mixed with starch [8]. Then, samples (at concentrations ranging from 1000 to 25 µg/mL) were added and left to react with the enzyme at room temperature for 5 min. The absorbance was read at 540 nm.

In the α-glucosidase inhibitory activity test, a maltose solution was mixed with α-glucosidase solution and *o*-dianisidine (DIAN) solution [8]. Samples (at concentrations ranging from 1000 to 25 µg/mL) were added to the mixture and left to incubate at 37 °C for 30 min. Then, perchloric acid was added, and the mixture was centrifuged. The super-

nantant was collected and mixed with DIAN and glucose oxidase peroxidase enzyme solution (PGO). After 30 min at 37 °C, the absorbance was read at 500 nm. Acarbose was used as a positive control in both tests.

Pancreatic lipase inhibitory activity was determined according to Leporini et al. [8]. 4-Nitrophenyl octanoate (NPC), an aqueous solution of porcine pancreatic lipase, and Tris-HCl buffer (pH 8.5) were prepared. Samples at different concentration from 2.5 to 40 mg/mL were added to a well with the enzyme, NPC, and buffer. The absorbance was measured at 405 nm after incubation of the mixture at 37 °C for 30 min. Orlistat was used as a positive control.

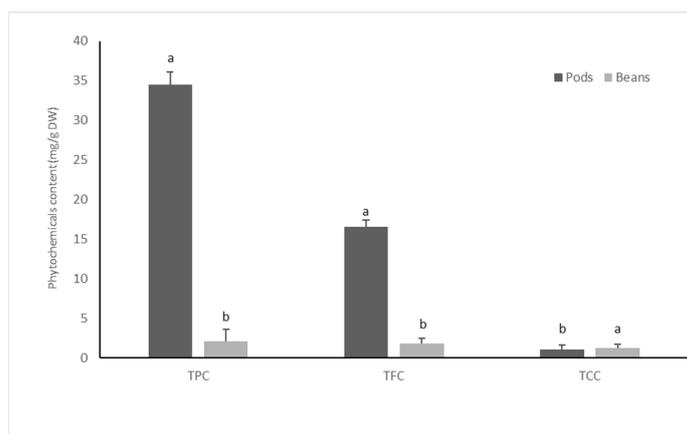
### 2.6. Statistical Analysis

Data are expressed as means  $\pm$  standard deviation (S.D.) ( $n = 3$ ). Prism GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA) was used to calculate the concentration that yielded 50% inhibition ( $IC_{50}$ ). Differences within and among groups were evaluated by one-way analysis of variance test (ANOVA) followed by a multicomparison Dunnett's test ( $\alpha = 0.05$ , \*  $p < 0.1$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$ ) in biological assays and Tukey's test to determine any significant difference in chemical parameters among investigated samples at  $p < 0.05$ . Studies of the Pearson's correlation coefficient ( $r$ ) and linear regression, assessment of repeatability, calculation of average, and relative standard deviation, were performed using Microsoft Excel 2010 software.

## 3. Results and Discussions

### 3.1. *V. faba* Beans and Pods Phytochemical Content and Phenolic Profile

Ethanol extract showed the highest TPC and TFC with values of 34.48 mg/g CAE DW and 16.58 mg/g QE DW, respectively, compared to the edible portion (2.06 mg/g CAE DW and 1.77 mg/g QE DW, respectively for TPC and TFC) (Figure 1). Previously, Chaieb et al. [9] evidenced pods TPC values from 56.97 to 149.21 mg gallic acid equivalent (GAE)/g in thirteen genotypes of cultivated *V. faba* pods. Differences were also recorded in TFC, where values ranging from 10.23 to 45.92 mg RE/g were observed. Lu et al. [10] evidenced that both TPC and TFC values are strictly dependent on the stage of maturity of the plant.



**Figure 1.** Comparative total phenols (TPC), flavonoids (TFC), and carotenoids (TCC) of *Vicia faba* beans and pods. Data are reported as mean  $\pm$  standard deviation ( $n = 3$ ). Differences were evaluated by a one-way analysis of variance (ANOVA) test completed with Tukey's test.  $p < 0.05$ . Means in the same column with different small letters differ significantly ( $p < 0.05$ ).

HPLC analysis revealed that (+)-catechin and (–)-epicatechin are the two dominant compounds in both samples. According to Lu et al. [10], these phytochemicals are the main abundant compounds; however, their content depended on varieties, plant maturation stages, and faba bean parts. Interesting also was the content of syringic acid.

### 3.2. *V. faba* Beans and Pods In Vitro Antioxidant Activities

Pods extract showed an ABTS radical scavenging ability (IC<sub>50</sub> value of 1.5 µg/mL) comparable to the positive control ascorbic acid (IC<sub>50</sub> value of 1.7 µg/mL), whereas beans extract was the most active in the protection of lipid peroxidation (IC<sub>50</sub> value of 17.6 µg/mL after 30 min of incubation). (Table 2). A value statistically comparable with the positive control was observed in the FRAP test for edible portions of faba beans extract (FRAP value of 70.16 µM Fe (II)/g). DPPH radical scavenging capacity from 3.1 and 4.73 µg TE/g DW for Cekin and Bauska samples, respectively, were found in pods by Valente et al. [2].

**Table 2.** *V. faba* beans and pods antioxidant activities.

Sample	DPPH Test IC <sub>50</sub> (µg/mL)	ABTS Test IC <sub>50</sub> (µg/mL)	β-Carotene Bleaching Test IC <sub>50</sub> (µg/mL)		FRAP Test (µM Fe (II)/g)
			t 30 min	t 60 min	
Pods	15.44 ± 2.2 ****	1.46 ± 1.48	123.49 ± 3.21 ****	147.89 ± 2.02 ****	49.56 ± 1.14 ****
Beans	74.71 ± 2.91 ****	2.35 ± 1.81 **	17.59 ± 1.8 ****	56.71 ± 1.14 ****	70.16 ± 1.57
<i>Positive control</i>					
Ascorbic acid	5.01 ± 0.80	1.72 ± 0.06			
Propyl gallate			0.09 ± 0.004	0.09 ± 0.004	
BHT					63.23 ± 4.31

DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP: Ferric Reducing Antioxidant Power Assay; BHT: Butylated hydroxytoluene. Data are expressed as means ± S.D. (n = 3). Differences within and between groups were evaluated by one-way ANOVA followed by a multicomparison Dunnett's test (α = 0.05): \*\* p < 0.01, \*\*\*\* p < 0.0001, compared with the positive controls.

### 3.3. *V. faba* Beans and Pods Hypoglycemic and Hypolipidemic Effects

As showed in Table 3 beans exerted a promising α-glucosidase inhibitory activity with an IC<sub>50</sub> value of 38.31 µg/mL, whereas pods were more active against α-amylase (IC<sub>50</sub> value of 38.31 µg/mL). No statistically significant differences were recorded against lipase with IC<sub>50</sub> values of 129.21 and 134.05 µg/mL for beans and pods, respectively. However, all values are higher than those reported for positive controls.

**Table 3.** *V. faba* beans and pods hypoglycemic and hypolipidemic effects [IC<sub>50</sub> (µg/mL)].

Sample	α-Amylase	α-Glucosidase	Lipase
Pods	188.94 ± 3.57 ****	742.91 ± 5.91 ****	134.05 ± 6.43 ****
Beans	313.21 ± 4.74 ****	38.31 ± 1.15 *	129.21 ± 5.28 ****
<i>Positive control</i>			
Acarbose	50.12 ± 1.36	35.51 ± 0.92	
Orlistat			37.42 ± 1.01

Data are expressed as means ± S.D. (n = 3). Differences within and between groups were evaluated by one-way ANOVA followed by a multicomparison Dunnett's test (α = 0.05): \*p < 0.1, \*\*\*\* p < 0.0001, compared with the positive controls.

Previously Mejri et al. [11] tested *V. faba* pods methanol extract in alloxan-induced diabetic mice. After oral administration at the dose of 500 mg/kg body weight (BW), for two weeks, animals showed a normal status of lipid profile and a mitigate the oxidative stress condition associated with diabetic disease. The presence of flavan-3-ols, such as catechin and epicatechin and flavonols (glycosides of quercetin and rutin), may be responsible for these activities. Moreover, Choudhary and Mishra [12] evidenced that *V. faba* beans acetone extract inhibited α-amylase with IC<sub>50</sub> value of 2.94 mg/mL). Kinetic analysis revealed that the extract displayed a mixed inhibition mode and that catechin and epicatechin, identified in both beans and pods, are able to interact with catalytic residues of the enzyme.

#### 4. Conclusions

This study confirmed that *V. faba* seeds and pods are rich in bioactive phytochemicals suggesting how this pulse crop is an interesting novel source of healthy compounds that should be considered not only for human nutrition but also by nutraceutical industries for new products development.

**Author Contributions:** M.R.L. carried out the overall project design experimental work, M.L. and T.F. analyzed results and wrote the manuscript. V.S., T.F., and M.B. assisted with experimental work. R.T. and M.R.L. conceptualized aspects of the project and assisted with reviewing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Conflicts of Interest:** The authors declare no conflict of interest.

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