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Phytochemical content and *in vitro* antioxidant activity of faba bean (*Vicia faba* L.) as affected by maturity stage and cooking practice

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Abstract

The effects of growing stage (immature and mature) and home cooking methods (boiling and steam cooking) on the phytochemical contents, and antioxidant capacity of faba bean were investigated. Different faba bean morphological fractions (whole pod, pod coat, whole seed, seed coat and cotyledon) were analyzed. The antioxidant capacity was evaluated by the DPPH radical scavenging capacity, total antioxidant capacity, and oxygen uptake inhibition. The results showed that the immature faba bean fractions had significantly higher phytochemical contents and displayed a better antioxidant activity than those of mature ones; the highest level of phytochemicals and the strongest antioxidant activity were recorded in the seed coat. This study demonstrates also that cooking caused significant decrease in the phytochemical bioactive contents, and antioxidant activity, depending on the morphological fraction and cooking practice. The results of this study revealed also that steam cooking would be preferred to preserve the bioactive phytochemicals of faba bean.

Introduction

Faba bean (*Vicia faba* L.) commonly named fava bean, broad bean, horse bean and field bean is a species of bean which belongs to the family of Fabaceae. It is a vegetable cultivated in many countries (Mediterranean region, China, India, South America and Middle-Eastern Europe) (Duc *et al*., 2010). In 2012, the world production of faba beans was of 3.7 million tons; Algeria is currently the 10th world producer. The Algerian production of faba bean has increased considerably during the last 20 years. Indeed, the production increased from 15500 tons in 1988 to 40507 tons in 2012 (FAO, 2014).

Faba bean is a major food and feed legume because of the high nutritional value of its seeds, which are rich in protein and starch (Duc *et al*., 2010; Asaduzzaman and Asao, 2012). It is also revealed that faba bean seed is a potential source of dietary fiber, and many macro and micro-elements for human and animal consumption (Hacıseferogulları *et al.*, 2003).

Phenolics have strong antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions, and chelate metals. Moreover, high phenol consumption has been correlated with reduced risks of cardiovascular diseases and cancers. Cooking significantly affects levels of particular substances, and their functionality. The phenolic content and antioxidant activity of fruits and vegetables are affected by several factors including maturity stage and cooking (Han and Baik, 2008; Wolosiak *et al.*, 2010; Maisarah *et al*., 2013). However, to our knowledge, there is no study carried out on the effect of cooking methods on the phytochemicals along with the antioxidant activity of faba bean parts at different maturity stage. Therefore, the aim of the present investigation was to evaluate and compare the effect of domestic cooking practices (boiling and steaming) on phenolic, flavonoid, and proanthocyanidin contents, as well as the antioxidant activity of faba bean fractions (whole pod, pod coat, whole seed, seed coat, and cotyledon) at both immature and mature stages.

Material and methods

Chemicals

Folin-Ciocalteu reagent, acetone and methanol were from Biochem, Chemopharma (Montreal, Quebec); aluminium chloride and sodium carbonate were from Biochem, Chemopharma (Georgia, USA); 2-2-diphenyl-1-picrylhydrazyl (DPPH), and ammonium molybdate were from Sigma-Aldrich (Sernheim, Germany); methyl linoleate (MeLo), a gift

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(Estorob 301.01) of Novance (Venette, France) was distilled in a glass oven (Kugelrohr, Büchi, Rungis, France) ; 2,2´-azobis-isobutyronitrile (AIBN) was from Fluka and oxygen from Air Liquide, France.

Plant material and sample preparation

The faba bean (*Vicia faba* L.) used for this study was cultivated in the region of Bejaia (North of Algeria) during the campaign 2012. The pods were obtained at two different stages of growth. Some pods (15 Kg) were collected in the immature stage when the plants were fresh, i.e. green (May); others (7 Kg) were harvested in the mature stage when the plants were completely dry (July).

Steaming process

The fresh pods were steamed over boiling water in the ratio of 1:3 (w/v) until they became soft (25 min).

Boiling process

The fresh pods and mature seeds were cooked in distilled water (100°C) in the ratio of 1:3 and 1:10 (w/v) until they became soft (25 and 85min, respectively).

After cooking, the samples were separated into five parts (whole pod, pod coat, whole seed, seed coat and cotyledon), lyophilized (Christ, Germany), ground with a crusher (IKA, Germany) and passed through a 500 μm sieve.

Preparation of faba bean extracts

A powder of each faba bean part (0.1 g) was extracted with 15 mL of 75% acetone as reported by Vioque *et al.* (2012). The mixture was shaken in a water bath shaker (Memmert, Germany) for 60 min at 37°C, centrifuged at 3000 rpm for 10 min (Nüve, Turkey), and paper filtered. The filtrates were subsequently used for the determination of phytochemicals and antioxidant activity.

Total phenolic contents (TPC)

The TPC were determined according to Singleton and Rossi (1965). Two hundred microliters of sample were mixed with Folin-Ciocalteu reagent (1000 μL) and 7.5% sodium carbonate (800 μL). The mixtures were allowed to stand at room temperature for 60 min. The absorbance was measured at 750 nm with a spectrophotometer (Uvline 9400, Secomam, Alès, France). The results were expressed as mg of gallic acid equivalent (GAE) per g of dry matter (DM).

Total flavonoid contents (TFC)

The flavonoid contents were estimated according to Quettier-Deleu *et al.* (2000). Equal volumes of extract and aluminum chloride solution (2%) were mixed. The absorbance of the mixture was measured at 430 nm, after 15 min of incubation. Flavonoid contents were expressed as mg quercetin equivalent (QE) per g of dry matter.

Proanthocyanidins (PA)

The proanthocyanidin contents were determined according to Skerget *et al*. (2005). The extract (200 μL) was mixed with 2 mL of iron sulphate solution. The mixture was incubated at 95°C for 15 min. The absorbance was determined at 540 nm. The results were expressed as mg cyanidin equivalent (CE) per g of dry matter, using a molar extinction coefficient of cyanidin (ε=34700 L/mol.cm).

Antiradical activity

The DPPH radical scavenging activity was measured according to Brand-Williams *et al.* (1995). Twenty microliters of extracts were added to 2 mL (60 μM) of DPPH solution. The absorbance was determined at 517 nm. The DPPH scavenging activity was expressed as mg gallic acid equivalent (GAE) per g of dry matter.

Phosphomolybdenum method

The total antioxidant capacity (TAC) was evaluated as described by Prieto *et al*. (1999). A volume of 200 μL of extract was added to 2 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95 °C for 90 min. After the samples had been cooled, the absorbance was measured at 695 nm. The results were expressed as mg gallic acid equivalent per g of dry matter.

Oxygen uptake inhibition method (OUI)

The antioxidant property was also investigated by evaluating inhibition of methyl linoleate (MeLo) oxidation. The induced oxidation by molecular oxygen was performed in a gas-tight borosilicate glass apparatus as previously reported (Diouf *et al.*, 2006). Reaction temperature was 60°C and initial conditions were as follow: 2mL of AIBN 18 mM; 1mL of MeLo 1.6 M; 1mL of extract 0.4 g/L; oxygen pressure 145 Torr. The oxygen uptake was monitored continuously by a pressure transducer (model 104, Grand Island, USA). In the absence of the extract (control), oxygen uptake is roughly linear, while in the presence of an antioxidant, oxygen consumption is slower. The antioxidative capacity (OUI) of the extracts was estimated by comparing the oxygen uptake after 3

h, in the absence (pressure variation ΔP control) and in the presence of the extracts (pressure variation ΔP sample) according to the following equation:

$$OUI\ (\%) = \frac{\Delta P\ control\ -\ \Delta P\ sample}{\Delta P\ control} \times 100$$

Error values (standard deviation) are about 5% for all the assays (El Hage *et al*., 2012).

Statistical analysis

All analyses were carried-out in triplicate and the results were expressed as means ± standard deviation of independent extractions. The analysis of variance (ANOVA) was performed using STATISTICA 5.5 to compare the means. Differences were considered statistically significant at $p<0.05$.

Results and Discussion

Total phenolic contents (TPC)

In the present study, different faba bean parts were investigated (Table 1). For the raw immature faba bean, seed coat showed the highest TPC (68.54 mg/g) followed by pod coat, whole pod, whole seed, and cotyledon (12.31mg/g). For the raw mature faba bean, seed coat also showed the highest TPC (41.46 mg/g) followed by pod coat, whole seed, and cotyledon (4 mg/g). Similarly, Gujral *et al.* (2013) recorded higher total phenolic amounts in seed coat fraction of some legumes including red kidney bean and chickpea than in whole seeds. This could be due to the physiological role of phenolic compounds in the first line of plant defense mechanism as UV protection and antimicrobial effects.

Data of the current study were higher than those reported by Zadernowski *et al*. (2001) for whole seed (276-794 mg catechin equivalent/kg) and seed coat (1761-5218 mg/kg) of broad bean varieties, Boudjou *et al.* (2013) for whole seed (9.53 mg GAE/g), seed coat (35.92 mg GAE/g) and cotyledon (2.2 mg GAE/g) of mature raw faba bean cultivated in Algeria. However, the phenolic contents recorded in the present study were lower than those found by Chaieb *et al.* (2011) for different parts of dry raw faba bean genotypes grown in Tunisia. Also, these authors found that for most genotypes investigated, pod of raw faba bean showed the highest TPC followed by seed coat, then whole seed and cotyledon. These differences could be due to various factors including the extracting conditions (solvent, temperature, and duration), cultural practices and geographical location.

The results of this work revealed that the total phenolic content of immature raw faba bean parts was significantly ($p<0.05$) higher than that of raw mature faba bean (Table 1). In samples harvested at the mature stage, the phenolic contents decreased by 40% in the seed coat to about 67% in the whole seed, and cotyledon. Similar observation was reported in our previous study (Benchikh *et al*., 2014) related to phytochemicals of carob (*Ceratonia siliqua* L.) as influenced by ripening. Phenolic contents were higher in immature fruits, because these compounds may protect the immature fruits and seeds from pathogens, predators, and abiotic stresses (Holderbaum *et al*., 2014). The depletion in phenolic content would be caused by the conversion of soluble phenolics into insoluble phenolics, which are bound to polysaccharides in the cell wall and/or the oxidation of phenolic compounds by polyphenol oxidase.

Traditionally, immature faba bean is steamed or boiled whereas mature faba bean is only boiled. Thus, it is important to investigate the effects of different cooking methods on phytochemical contents. After steaming, phenolic contents in immature faba bean decreased by 5% in cotyledon to 30% in seed coat (Table 1), while steamed pod coat was not affected. After boiling, TPC decreased by 10% in cotyledon to 47% in seed coat. Phenolic content in boiled mature faba bean was reduced by 57 and 62% in whole seed and seed coat, respectively, whereas it increased significantly in boiled mature cotyledon compared to raw one (Table 1). According to Wolosiak *et al.* (2010), steaming of immature faba bean resulted in a decrease of phenolic content by 16.22%.

The boiled mature seeds of faba bean investigated in the current study contained higher amounts of phenolics (5.15 mg/g) than those reported by Hernandez-Salazar *et al.* (2010) in cooked commonly eaten legume seeds cultivated in Mexico, including lentils, black bean and chickpea (1.4, 1.2 and 0.8 mg/g, respectively).

The loss of phenolics observed during cooking could be due to either the destruction of these compounds or chemical rearrangements such as the binding of phenolics with other organic substances (Satwadhar *et al.*, 1981). Volf *et al.* (2014) have reported a thermal decomposition of gallic acid with a degradation rate of 30% at 100°C over 4h of exposure. This decrease might be also explained by the leach of water-soluble phenolics into the cooking water (Han and Baik, 2008). The increase of TPC recorded in cotyledon could be due to the destruction of cell walls and subcellular compartments during boiling that induces the release of phenolics.

Total flavonoid contents (TFC)

Table 1. Phenolic contents of faba bean extracts

Faba bean part	Growing stage	Sample	Total phenolics (mg GAE/g DW)	Flavonoids (mg QE/g DW)	Proanthocyanidins (mg CE/g DW)
Whole pod	Immature	Raw	**57.18** ± 0.60^{a}	0.906 ± 0.015^{b}	**0.643** ± 0.014^{a}
		Steamed	42.14 ± 0.92^{b}	0.937 ± 0.029^{b}	0.413 ± 0.006^{b}
		Boiled	37.56 ± 0.89^{c}	**1.076**± 0.006^{a}	0.402 ± 0.017^{b}
Pod coat	Immature	Raw	**67.38** ± 1.95^{a}	1.079 ± 0.035^{c}	0.040 ± 0.004^{c}
		Steamed	**67.48** ± 0.96^{a}	1.133 ± 0.016^{b}	**0.192**± 0.006^{a}
		Boiled	39.15 ± 0.41^{b}	1.145 ± 0.005^{b}	0.129 ± 0.004^{b}
	Mature	Raw	30.22 ± 0.68^{c}	**1.613**± 0.021^{a}	0.024 ± 0.008^{d}
Whole seed	Immature	Raw	**36.35** ± 0.37^{a}	0.623 ± 0.019^{b}	**1.508** ± 0.020^{a}
		Steamed	28.33 ± 0.63^{b}	**0.709** ± 0.019^{a}	0.779 ± 0.024^{b}
		Boiled	20.16 ± 0.45^{c}	0.616 ± 0.010^{b}	0.553 ± 0.012^{c}
	Mature	Raw	12.00 ± 0.19^{d}	0.167 ± 0.013^{c}	0.423 ± 0.008^{d}
		Boiled	5.15 ± 0.59^{e}	0.131 ± 0.010^{d}	0.082 ± 0.003^{e}
Seed coat	Immature	Raw	**68.54** ± 2.39^{a}	**0.493** ± 0.046^{a}	**3.713** ± 0.070^{a}
		Steamed	48.18 ± 1.03^{b}	**0.528** ± 0.005^{a}	2.012 ± 0.048^{b}
		Boiled	36.28 ± 0.70^{d}	**0.524** ± 0.002^{a}	1.389 ± 0.056^{c}
	Mature	Raw	41.46 ± 0.78^{c}	0.372 ± 0.010^{b}	2.079 ± 0.054^{b}
		Boiled	15.72 ± 0.48^{e}	0.247 ± 0.009^{c}	0.428 ± 0.008^{d}
Cotyledon	Immature	Raw	**12.31** ± 0.59^{a}	0.540 ± 0.009^{c}	0.036 ± 0.007^{b}
		Steamed	11.68 ± 0.49^{ab}	**0.653**± 0.006^{a}	**0.151**± 0.006^{a}
		Boiled	11.11 ± 0.67^{b}	0.619 ± 0.010^{b}	**0.161**± 0.006^{a}
	Mature	Raw	4.00 ± 0.59^{d}	0.101 ± 0.010^{d}	0.018 ± 0.006^{c}
		Boiled	4.29 ± 0.26^{c}	0.079 ± 0.007^{e}	0.034 ± 0.006^{b}

Values are mean ± standard deviation (n = 3); Means in a column for each faba bean part with different letters indicate significant differences ($p<0.05$).

The flavonoid contents ranged from 0.079 in mature boiled cotyledon to 1.613 mg/g in mature raw pod coat (Table 1). For the immature faba bean, pod coat showed the highest TFC followed by whole pod, whole seed, and finally cotyledon and seed coat, with no significant difference ($p<0.05$). For different mature faba bean parts, the pod coat showed the highest TFC followed by seed coat, whole seed, and cotyledon.

In the current study, flavonoid levels of raw faba bean ranged from 0.10 (mature cotyledon) to 1.61 mg/g (mature pod coat). As for phenolics, Chaieb *et al*. (2011) reported higher flavonoid levels in different parts of dry raw faba bean genotypes grown in Tunisia (3.76-45.92 mg rutin/g).

The results of the current investigation revealed that flavonoid content in the mature pod coat was significantly ($p<0.05$) higher than that of immature pod coat; it increased by 49%. However, the TFC in mature samples of whole seed, seed coat and cotyledon declined by 73, 25 and 81%, respectively.

In immature bean, increase in TFC due to steam cooking differed significantly among faba bean parts (Table 1). The largest increase was observed in cotyledon (21%) and whole seed (14%) while the smallest was detected in pod coat (5%). For whole pod and seed coat, there are no significant difference ($p<0.05$) compared to immature raw parts. Boiling caused significant increases of flavonoid in whole pod (19%), cotyledon (15%) and pod coat (6%), but did not affect the flavonoid content in whole seed and seed coat (immature stage).

In mature faba bean, reduction in TFC due to boiling differed significantly ($p<0.05$) among faba bean parts (Table 1). The largest decrease was observed in seed coat (34%), whereas the smallest was recorded in whole seed and cotyledon (22%).

Proanthocyanidins

Proanthocyanidins (PA) have been shown to be effective antioxidants with even greater activity than simple phenolics (Hagerman *et al.*, 1999). In this study, PA contents ranged between 0.02 mg/g (mature cotyledon) and 3.71 mg/g (immature seed coat). Our data were higher than those reported by Zadernowski *et al.* (2001) for whole seed (145-525 mg CE/kg) and seed coat (500-2625 mg CE/kg) of broad beans. These differences would be related to the extraction solvent (methanol) and analytical method used (vanillin assay).

Table 2. Antioxidant capacity of faba bean extracts

Faba bean parts	Growing stage	Sample	DPPH (mg GAE/g DW)	TAC (mg GAE/g DW)
Whole pod	Immature	Raw	**31.74** ± 0.58^{a}	**319**.21 ± 3.87^{a}
		Steamed	24.01 ± 1.31^{b}	237.98 ± 1.48 ^{b}
		Boiled	20.48 ± 0.81^{c}	213.39 ± 6.91^{c}
Pod coat	Immature	Raw	**39.94** ± 0.32^{a}	**369.16** ± 1.80^{a}
		Steamed	35.95 ± 0.12^{b}	339.46 ± 4.73^{b}
		Boiled	19.77 ± 0.90^{c}	335.53 ± 2.73^{c}
	Mature	Raw	13.38 ± 0.71^{d}	135.71 ± 6.24^{d}
Whole seed	Immature	Raw	**19.10** ± 1.62^{a}	**258**.63 ± 0.34^{a}
		Steamed	15.46 ± 1.33^{b}	199.23 ± 2.07^{b}
		Boiled	10.34 ± 0.96^{c}	189.60 ± 3.25^{c}
	Mature	Raw	5.89 ± 0.48^{d}	60.72 ± 1.62^{d}
		Boiled	2.39 ± 0.27^{e}	13.97 ± 1.89^{e}
Seed coat	Immature	Raw	**40.47** ± 1.77^{a}	**473**.60 ± 4.35^{a}
		Steamed	30.47 ± 1.27^{b}	332.58 ± 3.25^{b}
		Boiled	19.49 ± 1.22^{d}	311.14 ± 1.48^{c}
	Mature	Raw	22.42 ± 1.16^{c}	191.56 ± 1.23^{d}
		Boiling	7.97 ± 0.43^{e}	28.05 ± 0.58^{e}
Cotyledon	Immature	Raw	2.85 ± 0.28^{b}	116.78 ± 0.23^{b}
		Steamed	**3.34**± 0.32^{ab}	**121.25**± 2.00^{a}
		Boiled	**3.91**± 0.11^{a}	**119.48**± 4.66^{ab}
	Mature	Raw	1.26 ± 0.00^{c}	34.02 ± 1.29^{c}
		Boiled	1.28 ± 0.67^{c}	12.09 ± 0.23^{d}

TAC : total antioxidant capacity; OUI : oxygen uptake inhibition.
Values are mean ± standard deviation (n = 3); Means in a column for each faba bean part with different letters indicate significant differences ($p < 0.05$).

For the immature faba bean, seed coat showed the highest PA followed by whole seeds and whole pod. For the mature faba bean seed coat showed the highest PA followed by whole seed (Table 1). There were no significant difference ($p<0.05$) in PA contents between pod coat and cotyledon of both immature and mature faba bean. The PA contents of mature faba bean decreased by 40 % (pod coat) to 72% (whole seed), when compared with those of immature samples.

Steaming significantly ($p<0.05$) decreased PA contents in whole pod (38%), whole seed (48%) and seed coat (46%), but significantly ($p<0.05$) increased in the pod coat and cotyledon compared to the corresponding immature raw faba bean parts. Wolosiak *et al*. (2010) reported that steaming caused a decrease of tannin concentrations by 13% in faba bean. Boiling resulted in decreased PA content by 37% in whole pod to 63% in whole seed and seed coat; however, this cooking practice increased the PA contents in pod coat and cotyledon compared to the immature raw faba bean. In mature faba bean, decreases in PA due to boiling differed significantly ($p<0.05$) among faba bean parts (Table 1). The largest decreases were observed in whole seed (81%) and seed coat (79%) whereas the largest increase in PA content was observed in the cotyledon (89%). It has been suggested that heat treatment possibly reduces extractability by increasing polymerization of tannins, which would show lower values on subsequent analysis (Van der Poel *et al*., 1992). Also, the reduction in PA levels might be due to the dissolution of these compounds in the cooking water, whereas the increase in PA content could be explained by their higher extractability, facilitated by thermal treatment.

Antiradical activity

Depending upon the faba bean fraction and cooking method, the DPPH scavenging activity of the investigated extracts varied from 1.26 to 40.47 mg/g. The highest values were obtained for the immature raw seed coat and raw pod coat, followed by the whole pod, whole seed and cotyledon (Table 2). In addition, the results showed the same order for the mature raw faba bean parts.

All different parts of the immature raw faba bean exhibited significantly ($p<0.05$) higher DPPH scavenging effect than the mature raw faba bean fractions (Table 2); when compared to the immature

raw faba bean parts, the antiradical activity of mature samples decreased by 69% in whole seed, 45% in seed coat and 56% in cotyledon. Our results revealed that the DPPH antiradical activity in immature faba bean was reduced after cooking and that this activity is more affected by boiling than steaming; the activity of the whole seed extracts was decreased by 19 (steaming) and 46 % (boiling). In contrast, in the case of the cotyledon extract (Table 2), this activity increased after steaming (17%) and boiling (37%). This significant increase in free radical scavenging effect may be caused by the disruption of the cell membranes and cell walls leading to the release of antioxidant compounds from the cell matrix.

In mature boiled faba bean, the DPPH scavenging activity decreased by 59 and 64% in whole seed and seed coat, respectively, while it shows no significant difference ($p<0.05$) among cooked cotyledons compared to raw ones (Table 2). Similar loss was recorded for yellow soybean and black soybean seeds (Yang *et al*., 2014). The results of the current work agree with those obtained by Gujral *et al.* (2013) who indicated that seed coat of red kidney bean, red lentil, green mung and chickpea had higher antioxidant activity than whole legume seeds.

Total antioxidant capacity by phosphomolybdenum method

The phosphomolybdenum method was also performed in order to evaluate total antioxidant capacity of faba bean parts. The total antioxidant capacity (TAC) ranged from 12.09 (mature boiled cotyledon) to 473.60 mg/g (immature raw seed coat), depending on the faba bean parts and cooking methods. The highest value was obtained for the immature raw seed coat followed by the pod coat, the whole pod, whole seed, and cotyledon. It is the same order of mature raw faba bean parts (Table 2). This result is in agreement with Annegowda *et al.* (2013) who indicated that the pods of an underutilized legume *Clitoria fairchildiana* (Howard) showed higher antioxidant capacity than the seed extracts. Total antioxidant capacity showed a similar trend to total phenolics. Therefore, the strongest reduction activity of Mo(VI) to Mo(V) might be attributed to the richness of the extracts in phenolics. The result was consistent with previous reports because some authors have shown that high total phenol content increases the total antioxidant capacity (Kumaran and Karunakaran, 2007). The total antioxidant capacity of all immature raw faba bean parts was significantly ($p<0.05$) higher than that of the mature raw faba bean (Table 2). Maturation resulted in a significant decline in TAC, i.e. 60% in seed coat and 77% in whole seed.

Table 3. Correlation coefficients between phenolic contents and antioxidant activity of faba bean extracts

	TPC	TFC	PA	DPPH	TAC	OUI
TPC	1					
TFC	0.558**	1				
PA	0.491*	-0.179	1			
DPPH	0.993***	0.492*	0.528*	1		
TAC	0.924***	0.511*	0.571**	0.919***	1	
OUI	0.781***	0.238	0.739***	0.807***	0.725***	1

TPC : Total phenolic contents; TFC : Total flavonoid contents; PA : Proanthocyanidins; TAC : total antioxidant capacity; OUI : oxygen uptake inhibition.
* Significant correlation at $p<0.05$; ** Very significant correlation at $p<0.01$; *** Extremely significant correlation at $p<0.001$.

After steaming or boiling, the TAC in immature faba bean was reduced by 9% in pod coat to 34% in seed coat (Table 2). On the contrary, in cotyledon, steam cooking as well as boiling led to a significant increase in TAC compared to raw sample (Table 2). TAC in mature faba bean after boiling was reduced by 77, 85 and 64% in whole seed, seed coat and cotyledon, respectively (Table 2). Therefore, steaming, for faba bean parts would be considered as most suitable method to preserve bioactive compounds and antioxidant activity. Hence, the decrease in antioxidant activities in cooked faba bean by steaming and boiling might be due to the softening of cell wall tissues which is usually accompanied by solubilisation of bound phenolics into the cooking water (Han and Baik, 2008).

Oxygen uptake inhibition

The study of the antioxidant capacity of different faba bean parts was also assessed using the inhibition of methyl linoleate (MeLo) oxidation. Figure 1 shows the autoxidation of MeLo in absence and in presence of seed coat extracts. It appears that the antioxidant power of seed coat extracts varied widely. The most efficient part is the immature raw seed coat followed by immature steamed, mature raw, immature boiled and mature boiled seed coat extract.

Oxygen uptake inhibition (OUI) is reported in Table 2. All extracts tested inhibit the oxidation of MeLo induced by AIBN. The immature raw seed coat exhibited the highest inhibition of MeLo oxidation (66%), while the lowest value was recorded for the mature raw cotyledon (14%). Data on the inhibition

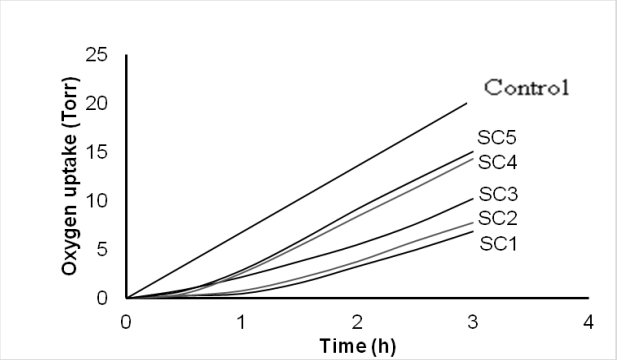

Figure 1. Oxygen uptake during the autoxidation of methyl linoleate induced by 2,2´-azobis-isobutyronitrile (AIBN) in the absence (control) and in the presence of seed coat extracts
SC1: Immature raw; SC2: Immature steamed; SC3: Mature raw; SC4: Immature boiled; SC5: Mature boiled.

of MeLo oxidation by legume extracts are not available in the literature.

Correlation between phenolic contents and antioxidant activity

Table 3 shows the relationship between phenolic, flavonoid, proanthocyanidin and DPPH scavenging activity, TAC, and oxygen uptake inhibition for all the parts of raw, steamed and boiled faba bean. The statistical analysis revealed high correlations between TPC and all antioxidant activity assays. The results presented in the current study showed a moderate correlation between flavonoid content and DPPH scavenging effect (r= 0.49) and TAC assay (r = 0.51). On the other hand, a weak correlation (r = 0.23) existed between the inhibition of linoleate oxidation and flavonoids, implying that flavonoids of the tested extracts are not the main components responsible for inhibition of linoleate oxidation. However, a high positive ($p<0.001$) correlation (r = 0.74) existed between proanthocyanidins and inhibition of linoleate oxidation.

Conclusion

To our knowledge, this is the first report of information regarding the effect of domestic cooking practices on phytochemicals and antioxidant activity of different faba bean parts, at immature and mature stages. This study showed that phytochemical contents and antioxidant activity of different immature and mature faba bean parts were reduced significantly by steam cooking and boiling. As boiling caused higher loss in phytochemicals than steaming, this latter should be the best method in retaining these bioactive compounds. Moreover, findings of the current work supported that immature faba beans have higher amounts of phenolic compounds and exhibited a better antioxidant activity than mature ones. For the maximum health benefits, consumption of whole pod of *Vicia faba* would be recommended.

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