

# Effects of Soaking, Cooking, and Steaming Treatments on the Faba Bean Seeds' Total Bioactive Compounds Content and Antioxidant Activity

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**Abstract** Faba bean (*Vicia faba* L.) is a winter-sowing grain legume with high-protein content utilized for human consumption and animal feeding. Faba beans contain high levels of polyphenol compounds with various nutritional benefits. Legume seeds, such as faba beans, are subject to different processing procedures before consumption, which decreases the levels of their bioactive compounds (polyphenols and flavonoids), resulting in a reduction in antioxidant activity. This study aimed to examine the influence of soaking, cooking, and steaming treatments on the total polyphenols, total flavonoid, and antioxidant activity levels of Broad (large) and Field (small) faba beans. Initially, the influence of the different extraction solvents on total phenolic content (TPC), total flavonoid content (TFC), and ferric reduction ability of plasma antioxidant activity (FRAP), variables were inspected, wherein the extraction efficiency. 70% acetic acetone treatment was found to be the best solvent for extraction. Then, we determined the soaking, cooking, and steaming effects on TPC, TFC, and FRAP levels compared to dry seeds as the control. It was found that the various processes resulted in a significant reduction in the level of these components in the processed seeds of both Broad and Field beans. Nevertheless, the soaking treatment resulted in the smallest reduction. In addition, steaming caused a more moderate decline among the cooking types. Therefore, to maintain a higher level of these three ingredients in the final product, it is better to steam the seeds than cook them.

**Keywords:** food processing, nutritional quality, Polyphenols, FRAP, *Vicia faba*

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## 1. Introduction

Faba bean (*Vicia faba* L.), also called Broad Bean, Horse Bean, or Field Bean, are a winter-sowing grain legume with high-protein content utilized for human consumption and animal feed [1]. Faba bean is one of the 7<sup>th</sup> the world's most important pulse crops, followed by bean, chickpea, cowpea, pea, pigeon pea, and lentil. Faba bean seeds are cultivated in more than 60 countries in East and North Africa, southern Europe, West, and South Asia, South and North America, and Australia. The leading producer countries are China (37%), Ethiopia (18%), and Australia (9%). It covers 3% (2.5 million hectares (ha)) of the world's pulse croplands and with a mean annual production of 4.8 million tons [2]. Faba bean seeds contain about 30% protein, 42% Starch, 10% fibers, 4% sugars, and 2% fat [3]. Faba bean cultivars can be divided into two main types: broad beans and field beans. Broad bean cultivars are large flattened seeds with a 100 seed weight between

100-200g, and field bean cultivars are small round seeds with a 100 seed weight between 40-80g [3].

Dry legumes, including Faba bean seeds, also contain high levels of polyphenol compounds that confer high levels of antioxidant activity (AA) [4,5,6,7]. These compounds correlate to several biological activities, including antiaging, anti-inflammation, apoptosis, antioxidation, anticancer, antiatherosclerosis, cardiovascular protection, and other chronic diseases [8,9,10]. Thus, faba bean seeds could be an excellent potential functional food source. Like other legume seeds, faba bean must be subject to different processing procedures before consumption, such as soaking and cooking. These processes improve seeds' flavor and palatability, increase their protein digestibility and eliminate raffinose carbohydrates responsible for the unpleasant feeling and flatulency [11,12]. Processing also reduces the bioactive compounds and AA levels [13,14,15]. Although studies of the effects of thermal processes on total phenolic content and AA of dry faba beans showed a reduction in their levels [8,15,16], the data on the amount of polyphenols in the surrounding water is

limited and was done on frozen beans [17] or ground seeds [18]. This study aimed to examine the influence of soaking, regular, pressure, steaming, and pressure steaming cooking on the total polyphenols and flavonoid compounds and antioxidant activity levels of broad (large) and field (small) faba bean seeds.

## 2. Materials and Methods

### 2.1. Plant Material

This study used two *Vicia faba* seed types: one large, broad bean type with a light green color and one small field bean type with a brown-colored seed. Both bean types were purchased at a retail food store.

### 2.2. Extraction and Determination of Phenolic Compounds and Antioxidant Activity from Seeds

For total phenolic compounds (TPC) and total flavonoid content (TFC) extraction, seeds of both faba bean types were ground into a fine powder of 60 mesh in a grinder (Retsch MM301). Total polyphenols were extracted from 0.1g powder with 1000 $\mu$ l of the extraction solvents (Bio-Lab): 70% methanol, 50 and 80% acetone, 70% acid acetone, and 66% acid, as previously described [19]. All extractions were conducted in triplicate. Determination of TPC, TFC, and ferric reduction ability of plasma AA (FRAP AA) was as previously described [19].

### 2.3. Soaking, Cooking, and Steaming Treatments

For soaking, raw faba bean seeds (1200g) were immersed in 3-4L of tap water in a plastic container for 16hr at room temperature. For regular cooking, 200g of soaked seeds were added to 1L of fresh tap water and brought to a boil in a 2.5L pot. After boiling, the seeds were continued to be cooked at 100°C for 80min. For pressure cooking, 200g soaked seeds were added to 1L fresh tap water and autoclaved at 121°C 1.5 atmospheres for 45min. For TPC, TFC, and FRAP AA determination in soaking and cooking water, three samples of 1.5ml of soaking or boiling water were sampled. For steaming, 200g soaked seeds were steamed in a steaming cooker VC100 (Tefal) for 180min. For pressure steaming, 200g soaked seeds were put into a plastic bag and autoclaved at 121°C 1.5 atmospheres for 45min. After each treatment, seed (100g) and water samples were cooled to room temperature and freeze-dried for five days. After drying the seed, grind it into a fine powder as above.

### 2.4. Statistical Analysis

Data were subjected to Tukey's HSD test utilizing JMP version 5 software (SAS Institute, Inc. Cary, NC, USA). Correlation among variables was done using the Pearson correlation test. Significant levels were defined using  $P \leq 0.05$ . All analyses were performed in triplicate.

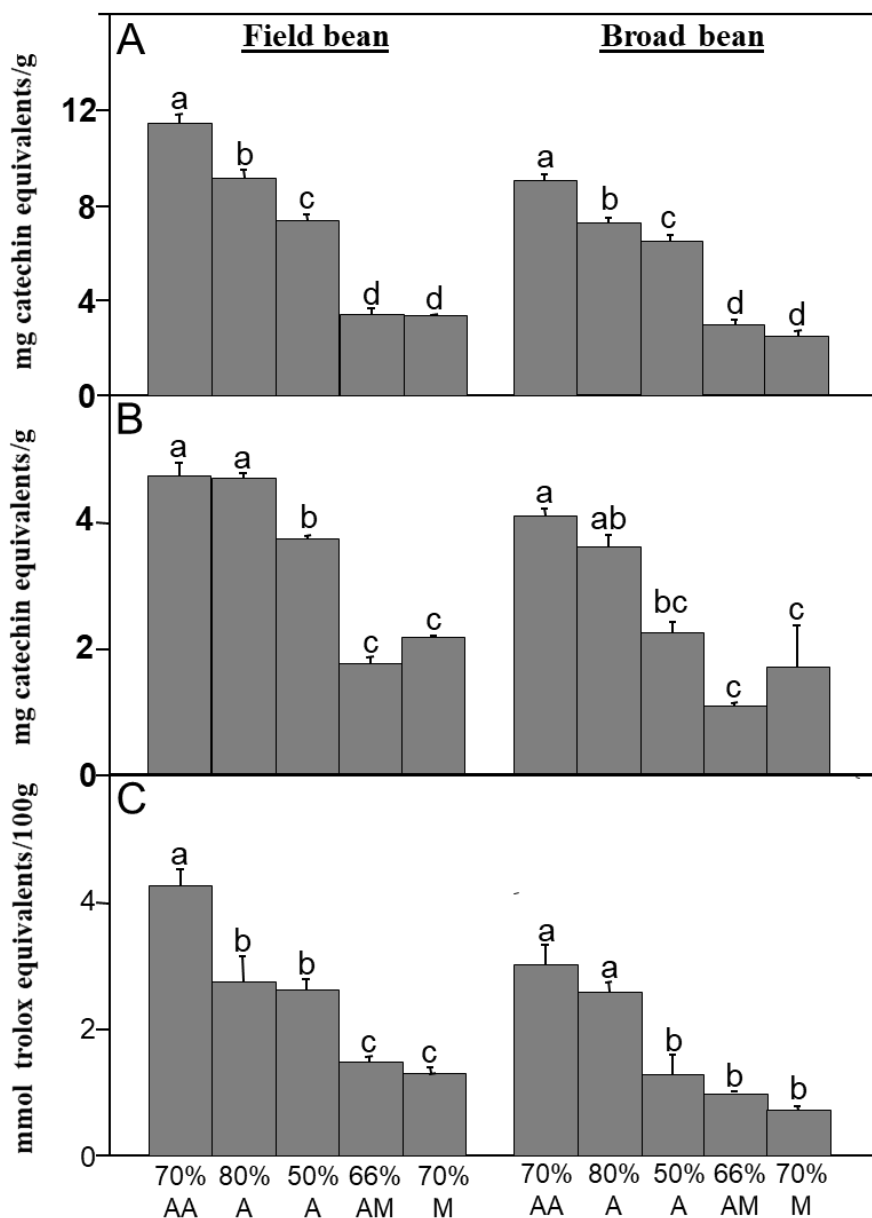
## 3. Results and discussion

### 3.1. Influence of Extraction Solvents on TPC, TFC, and FRAP AA

Figure 1 shows the effect of extraction solvents on TPC, TFC, and FRAP AA levels extracted from both broad and field bean types. High variation was observed between the solvents in the TPC, TFC, and FRAP AA extraction yield. In both bean types, 70% acidic acetone was significantly the best solvent for the extraction of both TPC (Figure 1A) and TFC (except for 80% acetone) (Figure 1B). The difference in the extraction yield of TP and TFC results in higher levels of FRAP AA (Figure 1C). As in the current study, the extraction yield of TPC, TFC, and FRAP AA from other legume seeds was greatly affected by the extraction solvents. 50% acetone was the best extraction solvent for both TPC, TFC, and FRAP AA from chickpeas [19], peanuts [20], and yellow soybean [21]. 70% acidic acetone was the best extraction solvent for lentils, black soybean, red and black kidney beans [21]. In faba beans, different solvents have been used to extract TPC. Siah *et al.* (2019) [7] found that TPC and antioxidant activities with 70% acetone extract were 2 and 2-6 fold than those of 80% methanol extract. Amarowicz and Shahidi (2017) [4] used 80% acetone, Kwon *et al.* (2018) [6] used 100% methanol, and Mehanni *et al.* (2017) [22] used 60% acidic methanol. Our results showed that 70% acidic acetone is the most efficient solvent for faba beans, therefore, further analyses were made only on 70% acidic acetone extractions.

### 3.2. TPC, TFC, and FRAP AA in Dry Seeds

TPC, TFC, and FRAP AA in dry seeds of Field and Broad beans are shown in Figure 2A-C (columns DS). Field bean type contains 30% more TPC, 20% more TFC, and 50% more FRAP AA than the Broad bean type. This may be due to the difference in seed color as previously reported in other crops [4,5,6,7,23-27]. Field faba bean contained 11.5mg catechin equivalents/g (mce/g) TPC, 4.8mce/g TFC, and 4.3mmol Trolox equivalents/100g (mte/100g) FRAP AA. Broad faba bean contained 8.7mce/g TPC, 4.1mce/g TFC, and 2.9mte/100g FRAP AA Figure 2A-C (columns DS). The levels of TPC, TFC, and FRAP AA in dry seeds were lower than those found by some reports [4,23,24] and higher than those found by others [7,26,27,28]. This may be due to different extraction conditions, different cultivars, or different reference standards. Dry faba bean seeds contain high TPC, TFC, and FRAP AA levels, as found in other colored seed legumes [19,21,29,30], including faba bean [4,5,15,23,24,27,28]. The TPC and FRAP AA levels of dry seeds were similar to or even higher than those of most fruits, vegetables, beverages, chocolates, and nuts [31,32]. This supports that dry-colored pulses are a good source of polyphenols and antioxidant activity [19]. High significant correlations were found between TPC, TFC, and FRAP AA (data not shown) with R values of 0.92-0.95, indicating that most antioxidant activity of faba bean seeds is derived from polyphenol compounds. Similar results were obtained in other seed legumes [15,19,21].



**Figure 1.** Total phenolic content (A), total flavonoid content (B), and FRAP antioxidant activity (C) of Field and Broad bean types extracted with different solvents. 70% acidic acetone (70% AA), 80% acetone (80%A), 50% acetone (50%A), 66% acidic methanol (66%AM) and 70% methanol (70%M). The bar represents means  $\pm$  standard error from at least three replicates. Columns of each seed type marked by the same letter are not significantly different ( $P > 0.05$ )

### 3.3. Effects of Soaking, Cooking, and Steaming on TPC, TFC, and FRAP AA

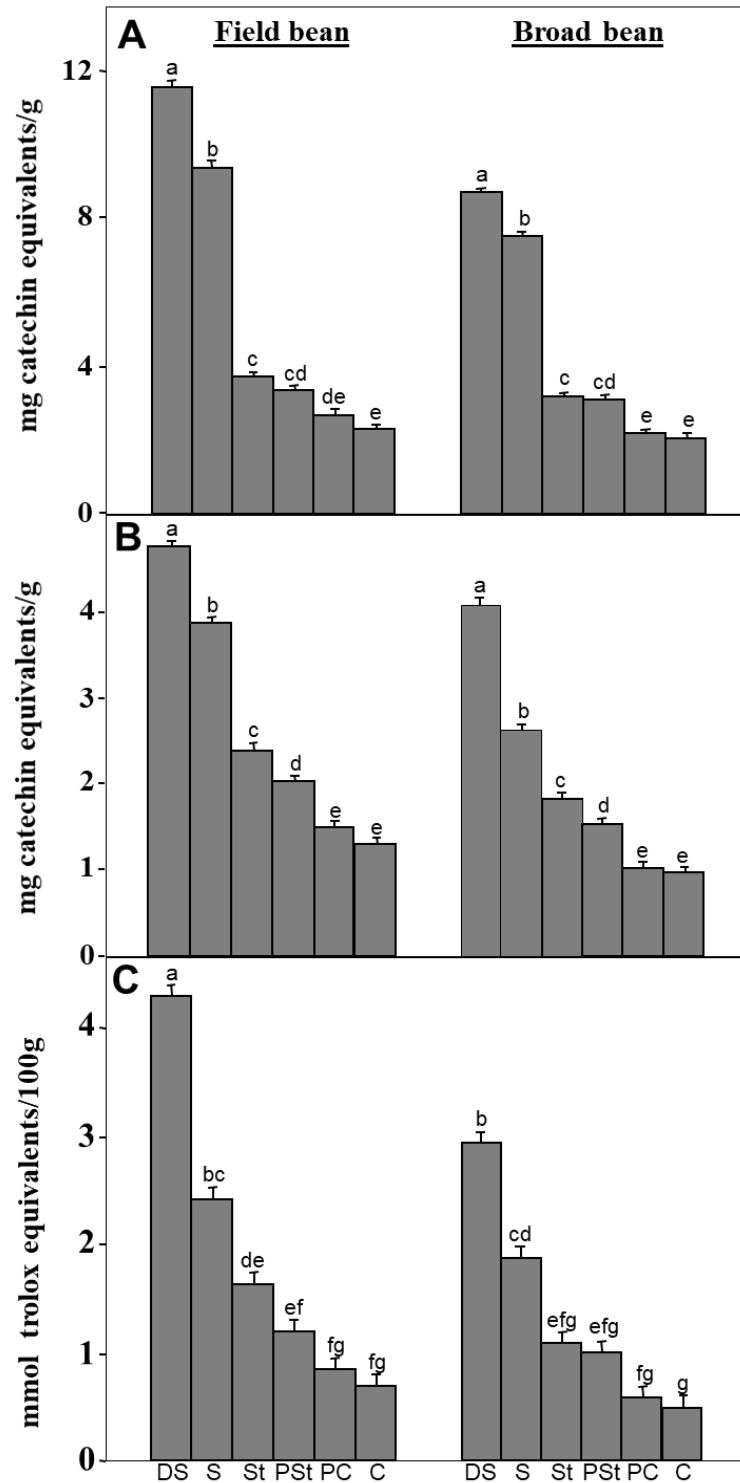
Soaking in water or alkali salts is a standard cooking pretreatment of chickpea or legume seeds that decreases energy costs due to a reduction in cooking times [33]. Therefore, we also checked the soaking effect on faba beans. Soaking treatment's effect on the TPC, TFC, and FRAP AA levels in the field and Broad faba beans are presented in Figure 2A-C (columns S) and Table 1A. In both Field and Broad seeds, soaking reduced TPC, TFC, and FRAP AA levels by 13-18%, 19-36%, and 36-44%, respectively.

The effect of regular cooking (C), pressure cooking (PS), steaming (St), and pressure steaming (PSt) treatments on the level of TPC, TFC, and FRAP AA in the field and broad beans are shown in Figure 2A-C (columns C, PC, St, and PSt). Both bean types' cooking and

steaming treatments reduced TPC, TFC, and FRAP AA levels. TPC decreased by about 67% during St and PSt and by about 77% during C and PS treatments (Figure 2A (columns C, PC, St, and PSt)). TFC was reduced by about 56% during St and PSt and by about 73% during C and PS treatments (Figure 2B (columns C, PC, St, and PSt)). FRAP AA was reduced by about 65% during St and PSt and by about 82% during C and PS treatments (Figure 2C (columns C, PC, St, and PSt)). A similar decrease in TPC, TFC, and AA after soaking were also reported in other legumes [13,34,35], including faba beans [22,28,36]. In many cases, the decrease in TPC, TFC, and AA are caused by the leakage of polyphenols and flavonoids into the soaking or cooking water [10,13,34,35,37]. Thus, we examined the soaking and cooking water for the presence of polyphenols, flavonoids, and FRAP AA (Table 1A-C). As shown in the table, 39-77% of the disappeared TPC, 28-42% of the disappeared TFC, and

16-36% of the disappeared FRAP AA were found in the soaking water after soaking. Thus, 23-61% of the disappeared TPC, 58-72% of the disappeared TFC, and 64-84% of the disappeared FRAP AA were broken (Table 1A). After pressure cooking, 46-83% of the disappeared TPC, 74-91% of the disappeared TFC, and 91% of the disappeared FRAP AA were found in the cooking water. Thus, 17-54% of the disappeared TPC, 9-26% of the disappeared TFC, and 9% of the disappeared FRAP AA were broken (Table 1B).

After regular cooking, 57-62% of the disappeared TPC, 71-92% of the disappeared TFC, and 67-106% of the disappeared FRAP AA after presser cooking were found in the cooking water. Thus, 38-43% of the disappeared TPC, 8-29% of the disappeared TFC, and (-6)-33% of the disappeared FRAP AA were broken (Table 1C). The breakage in TPC, TFC, and AA might be due to germination processes [22,38], the enzymatic activity of polyphenol oxidase [39,40], or decreases in extractability [41].



**Figure 2.** Total phenolic content (A), total flavonoid content (B), and FRAP antioxidant activity (C) in several treated conditions of two faba bean types. DS = dry seed, S = soaking, St = steaming, Pst = pressure steaming, PC = pressure cooking and C = cooking. All samples were extracted with 70% acidic acetone. The bar represents the means  $\pm$  standard error from at least three replicates. Columns marked by the same letter are not significantly different ( $P > 0.05$ )

**Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and FRAP antioxidant activity (AA) in the seeds and water of soaked and cooked faba bean treatments**

Treatment	Seed Type		Disappeared From seeds <sup>1</sup>	Found in water <sup>2</sup>	Break up
A. Soaking <sup>1</sup>	Field	TPC	18	39	61
		TFC	19	42	58
		FRAP AA	44	16	84
	Broad	TPC	13	77	23
		TFC	36	28	72
		FRAP AA	36	36	64
B. Presser cooking <sup>2</sup>	Field	TPC	79	46	54
		TFC	70	74	26
		FRAP AA	75	91	9
	Broad	TPC	75	83	17
		TFC	66	91	9
		FRAP AA	76	91	9
C. Regular cooking <sup>2</sup>	Field	TPC	82	57	43
		TFC	76	71	29
		FRAP AA	79	67	33
	Broad	TPC	79	62	38
		TFC	74	92	8
		FRAP AA	67	106	-6

1- 100% = TPC, TFC, and FRAP AA in dry seeds.

2- 100% = TPC, TFC, and FRAP AA in seeds after soaking treatment.

## 4. Conclusion

Dry faba beans of both sizes (types) contain high levels of polyphenols, flavonoids, and antioxidant activity. Therefore, the faba bean seeds can be a good source of these components, which have been linked to the prevention of various chronic diseases such as cancer and heart disease. Despite the high level of these three compounds in dry seeds, various processes such as soaking, cooking, cooking with pressure, and evaporation, which are necessary for preparing these seeds for eating, caused a significant decrease in the level of these three components in the processed seeds. We also found that the soaking treatment resulted in the smallest reduction of these compounds than any other processing treatments. In addition, among all types of cooking, evaporation caused a more moderate decline than cooking and cooking pressure. Therefore, to maintain a higher level of these three ingredients in the final product, it is better to vaporize the seeds than cook them.

## Conflict of Interest

The authors declare no conflict of interest.

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