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Tunisian *Ceratonia siliqua*: Phytochemical Analysis, Antioxidant Activity, Preparation and Characterization of Carob Emulsion System

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Ceratonia siliqua were screened for their polyphenol content and antioxidant ability. A formulation of emulsion using the seed oil and galactomannans was assessed. Results showed that maceration contained the greatest amount of phenolics in organs. The best antioxidant capacity was found in seeds extract using soxhlet method. A chromatographic analysis of carob organs showed the predominance of gallic acid in fruits and pods.

Fatty acid composition was dominated by palmitic, oleic and linoleic acids with 16.04, 38.08 and 38.85%, respectively. Finally, characterization of emulsions stabilized with the galactomannan from seeds proves that this biopolymer is an excellent food emulsifier. In fact, the production of emulsions having an average diameter of the dispersed droplets of a few micrometers and a creaming index greater than 80% reflects the very high stability. Overall, the results obtained indicated that *C. siliqua* can be valued as an emulsifier in several foods, pharmaceutical and cosmetic industries.

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Keywords: Antioxidant activity; Ceratonia siliqua; emulsion; phenolic compounds; phytochemical analysis.

1. INTRODUCTION

Carob tree (*Ceratonia siliqua* L.) is evergreen specie belongs to the Fabaceae family and presented in several countries of the Mediterranean basin. The world's average carob pod output has decreased in recent years, from 165,990 tonnes in 2013 to 136,612.75 tonnes in 2018 [1]. Carob fruit seeds and pods are employed in a variety of industries, including food, pharmaceuticals, and cosmetics [2]. Besides, *Ceratonia siliqua* can be used in emulsion which is defined as colloidal systems consisting of two liquid phases, oil and water in which one of these dispersed into the other [3]. So, this domain have potent and high prospective for food industries, cosmetic and agriculture [4]. Actually, the main interest of carob is extraction of carob gum (E140) used as a growth medium, a thickener and as a food stabilizer (E410) [5], also called locust bean gum (LBG) containing an important content of galactomannans which are found in the endosperm [6]. Carob powder is also a natural sweetener with a flavour and appearance similar to chocolate; as a result, it is frequently used as a cacao alternative in the creation of sweets, biscuits, and processed drinks [2]. LBG is regarded as the first galactomannan to be employed as an addition in industries such as paper, textiles, pharmaceuticals, cosmetics, and food [5].

"This evergreen specie is considered one of the most effective fruit and forest trees, since all its organs (leaves, flowers, fruits, wood, bark and roots) are precious and have values in several fields. The two main carob pod constituents are pulp (90%) and seed (10%), Carob pulp has a high content in total sugar, consisting of mainly sucrose, glucose, fructose and maltose. In addition, it contains about 18% of cellulose and hemicelluloses. However, constituents of the carob seed are coat (30-33%), endosperm (42-46%) and embryo or germ (23-25%)" (El Hajaji et *al*., 2010). The carob pods is characterized by the high amounts of carbohydrates (40– 60%), dietary fibers (27–50%), phenolic compounds, particularly tannins (18–20%), minerals (potassium, sodium, iron, copper, manganese and zinc) and 3 to 4% protein [7].

Carob pods are a rich source of natural antioxidants which are affected by numerous parameters such as development stage, organs, region and extraction methods. In this context, several techniques have been used for phenolic compounds extraction including supercritical fluid, ultrasound and conventional extractions, soxhlet apparatus. In fact, its richness on phenolic compounds such as gallic, syringic, cinnamic and *p*-coumaric acids, quercetin derivates and flavan-3-ols [8] are related by the potent biological activities of this specie. *Ceratonia siliqua* extracts was known for their antioxidant properties [6] which are directly associated to the ability to inhibit reactive oxygen species production. These compounds are able to ensnare free radical species derived from oxygen or nitrogen and neutralize non-radical species such as hydrogen peroxide [9]. Besides, carob pod exhibit anti-diarrheal and anti-bacterial activities especially against *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [10]. Then, carob extracts have also anti-inflammatory and anti-ulcer effects [10]. All these potentialities are related to their phenolic compounds mainly flavonoids.

To improve and maintain the products and especially territorial product, the aim of this study was to determine the variability of phenolic content and antioxidant activities of *C. siliqua* L. depending on the organ (fruit, pulp and seeds) and on extraction method (maceration and soxhlet system). Then, evaluation of antimicrobial activity, identification of main phenolic compounds by RP-HPLC and carob seed oil extraction and identification by CPG were carried out. Finally, a valorization of locust bean gum from seeds as an emulsion concept has been developed.

2. MATERIALS AND METHODS

2.1 Plant Sampling

Ceratonia siliqua fruits were collected from Grombalia (Northeast of Tunisia), superior semiarid bioclimatic stage; Plant identification was carried by Professor Abderrazek Smaoui (Biotechnology Center in Borj-Cedria Technopole, Tunisia).

Once brought back to the laboratory, the fruits were washed, dried in the open air for 48 hours, and then the seeds were separated from the pulp of the pods. The whole fruit, the pulp and the separated seeds were dried in an oven at a

temperature of 40 ° C for 72 hours in order to obtain dry samples. These were directly ground using a 400MM type ball mill. The powders obtained were stored in glass boxes for further analysis.

2.2 Preparation of Plant Extract

2.2.1 Extraction by traditional maceration

Three g of the corresponding dried and ground fruit, pulp and seeds were weighed, to which 30 ml of the solvent was added. The solvent was used: 30% aqueous ethanol (EtOH 30%) [11], subsequently, it was constantly stirred in the dark for 30 min at room temperature, which followed by filtration through Whatman No. 4 filter paper. The filtrate is combined, centrifuged at 4,000 rev/min for 20 min and stored at 4°C until use.

2.2.2 Extraction using soxhlet apparatus

20 grams of plant material was put in the presence of 200 ml of 30% ethanol. The assembly is carried out with a temperature which has not exceeded 40°C for 6 hours. With these parameters we were able to more or less reach the 18 cycles. The extracts thus obtained were stored in the dark at 4°C for further handling.

2.3 Total Phenolic Contents

Total polyphenols were measured using the Folin–Ciocalteu reagent, as described by Oueslati et al. [12]. Total phenolic contents were expressed as milligrams gallic acid equivalents per gram dry weight (mg GAE/g DW) through the calibration curve with gallic acid, ranging from 0 to 400 µg/ml. All samples were analysed in triplicate. The absorbance was read at 760 nm versus a prepared blank.

2.4 Total Flavonoid Contents

According to Oueslati et al. [12], total flavonoids were assessed. “Absorbance of the mixture was determined at 510 nm against the blank where the sample was omitted. Flavonoid contents were expressed as mg catechin equivalent per gram of dry weight (mg CE/g DW), through the calibration curve of (+)-catechin, ranging from 0 to 400 µg/ml. All samples were analysed in triplicate.”

2.5 Condensed Tannin Contents

The analysis of condensed tannins (proanthocyanidins) was carried out according to the Oueslati et al. [12]. Contents were expressed as mg CE per gram of dry weight (mg CE/g DW). The calibration curve range of catechin was established between 0 and 400 µg/ml. The absorption was measured at 500 nm. Samples were analyzed in triplicate.

2.6 Determination of Antioxidant Activities

2.6.1 Total antioxidant capacity

Total antioxidant ability was evaluated through the bio-assay of a green phosphate/Mo^{5+} complex according to the method described previously by Oueslati et al. [12]. The absorbance was measured at 695 nm against blank. Antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). All samples were analyzed in triplicate.

2.6.2 DPPH radical-scavenging activity

Oueslati et al. [12] investigated the efficacy of plant extracts to quench DPPH•. The potential of extracts to reduce the free DPPH radical (1,1-diphenyl-2-picrylhydrazyl) was expressed as IC_{50} (µg/ml), the antiradical dose required to cause a 50% inhibition. Antioxidant (BHT) was used as standard. For that, samples at different concentrations were added to DPPH methanolic solution (0.2 mM). The absorbance was measured at 517 nm.

2.6.3 Iron reducing power

The reducing power of *Ceratonia siliqua* extracts was determined through the transformation of Fe^{3+} to Fe^{2+}. The intensity of the appearing blue-green colour was measured at 700 nm. Reduction power was expressed as EC_{50} (µg/ml), is the ability of extract at which the absorbance was 0.5 and ascorbic acid was used as a positive control. All samples were analysed in triplicate [12].

2.7 RP-HPLC Evaluation of Phenolic Compounds

Ceratonia siliqua extracts was filtered through a 0.45 mm membrane filter and injected into a high-performance liquid chromatography (HPLC) system. The phenolic compounds were analyzed using a reverse-phase-HPLC system (RP-HPLC; Agilent Technologies 1100 Series; Agilent Technologies, Santa Clara, CA) that was coupled

with an ultraviolet/visible spectrum multi-wave length detector. The separation was carried out on a reverse phase ODS C18 (4 µm, 2509 4.6 mm, Hypersil) column used as stationary phase (Thermo Fisher Scientific Inc., Waltham, MA) at ambient temperature. The mobile phase consisted of acetonitrile (Solvent A) and water with 0.2% sulfuric acid (Solvent B). The flow rate was maintained at 0.5 ml/min. The gradient program was as follows: 15% A/85% B for 0–12 min; 40% A/60% B for 12–14 min; 60% A/40% B for 14–18 min; 80% A/20% B for 18–20 min; 90% A/10% B for 20–24 min and 100% A 24–28 min. The injection volume was 20 µl and peaks were monitored at 280 nm. The retention periods and spectral properties of phenolic compounds' peaks were compared to those of standards to identify them. Triplicate analyses were carried out.

2.8 Oil Extraction

For 6 hours, ten grammes of each ground sample were extracted with 100 ml hexane (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) in a soxhlet device. The extraction was shielded from light. After filtering of the mixture and solvent evaporation at reduced pressure, the oil was extracted.

2.9 Fatty Acid Methylation and Analysis

Cecchi et al. [13] proposed a technique for converting total fatty acids into their methyl esters using 3 percent sodium methylate in methanol. To measure fatty acids, the methyl ester of neodecanoic acid (C10:0) was utilised as an internal standard. Prior to analysis, the superior phase containing fatty acid methyl esters (FAMEs) was aspired and the solvent volume was decreased under a stream of nitrogen. A Hewlett-Packard 6890 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame-ionization detector (FID) and an electronic pressure control (EPC) injector was used to analyse FAMEs utilising gas chromatography. A RT-2560 capillary column was used to separate them (100m length, 0.25mm i.d., 0.20 mm film thickness). The oven temperature was maintained at 170°C for 2 minutes, followed by a 3°C min-1 ramp to 240°C, which was then maintained for another 15 minutes. At a flow rate of 1.2 ml min-1, nitrogen (U) was used as the carrier gas. Lastly, the injector and detector temperatures were kept at 225°C, and the retention durations of the FAMEs were compared to those of co-injected legitimate standards to aid identification.

2.10 Gas Chromatography (GC-FID)

Bettaieb Rebey et al. [14] conducted a study on volatile chemicals. An HP-INNOWAX polyethylene glycol capillary column connected to an Agilent 6980 gas chromatograph with a flame ionisation detector (FID) and an electronic pressure control (EPC) injector (30m 0.25 mm). The flow of the carrier gas (N2) was 1.6 ml min^{-1}. The split ratio was 60:1. The following temperature programme was used: oven temps isotherm at 35°C for 10 minutes, from 35 to 205°C at a rate of 3°C min1, and isotherm at 205°C for 10 minutes. The injector and detector temperatures were kept at 250 and 300°C, respectively. One microliter of the material was introduced into the system (dissolved in hexane at 1/50 v/v). Individual peaks were determined by comparing their retention indices relative to (C6-C22) n-alkanes to those reported in the literature and/or those found in our lab. The total ion current was used to calculate the percentage compositions of samples based on the area of the chromatographic peaks.

2.11 Emulsification and Spray Drying

Oil-in-water emulsions were prepared by mixing 0.5 or 1% carob seed oil as oil phase with 95 or 99% (w/w) aqueous phase containing 5% (w/w) galactomannan extracted from carob as emulsifier. Emulsions were produced by high shear mixer using an Ultra Turrax T25 (IKA Werke GmbH & Co., Sigma-Aldrich, St.Louis, MO) equipped with a S25 N18 G rotor operated at 10.000 rpm for 5 min at 4°C twice. The resultant emulsions were spray dried in a Buchi-B90 model mini spray dryer (Buchi, Switzerland) with a 0.5 mm diameter nozzle (inside chamber dimension: 100 cm height, 60 cm diameter). For the spray flow, the compressed air pressure was adjusted to 2 bars. The temperatures at the entrance and outflow were kept at 85±2°C and 75±2°C, respectively. And the feed rate was 20 millilitres per minute. The microcapsules were extracted from the collection chamber and kept until they were needed.

2.11.1 Emulsions characterization

Creaming Index: Creaming index was measured to evaluate the physical stability of emulsions against centrifugation test. Emulsion samples (10 ml) were centrifuged for 1h at 5000 rpm and 25°C using a refrigerated centrifuge (PK

130R, ALC International, Italy). The creaming index was calculated according to equation (1)

$$CI(\%) = \frac{V_{sep}}{V_{tot}} x100$$

Where V_{sep} is the volume of the separated oil layers and V_{tot} is the total volume of emulsion.

Droplet Size Measurements: The droplet size of each emulsion was measured using a photon correlation spectrometer (HPPS, Malvern Instruments, Malvern, UK), whose characteristic size was within the instrument sensitivity range (1–6000 nm). Donsi et al. [15] previously defined the droplet size distribution in terms of the mean droplet size (Z-average diameter), which was found by cumulant analysis of the intensity-intensity autocorrelation function G(q,t). The samples were diluted to an appropriate concentration using bidistilled water and the polydispersity index (PDI) (usually a 1: 100 dilution was applied).

2.12 Statistical Analysis

Results were expressed as mean ± standard deviation (SD) of six replicates. Data were subjected to statistical analysis using statistical program STATISTICA. Analysis of variance (ANOVA) followed by Duncan's multiple comparison test ($p < 0.05$) were used.

3. RESULTS AND DISCUSSION

3.1 Fatty Acid Composition

Fatty acid composition of *Ceratonia siliqua* seeds was determinate in Table 1. Comparison of the analytical data revealed that a total of 12 different fatty acids were identified in the seed oils. The total unsaturated fatty (UFA) acids recorded 79.11%, while the total saturated fatty acids (SFA) recorded 20.83%. Unsaturated fatty acids are classified as monounsaturated or polyunsaturated fatty acids. The predominant fatty acids in Tunisian carob seed oils were linoleic acid (C18:2n6) (38.85%), oleic acid (C18:1n9) (38.08%), palmitic acid (C16:0) (16.04%) and stearic acid (C18:0), (3.9%). Our results are in accord with values previously reported by Matthaus and Özcan [16], they reported that oil seeds of Turkish carob contain linoleic acid (49.1%), oleic acid (30.4 %), palmitic acid (10.3%) and stearic acid (3.5%). These results are better than those found in *Pimpinella anisum* which oleic and linoleic acids contents are 13.5 and 0.09%, respectively [14]. In other study, Tlili et al. [17] reported that carob seed oil contained linoleic, oleic and palmitic acids with 45.43%, 32.49% and 13.13% acids, respectively. According to the findings, carob fruit seed contains a significant amount of unsaturated fatty acids, hence the oil should be kept at a low temperature and kept away from air. Arachidic, eicosenoic, myristic, lauric, behenic, erucic and stearic acids were also present in carob seeds (Table 1).

3.2 Total Phenol, Flavonoid, and Condensed Tannin Contents

The contents of total phenols was determined as gallic acid equivalent in milligrams per gram dry weight (mg GAE/g DW) while flavonoid and condensed tannin contents were calculated as catechin equivalent in milligrams per gram dry weight (mg CE/g DW). As shown in Fig. 1a, maceration extracts showed the highest polyphenol contents in comparison with soxhlet extraction. In fact, the highest phenolic content was detected in fruits and pods with 11.65 and 11.51 mg GAE/g DW, respectively with maceration. While the lowest one was observed in seeds with 1.59 mg GAE/g DW (soxhlet extraction). Concerning flavonoid content, seeds extract with maceration exhibit the highest amount (1.98 mg CE/g DW) (Fig. 1b). In fact, the total condensed tannin varied from 0.49 (seeds extract using soxhlet method) to 2.71 mg CE/g DW (pods extract using maceration) (Fig. 1c). Several studies have shown that the extraction technique is an important factor influencing total polyphenol content [18]. These changes observed could occur from an increase in the activity of phenylalanine ammonialyase (PAL) enzyme implicated in secondary metabolites biosynthesis [19]. In this context, "the level of secondary metabolites is reported to vary among the organs of a plant species and according to the environmental conditions and plant development stage" [20]. Our results are in accord with those demonstrated by Jalleli et al. [21], they reported that "the comparison of the two extraction methods revealed that total phenolic contents of maceration extract of *C. maritimum* are higher than those of Soxhlet extracts." Besides, Tunisian carob extracts seems to be more efficient than carob extracts from Morocco which shows polyphenol contents of the order of 0,77 mg EAG g^{-1} MS [7]. Besides, [1] reported that the phenolic contents of carob pods of three Algerian varieties at ripe stage were ranged from 1.35 ± 0.01 to 2.34 ± 0.01 g GAE/100 g DW.

Table 1. Fatty acid composition of carob seeds

Fatty acids	Carbon chain	Oil yield (%)
Lauric acid	C 12: 0	0.03
Myristic acid	C 14: 0	0.16
Palmitic acid	C 16: 0	16.04
palmitoleic acid	C 16:1n7	0.27
stearic acid	C 18: 0	3.91
oleic acid	C 18: 1n9	38.08
Linoleic acid	C 18: 2n6	38.85
Linolenic acid	C 18: 3n3	1.40
Arachidic acid	C 20: 0	0.36
Eicosenoic acid	C 20: 1n9	0.35
Behenic acid	C 22: 0	0.33
Erucic acid	C 22: 1	0.16
SFA (%)		20.83
UFA (%)		79.11
PUFA (%)		40.25
SFA/PUFA		0.52

Table 2. Antioxidant activity of carob

		Antioxidant assays		
		AAT (mg EAG g^{-1}DW)	**DPPH** CI_{50} (µg ml^{-1})	**Reducing power** CE_{50} (µg ml^{-1})
Maceration	**Fruits**	15.07 c	68a	2400a
	Pods	19.64 a	72a	1700b
	Seeds	17.98 b	46b	1100d
Soxhlet	**Fruits**	13.13d	38c	1300c
	Pods	13.44 d	37c	1150d
	Seeds	10.53 e	32d	520e

3.3 Antioxidant Potentialities

Antioxidant activities of *Ceratonia siliqua* extracts using two extractions (maceration and soxhlet) during three ripening stages were evaluated by three complementary tests *via* total antioxidant ability (AAT), DPPH and reducing power assays (Table 2). Based on AAT, results showed that maceration is the potent extraction method then soxhlet one. Pods and seeds using maceration exhibited the highest capacity with 19.64 and 17.98 mg GAE/g DW, respectively, this result is directly related to the highest level of polyphenol content in these organs. For the antiradical activity, this ability depends on the extraction method and organ of the plant. Seeds extract showed the higher capacity to quench DPPH radical than the other organs whether by maceration or by soxhlet method. In fact the potent activity was observed with soxhlet with IC_{50}=32 µg/ml. Concerning reducing power, the extracts obtained by soxhlet exhibit the lowest values of EC_{50}, showing a high antioxidant power of these extracts to reduce iron. Seeds extracts obtained by soxhlet show the lowest EC_{50} value which is of the order of 520 µg ml^{-1}. This activity

is twice as high as that observed for the extracts of the seeds obtained by maceration. The same trend was observed for the fruits and pulps of *C. siliqua* L. However, the extracts obtained by soxhlet reveal the best antioxidant potentialities than those found by maceration. Thus the extracts of the seeds obtained by soxhlet exhibit the best activity in neutralizing the DPPH radical with the lowest IC_{50} (32 µg ml^{-1}) in comparison with that found by maceration (46 µg ml^{-1}). Our results corroborate those found by Shukla et al. [22] who showed that the extract of the leaves of *Casearia tomentosa* obtained by soxhlet has the best antioxidant potential with the lowest IC_{50} (280 µg ml^{-1}) in comparison with the extract obtained by maceration (480 µg ml^{-1}). The same is true for the reducing iron activity, the lowest EC_{50} were marked in the extracts obtained by soxhlet. These results were confirmed by Murugan and Parimelazhagan [23] who revealed that the extract of *Osbeckia parvifolia* obtained by soxhlet has the lowest EC_{50} (113 µm Fe / g) in comparison with the extract obtained by maceration which reveals an EC_{50} of the order of 538 µm Fe/g. This clearly shows that the extraction by soxhlet makes it possible to obtain lower levels of phenolic compounds but of better qualities since it determines the extent of their biological properties [21]. In addition, secondary metabolites particularly phenolic compounds, depending on their diverse properties, contribute effectively to the antioxidant capacity in a dose-dependent manner until a maximum of activity.

3.4 Identification of Phenolic Compounds in *ceratonia siliqua* organs by RP-HPLC

The chromatographic profile of several carob organs demonstrates eleven phenolic compounds in pods extracts (Fig. 3A), nine phenolic compounds (PC) were identified in fruits extract (Fig. 3B) and 17 PC in seeds (Fig. 3C). The comparison of the retention times and the spectral characteristics with the reference standards made it possible to identify that the seed seems to be the richest in phenolic compounds. In fact, the main phenolic compound in pods and fruits extracts was gallic acid. However, seeds extract was rich mainly in phenolic acids (11 acids) and flavonoids (9 compounds). The major compound was cinnamic acid followed by gallic acid and Kampferol 3-O-rutinoside. Other compounds such as

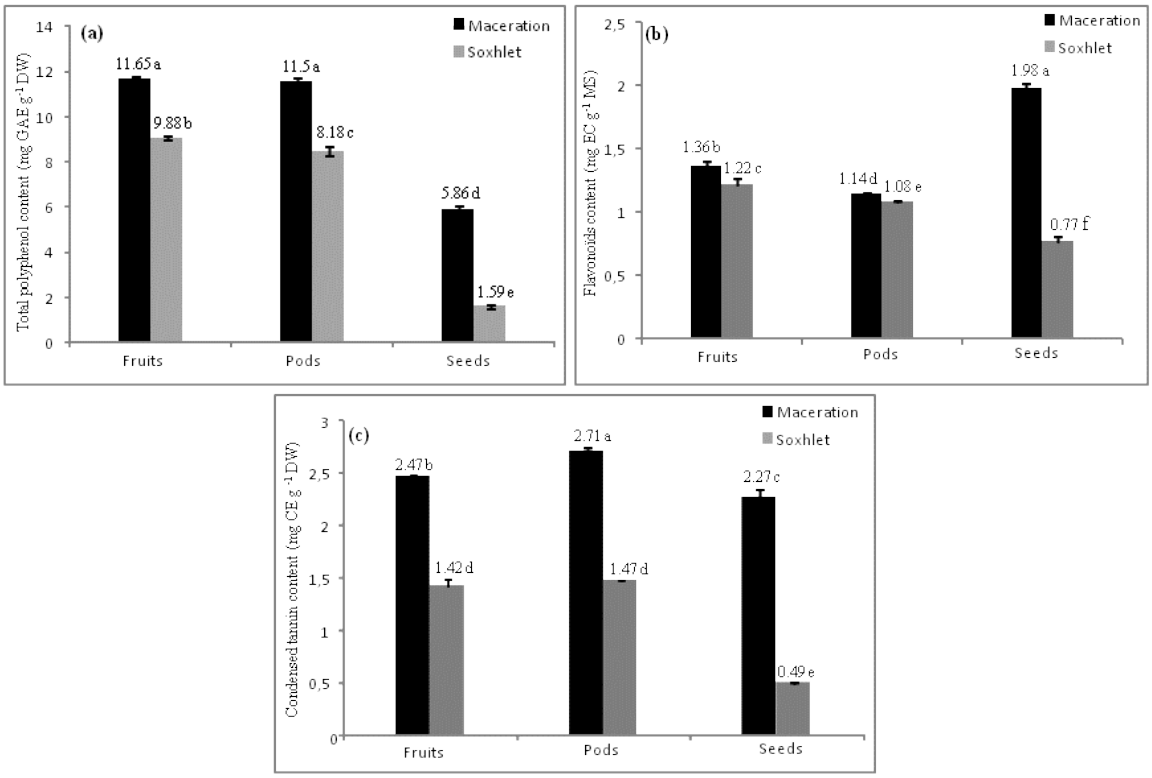

Fig. 1. Total phenolic (a), flavonoid (b) and condensed tannin contents (c) from *Ceratonia siliqua* extracts (fruits, pods and seeds). Values are the means of three replicates and standard deviation. Values with different superscripts are significantly different at P<0.05

epigalloctechin, catechin, *p*-coumaric and sinapic acids were also identified in seeds extract. Previous studies indicated that gallic acid was the most abundant phenolic acid and it is very dominating in both unripe and ripe carob pods [24]. These compounds might be considered as interesting bioactive natural substances that may be used in several fields, such as nutraceuticals, cosmetics and agro-food industry. Besides, recent study showed "a variability of qualitative and quantitative compounds. Extracts of pure solvents (acetone, ethyl acetate, methanol, and ethanol) contained lower amounts of flavonoids. This means that the carob fruit comprises more glycosidic forms of flavonoids than aglycones" [25].

In fact, the chemical substances in carob pods differ widely according to carob species, climate and the stage of maturity as well as to different parts of tree. Indeed, the HPLC analysis was showed that the principal compounds are: pyrogallol (48.02±3.55%), catechin (19.10±2.11%) and tannic acid (9.01±1.40%) in mature carob pods [8]. However, in immature carob pods the proportions are different and they are in the following order, the pyrogallol (26.45± 3.03%), catechin (16.52 ±2.34%), gallic acid (15.12 ±2.31%), chlorogenic acid (15.01±1.72%) and epicatechin (12.26±1.04%) [26]. In addition, the chromatogram revealed the presence of many phenolic compounds in leaves as kaempferol (77±2.43%), tannic acid (13±0.45%), catechin hydrate (4.30±0.34%) and polydatin (0.85 ±0.22%) [26]. All of these chemicals have a variety of pharmacological effects, particularly in the digestive system, such as antioxidant, antidiarrheal, antibacterial, anti-ulcer, and anti-inflammatory properties. Aside from that, Rtibi et al. [26] proposed that carob tree could be used as a dietary natural antioxidant supplement to prevent free radical-related disorders.

The two main carob pod constituents are pulp (90%) and seed (10%). Chemical composition of the pulp depends on cultivar, origin and harvesting time [8].

3.5 Emulsifying Properties

3.5.1 Creaming index

The emulsifying ability of the galactomannan extracted from carob seeds was assessed in terms of physical stability of the resulting emulsions under centrifugation. Creaming measures the tendency of the oil phase to separate. As expected, higher oil phase concentration increased the stability of emulsions by decreasing the creaming index. Indeed, the use of 0.5% carob seed oil resulted in the emulsion with lower physical stability, with the

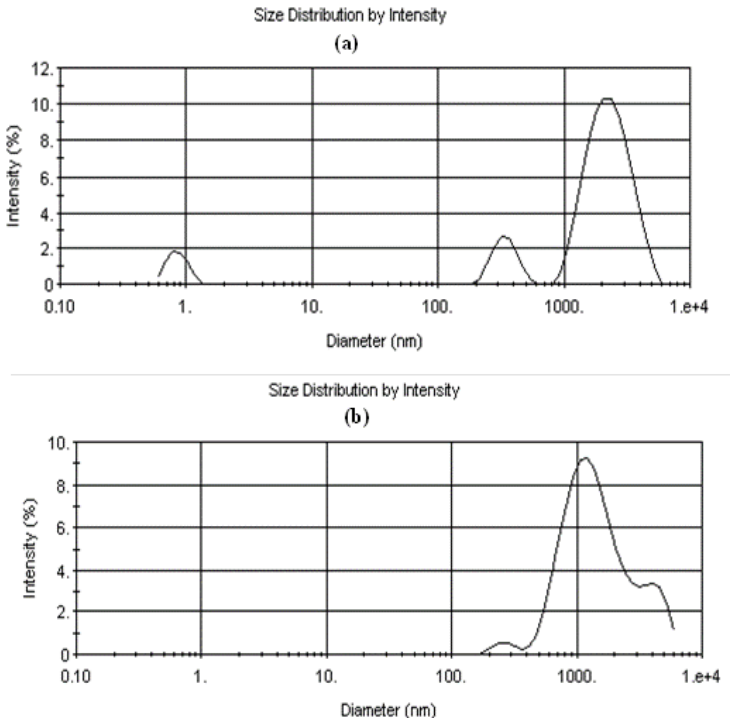

Fig. 2. Profile of droplet size of emulsions prepared on 0.5% (a) and 1% (b) of carob seeds oil

creaming index of 91%. However, at higher concentration of oil phase (1%), galactomannan stabilized emulsions results in the reduction of the creaming index (83%). These results showed that an increase in the concentration of the oil phase causes a significant stability of emulsions. Therefore, an increase in oil phase concentration resulted in a decrease in creaming stability. Similar results were observed by Sun and Gunasekaran [27-29] who showed that oil phase volume fraction has a significant effect on the creaming of emulsions.

These results could be explained by the effect of higher oil phase concentration on increasing emulsion viscosity which will have a significant

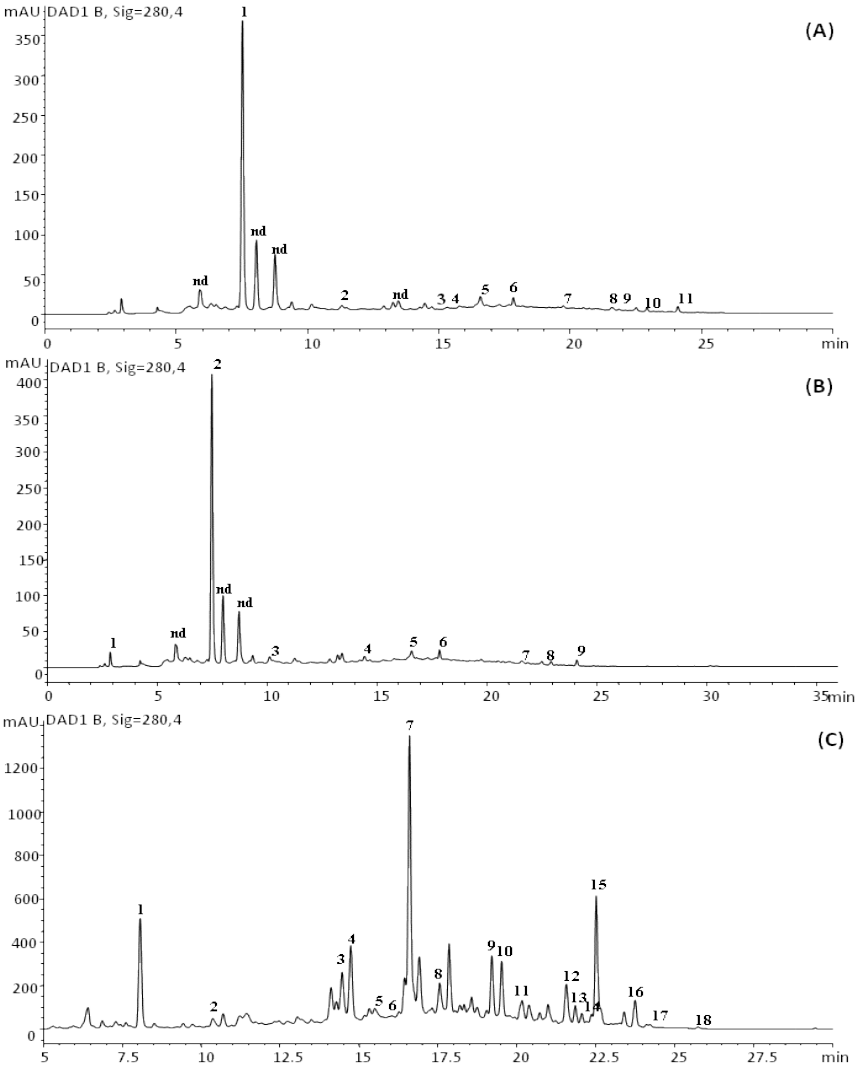

Fig. 3A. Chromatogram obtained for pods extract. Detection at 280 nm. Pics correspond to: 1, gallic acid; 2, Resorcinol; 3, Epigallocatechin; 4, chlorogenic acid; 5, Epicatechin 3-O-gallate; 6, syringic acid; 7, coumaric acid; 8, rosmarinic acid; 9, protocatechuic acid; 10, Kampferol 3-O-rutinoside; 11, quercitin

Fig. 3B. Chromatogram obtained for fruits extract. Detection at 280 nm. Pics correspond to: 1, ascorbic acid; 2, gallic acid; 3, Resorcinol; 4, Epigallocatechin; 5, Epicatechin 3-O-gallate; 6, syringic acid; 7, rosmarinic acid; 8, myrictin; 9, quercitin.

Fig. 3C. Chromatogram obtained for seeds extract. Detection at 280 nm. Pics correspond to: 1, gallic acid; 2, Resorcinol; 3, Epigallocatechin; 4, Catechin hydrate; 5, chlorogenic acid; 6, Epicatechine 3-O-gallate; 7, cinnamic acid, 8, acid syringique; 9, *p*-coumaric acid; 10, sinapic acid, 11, ferulic acid; 12, rosmarinic acid, 13, isoquercetin, 14, ellagic acid, 15, Kampferol 3-O-rutinoside; 16, Quercetin; 17, transcinnamic acid; 18, Isorhamnetin

effect on the stability of emulsions. Indeed, creaming behavior correlates with the viscosity of the emulsion systems, where emulsions with higher viscosity show higher stability against creaming. As a result, in emulsions prepared with higher concentration of oil phase, droplets are more densely packed, which increases emulsion viscosity which decreases the creaming rate [30].

3.5.2 Droplet size measurement

Mean droplet size of emulsions was measured after homogenization using the ultra turrax. Higher sizes were observed when oil phase concentration was increased from 0.5 to 1% (1.21µm to 2.36 µm respectively) (Fig. 2). These results could be explained by the effect of the emulsifier, the extracted galactomannan from carob, on the stability of emulsions. So the used concentration of emulsifier (5%) was not enough to cover the surface of oil droplets when the oil phase concentration increased from 0.5 to 1%. Similar results were observed by Jacome-Guth et al. [31] who found that the concentration of gum arabic used to stabilize beverage emulsions has an important influence on their stability. Indeed, they proved that we should increase gum arabic concentration if we increase the oil phase concentration in order to obtain stable emulsions and to reduce the droplet size. In general, emulsion properties as stability, appearance and rheology are determined by droplet size. Indeed, when the droplet size decrease the apparent viscosity of the emulsion increase, which increases the emulsion stability.

4. CONCLUSION

The current study highlighted that extraction method of organ carob act phenolic content and antioxidant activity. A great variability in phenolic composition was shown in carob organs.

According to our data, the seeds carob extracts exhibits potent radical scavenging and reducing power. The potentiality of *Ceratonia siliqua* organs as effective natural antioxidants that can be considered as substituent source in the food and pharmaceutical fields.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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