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Chemical profile and in vitro bioactivity of Vicia faba beans and pods

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INTRODUCTION

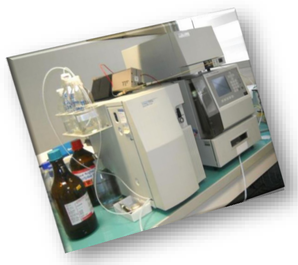
***Vicia faba* L. (fava bean) is member of Fabaceae family. It is of uncertain origin and cultivated worldwide as a crop for human consumption [1]. In this study, beans and pods are investigated for their phytochemical content and nutraceutical properties as strategy to counteract metabolic syndrome (MetS), a group of risk factors, including insulin resistance and consequently impaired glucose tolerance, dyslipidaemia and obesity. In this contest, the oxidative stress plays a key role [2].**

The efficacy of phytochemicals from foods is a topic of great interest not only to cure but also to prevent the onset of the MetS. Moreover, pods represents a fava bean industrial processing by-products. The possible reuse of by-products can contribute to innovation and growth in the functional food and nutraceutical industry due their high bioactive phytochemical content [3].

MATERIALS AND METHODS

Sample and extraction procedure

Commercial fava beans were bought in the market in Cosenza, Calabria (Italy). Beans and pods were manually separated. Samples (50 g) were exhaustively by ultrasound assisted maceration process with ethanol (48h x 3 times). The resultant solutions were dried under reduced temperature and pressure using a rotary evaporator to give extraction yield of 4.22 and 5.22 % for pods and beans, respectively.



Total Phenols, flavonoids and carotenoids content

Total phenols, flavonoids and carotenoids content was determined as previously reported by Leporini et al. [4]

HPLC quantification of selected phenolic compounds

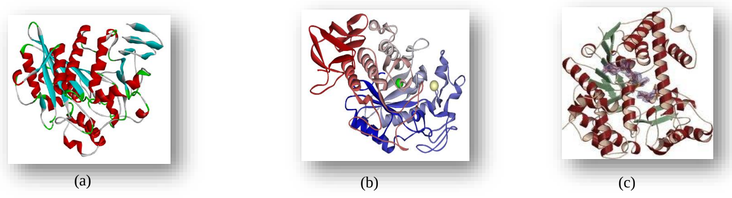
Selected phytochemical markers were quantified by HPLC following the method previously described by Leporini et al. [4]

Antioxidant activity

Antioxidant compounds may act *in vivo* through different mechanisms of action. For this reason no single method can fully evaluate the antioxidant capacity of food since levels of single antioxidant in food do not necessarily reflect their antioxidant activity. Therefore, to investigate the antioxidant activity of chemicals choosing an adequate assay based on chemicals of interest is critical. A multi-target approach was used to test the antioxidant activity by using DPPH, ABTS, β-carotene bleaching, and FRAP assays [4].

Hypoglycaemic and hypolipidemic effects

The hypolipidemic potential was investigated through inhibition of lipase while, the inhibition of carbohydrate hydrolysing enzymes, α-amylase and α-glucosidase, was used to evaluate the hypoglycaemic activity [3].

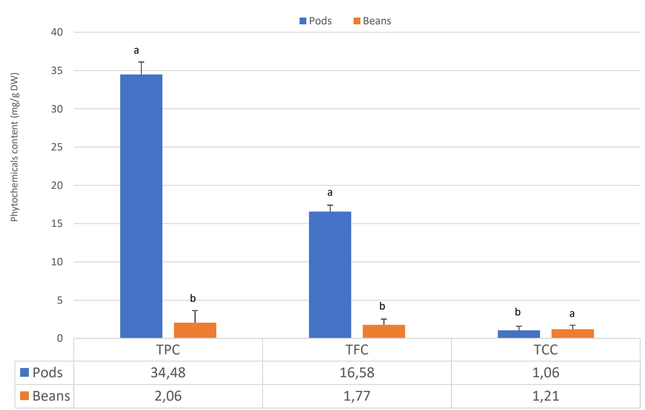


Lipase (a), α-Amylase (b) and α-Glucosidase (c)

RESULTS AND DISCUSSION

It is interesting to note that pods ethanol extract showed the highest total phenols and flavonoids content with values of 34.48 mg/g chlorogenic acid equivalent (CAE) DW and 16.58 mg/g quercetin equivalent (QE) DW, respectively compared to edible portion (2.06 mg/g CAE DW and 1.77 mg/g QE DW, respectively for TPC and TFC).

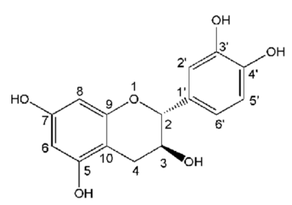
TPC, TFC and TCC in V. faba samples

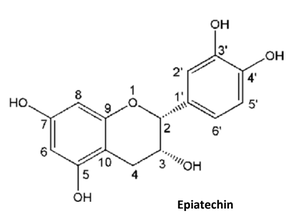


	TPC	TFC	TCC
Pods	34,48	16,58	1,06
Beans	2,06	1,77	1,21

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

HPLC analysis revealed that in both samples (+)-catechin and (−)-epicatechin were the two main abundant compounds. Interesting also, was the content in syringic acid. Among flavonoids rutin was the main abundant compounds in pods (33,68 mg/Kg)





Selected markers quantification

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

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

Antioxidant activity of V. faba samples

	DPPH test (IC_{50} µg/mL)	ABTS test (IC_{50}µg/mL)	β-carotene bleaching test IC_{50} (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
Positive control					
Ascorbic acid	5.03 ± 0.81	1.71 ± 0.06			
Propil gallate			1.01 ± 0.04	1.01 ± 0.06	
BHT					63.20 ± 4.03

Data are express as ± S.D. (n= 3). DPPH Radical Scavenging Activity Assay; Antioxidant Capacity Determined by Radical Cation (ABTS+); Ferric Reducing Antioxidant Power (FRAP). Ascorbic acid. BHT and Propyl gallate were used as positive control in antioxidant tests. Differences within and between groups were evaluated by one-way ANOVA followed by a multicomparison Dunnett's test (α= 0.05): ****p< 0.0001, **p< 0.01. compared with the positive controls.

Pods extract showed an ABTS radical scavenging ability (IC_{50} value of 1.5 µg/mL) comparable to the positive control ascorbic acid (IC_{50} value of 1.7 µg/mL) whereas, beans extract was the most active in protection of lipid peroxidation (IC_{50} value of 17.6 µg/mL after 30 min of incubation).

Hypoglycaemic and hypolipidemic effects of V. faba samples

	α-Amylase IC_{50} (µg/mL)	α-Glucosidase IC_{50} (µg/mL)	Lipase IC_{50} (µg/mL)
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****
Positive control			
Acarbose	50.12 ± 1.36	35.51 ± 0.92	
Orlistat			37.42 ± 1.01

Data are expressed as means ± S.D. (n= 3). Acarbose used as positive control in α-amylase and α-glucosidase tests. Orlistat used as positive control in lipase test. Differences within and between groups were evaluated by one-way ANOVA followed by a multicomparison Dunnett's test (α= 0.05): ****p< 0.0001. *p< 0.1. compared with the positive control.

Value statistically comparable with the positive control was observed in FRAP test for edible portions of fava beans extract (IC_{50} value of 70.16 µM Fe (II)/g).
The same sample exerted also a promising α-glucosidase inhibitory activity with an IC_{50} value of 38.31 µg/mL.

CONCLUSION

Collectively, our results demonstrated the potential health properties of *V. faba* edible and inedible portions. However, further *in vivo* studies will be needed to confirm the potential in humans and prove the safety of the products.

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