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Review

Polyphenols in carobs: A review on their composition, antioxidant capacity and cytotoxic effects, and health impact

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ABSTRACT

Carob (Ceratonia Siliqua L., tree of the pea family Fabaceae) and its products have recently attracted great interest due to their polyphenolic composition. This review summarizes the polyphenolic compounds that are contained in different carob parts (leaves, pod, seeds, barks) and products (syrup, flour, fiber). It also states the main differences of polyphenolic composition due to environmental and natural reasons, such as region, variety, and gender, and due to the processes used for preparation, extraction and analysis. The gender, along with the extraction procedure, proved to be the most important factors affecting the polyphenolic composition. Supercritical fluid extraction is the most efficient technique used because it protects polyphenols from decomposition. Due to the relatively low number of publications, it is concluded that further development of optimum methods for extraction, analysis and isolation of polyphenols should be carried over to assess their antioxidant capacity and their food technological and pharmaceutical industry.

1. Introduction

The carob tree (Ceratonia siliqua, tree of the pea family Fabaceae) is found in Mediterranean countries, such as Greece, Italy, Spain, Morocco, Turkey and Syria. The ancient Greeks brought it from Middle East to Greece and Italy, while the Arabs spread it along the North African coast and north into Spain and Portugal. In recent times, the carob tree has been spread along some areas where the climate is similar to the Mediterranean climate, such as California, Arizona, Mexico, Chile, Argentina, Australia, South Africa and India (Battle & Tous, 1997). The world carob production is approximately 315,000 tons per year, with Spain being the main producer and exporter followed by Italy, Morocco, Portugal, Greece, Turkey and Cyprus (Battle & Tous, 1997; Biner, Gubbuk, Karhan, Aksu, & Pekmezci, 2007). Based on the Food and Agriculture Organisation of the United Nations data (FAO) for the period 1994–2014, Spain produced 74,802.81 tons per year, Italy around 30,000 tons, Morocco and Portugal around 22,000 tons, Greece and Turkey around 15,000 tons, and Cyprus approximately 7000 tons per year (FAO, 2017).

Carob trees can be cultivated in areas with low rainfall, they don't require any significant attention and they live up to 150 years (Hajaji et al., 2011; Iipumbu, 2008; Marakis, 1996). Due to these characteristics, carobs, have, over the years, been considered as a cheap source for both human and animal nutrition (RamÓN-Laca & Mabberley, 2004). Nowadays, carobs are used in the food, pharmaceutical and cosmetic industries (Kotrotsios, Christaki, Bonos, & Florou-Paneri, 2012; Tous et al., 2009) (Fig. 1).

The carob tree is an evergreen tree and its leaves have a very thick single-layered upper epidermis, the cells of which contain phenolic compounds (Coit, 1951; Davies, 1970). The fruit, which is the main raw material used in industry, is a brown pod, with a wrinkled surface that becomes leathery when ripe (Fig. 2). The pod consists of the pulp and the seeds. Pulp is actually the seedless part of the carob pod, of which the outer leathery layer is called pericarp and the inner region is called mesocarp. Seeds are found transversely to the pod and are separated by mesocarp. They are very hard with a compressed ovate-oblong shape (Battle & Tous, 1997; Naghmouchi, Khouja, Romero, Tous, & Boussaid, 2009). Carob pulp has a high content of sugar and a relatively low content of fats and protein. The seeds were found to have lower sugar content and more fat compared to the pulp. Minerals, such as calcium, phosphorus and potassium have also been detected in carob pods, proposing carob pods as an alternative source of minerals (Ayaz et al., 2007). Polyphenols, another group of compounds well known for their health impact, are also contained in carobs. Briefly, antioxidants are found in both the carob pods and the carob tree leaves. This urged the scientific community to investigate the antioxidant potential of the carob trees, along with their antioxidant capacity and their health impact.

It is worth here to mention that different carob populations grown in different regions and the different growing stages affect the

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Carob Products	Carob Health Benefits
Carob Syrup	Gluten free (celiac disease)
Carob Powder (cocoa substitute)	Improves digestion (may help obesity)
Carobolate	Lowers cholesterol level in the blood
Carob Cream –Carob Spread	Acts as an antioxidant
Carob Snack Bar	Can be used to treat diarrhea
Carob Coated Products (dried fruit, nuts, etc)	Caffeine free
Soy Carob Products	Contains vitamins A, B1, B2, B3, D and E
Carob Flour	High in potassium and magnesium
Carob Tea	Rich in phosphorus and calcium (fight against osteoporosis)
Carob Breadsticks and Biscuits	
Carob supplements	Contains iron, manganese, barium, copper and nickel
Carob Pasta	
Caroffee (coffee substitute)	Helps with IBS (Irritable Bowel Syndrome, spastic colon)
Carob Hazelnut Spread	
Carob-Coated Rice Cakes and Cups	Carob tannins contain gallic acid (an anti-allergic, anti-bacterial, antioxidant, anti-viral and anti-septic)
Carob Liqueur	
Carob Soap and Moisturizer	

Fig. 1. Carob products used in food, pharmaceutical and cosmetic industries and their properties.

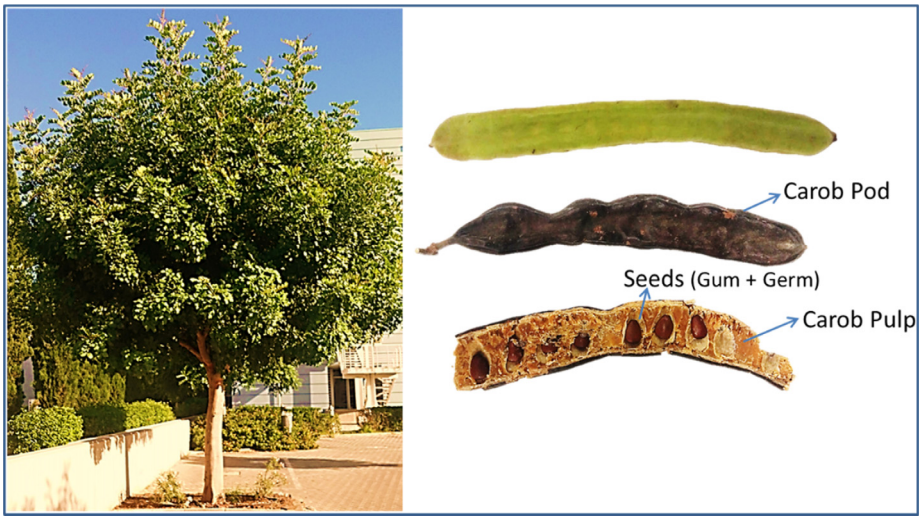


Fig. 2. Carob tree, immature and mature carob pod and its constituents.

morphological variability and chemical composition (Battle & Tous, 1997; Gharnit & Ennabili, 2016; Naghmouchi et al., 2009). Moisture, sugars, dietary fiber, protein, fat, ash and polyphenols are the quality characteristics that are mainly affected. Despite the fact that carob fruit has been used since ancient times as food and animal feed, the development of food industry and the economic prosperity are probably the reasons for the decrease in carob production. Based on FAO data, the carob production has been significantly reduced during the last decades (Fig. 3). In 1994, the world's total harvested area was approximately 140,849 ha and the production about 219,541 tons, while, in 2014, were 71,374 ha and 156,798 tons, respectively.

However, carob product research and development has recently received attention due to the nutritional potential of carob pods (Fig. 4). Because of the high sugar content, the pulp (seedless carob pod) is used to make syrups or molasses (Diaz, 1997; Marakis, 1996). Moreover, unroasted and roasted powder (flour) is used as cocoa substitute and this can be attributed to the fact that carob powder contains no caffeine, thiobromine or oxalic acid, and has low fat content (Aydın & Özdemir, 2017; Biner et al., 2007; Srour, Daroub, Toufeili, & Olabi, 2016; Yousif & Alghzawi, 2000). The seeds are mainly composed of galactomannans, which are found in the endosperm. This source of gum (locust bean gum, LBG) is used as a growth medium, a thickener and as a food stabilizer (E410) (Barak & Mudgil, 2014; Battle & Tous, 1997; Calixto & Cañellas, 1982). Moreover, LBG has been used for pharmaceutical and medicinal purposes (Kaity, Isaac, & Ghosh, 2013). The carob germ is used to make germ flour, which contains proteins, unsaturated oil at high levels, and it is proposed as a dietetic human food (Dakia, Wathelet, & Paquot, 2007). Caroubin, a protein of the carob germ, has similar properties to gluten; so, gluten-free products have been developed for celiac people by using carob germ flour (Smith et al., 2010; Tsatsaragkou, Gounaropoulos, & Mandala, 2014; Tsatsaragkou, Yiannopoulos et al., 2014). Moreover, germ flour

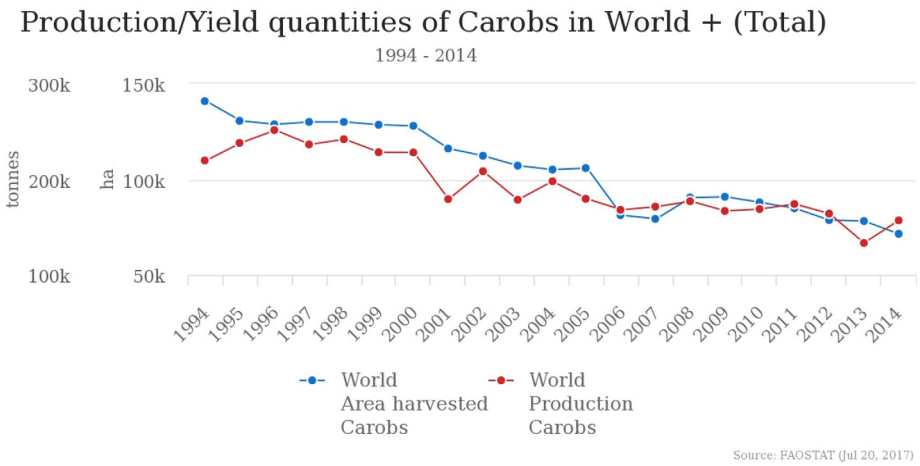


Fig. 3. Graph demonstrating the fluctuation on the worldwide production and yield quantities of carobs for the period 1994–2014 (FAO, 2017).

contains polyphenols, proanthocyanidins and ellagi- and gallotannins, compounds that are well known for their antioxidant capacity and their impact on human health (Martin-Diana et al., 2017; Sęczyk, Świeca, & Gawlik-Dziki, 2016). However, most carob pods are discarded, and they have not been sufficiently utilized yet.

As mentioned earlier, carob pods and leaves contain polyphenols. Therefore, carob-based products show important properties. For example, the de-sugared carob pulp powder (also referred as carob fiber or CAROBMAX®) is rich in insoluble tannins. They have been used as anti-diarrheic products, while carob pod extracts showed anti-cardiovascular and antioxidant properties. However, as noted earlier, the polyphenol composition varies due to the different carob cultivars and the different carob growing stages. The region, the tree gender, the domestication of wild carob trees and the fruit development stages are the main factors that actually affect the phenolic composition. In addition to this, the temperature, along with the production processes, seem to have an important influence on the polyphenolic patterns and quantities of several products (Daglia, Papetti, Gregotti, Bertè, & Gazzani, 2000; Garrido, Monagas, Gómez-Cordovés, & Bartolomé, 2008; Srour et al., 2016). Therefore, different polyphenolic compounds can be detected, not only in different carob cultivars, but also in carob products that have been processed under different conditions (e.g. temperature/roasting). A lot of research has, so far, been performed on the identification and quantification of polyphenols in carob fruits and derived products. However, an important issue is raised here, which involves the extraction method that affects the phenolic profile and the antioxidant capacity (Khan, Abert-Vian, Fabiano-Tixier, Dangles, & Chemat, 2010; Spigno, Tramelli, & De Faveri, 2007). The use of different extraction methods provides different patterns and compositions. Therefore, the results obtained are not comparable, and they may not be in agreement (Alothman, Bhat, & Karim, 2009; Kumazawa et al., 2002).

This review focuses on the composition of polyphenols in carobs and their products, their antioxidant capacity and the impact on human health, while, at the same time, it points out the main factors that affect the polyphenolic profile and the antioxidant capacity.

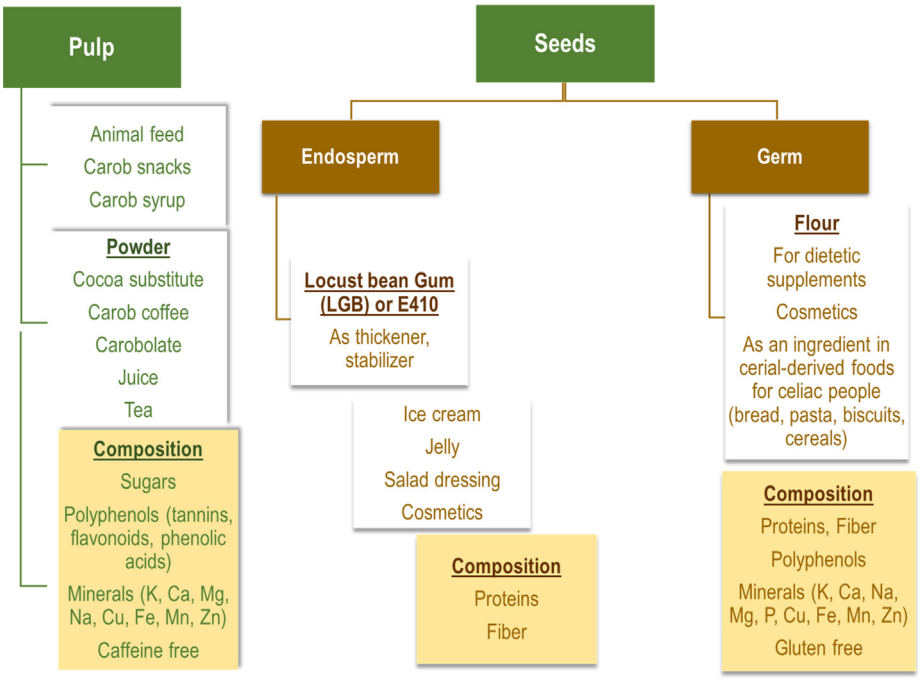


Fig. 4. Parts of carob fruit, their products and composition.

2. Polyphenolic composition and antioxidant capacity

Phenolic compounds are plant metabolites that determine the quality of fruits, vegetables and other plants (Ignat, Volf, & Popa, 2011). They consist of an aromatic ring with one or more hydroxyl groups. Some phenolic compounds are simple molecules with low molecular weight, while some others are polymers (Kim, Quon, & Kim, 2014; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). The main sources of polyphenols are fruits, such as grapes, citrus, berries, cherries, apples, plums, peaches, along with coffee, cocoa and some vegetables (Manach et al., 2004). The polyphenolic compounds are usually divided into several classes, according to the phenol rings they contain and the structural differences on the binding between these rings. Polyphenols are mainly classified into flavonoids, phenolic acids,

Table 1
Structures of the main classes of polyphenols.

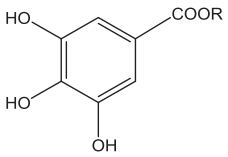
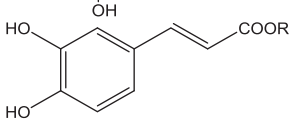
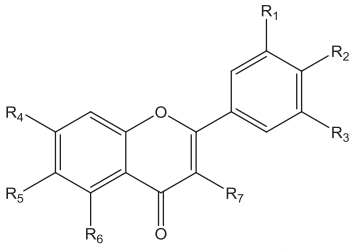
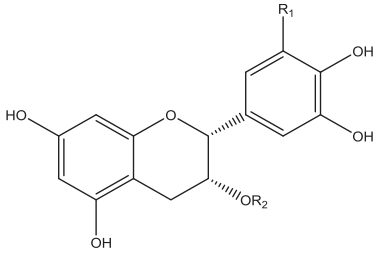
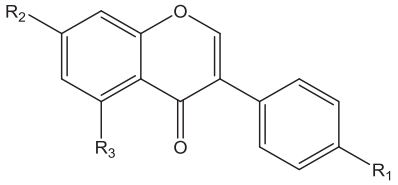
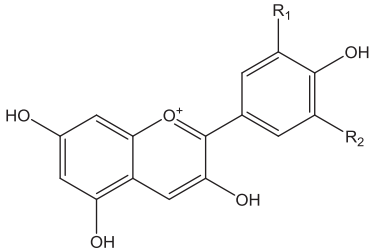
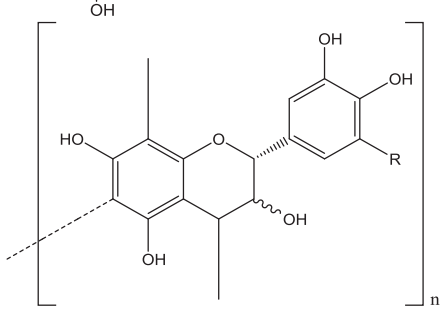
Class	Structure	Representatives
Phenolic acids: Hydroxybenzoic acids and derivatives		Gallic acid Gentisic acid Ellagic acid Protocatechuic acid Syringic acid
Hydroxycinnamic acids and derivatives		Caffeic acid Chlorogenic acid Ferulic acid Sinapic acid
Flavonoids: Flavonols Flavones Flavanones		Quercetin Apigenin Naringenin Kaempferol Myricetin Luteolin Chrysoeriol
Catechins		(+)-Catechin (−)-Epicatechin (−)-Epigallocatechin (−)-Epigallocatechin gallate (−)-Epicatechin gallate
Isoflavones		Genistein Daidzein
Anthocyanidins		Delphinidin Pelargonidin
Tannins: Condensed tannins- procyanidins		Procyanidin B1 Procyanidin B4

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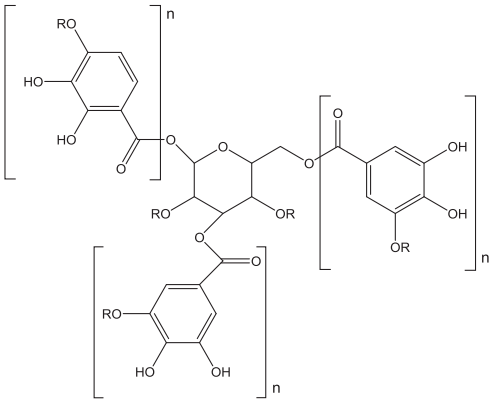
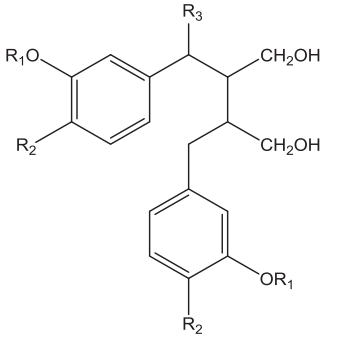
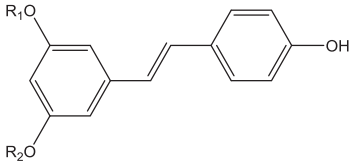
Table 1 (*continued*)

Class	Structure	Representatives
Hydrolyzable tannins: Gallotannins Ellagitannins		Theogallin Punicalagin
Lignans		Pinoresinol Isolariciresinol Lariciresinol Secoisolariciresinol
Stilbenes		Piceatannol Pterostilbene Resveratrol

tannins (hydrolysable and condensed), stilbenes and lignans. The chemical structures and typical representatives of the most investigated polyphenolic classes are presented in Table 1.

Due to their antioxidant properties and their ability to modulate several proteins, polyphenols have beneficial effects on human health (Fraga, Galleano, Verstraeten, & Oteiza, 2010; Yao et al., 2004). Consumption of polyphenols prevents from coronary heart diseases and cancer, promotes anti-allergy effects, and vaso-relaxation (Higdon & Frei, 2003; Kim et al., 2014; Yao et al., 2004). Therefore, a lot of research has, so far, been performed in order to characterize the phenolic content in different plants, vegetables and fruits (Gómez-Caravaca, Verardo, Segura-Carretero, Fernández-Gutiérrez, & Caboni, 2014; Ignat et al., 2011; Kontogianni, 2014).

3. Methodology for analysis: extraction and identification

The extraction of polyphenolic compounds is a crucial step for both the identification/quantitation and the isolation and assessment of the antioxidant capacity and cytotoxicity of polyphenol extracts. Although traditional and conventional methods have been widely used over the years, during the last decades, more advanced methods have been utilized (Kalia, Sharma, Singh, & Singh, 2008). Phenolic compounds are extracted from fresh, frozen or dried plant samples by applying first a pre-treatment of milling, grinding, drying and homogenization. The drying can be performed by the use of air or lyophilizer. The second technique seems to be preferred since it retains higher phenolic content levels in the samples than air drying (Abascal, Ganora, & Yarnell, 2005). Moreover, the extraction parameters, such as extraction time, temperature, solvent-to-feed ratio, the number of repeated extractions of the sample, as well as the choice of extraction solvents, affect the extraction yield (Kalia et al., 2008; Lapornik, Prošek, & Golc Wondra, 2005; Spigno et al., 2007).

Some of the most commonly used extraction methods are liquid-liquid extraction, solid-liquid extraction and Soxhlet technique. However, these methods usually require the use of organic solvents, such as methanol, acetone, diethyl ether and ethyl acetate, which are not only hazardous compounds but some residues may remain in the final extracts as well. In order to remove the remaining solvents, time-consuming purification steps are required. Moreover, high temperatures are sometimes used by using conventional methods for the extraction. The heating, boiling, and/or refluxing, lead to the loss of polyphenols due to ionisation, hydrolysis and oxidation during the extraction process. Consequently, alternative and more advanced methods have, over the years, been developed in order to overcome the above drawbacks.

Some of the modern techniques are ultrasound-assisted extraction, microwave-assisted extraction, ultrasound-microwave-assisted extraction and supercritical fluid extraction. These methods are simple, with relevant short extraction times and low solvent consumption. Extraction by using microwave energy provides mild conditions and achieves a superior effect of the extraction, while ultrasound-assisted extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques (Garcia-Castello et al., 2015). The ultrasonic extraction warrants a sufficient contact of the sample matrix with the extraction solvent, leading, thus, to a more efficient extraction than microwave-assisted technique (Garcia-Castello et al., 2015;

Table 2
Polyphenolic compounds found in different carob plant parts and products.

Plant parts	Polyphenol class	Polyphenol	Method of extraction (conditions)	Method of analysis	References
Carob Pulp	Benzaldehydes	*p*-Hydroxybenzaldehyde Dimethoxybenzaldehyde Dimethoxy-*p*-hydroxy-benzaldehyde Methoxybenzaldehyde Methoxy-*p*-hydroxy-benzaldehyde	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
	Flavanol galloyl esters	(−)-Epicatechin gallate	Infusion (water, 100 °C)	HPLC-UV	Corsi et al. (2002)
			Ultrasound assisted extraction (90% methanol/0.5% acetic acid)	HPLC-UV	Sakakibara et al. (2003)
		(−)-Epigallocatechin gallate	Solid-liquid extraction (1.water, 30 °C, 2.water, 100 °C)	CZE-UV	Almanasrah et al. (2015)
			Infusion (water, 100 °C)	HPLC-UV	Corsi et al. (2002)
			Decoction (water, 98.5 °C)	CZE-UV	Roseiro, Tavares et al. (2013)
			Ultrasound assisted extraction (90% methanol/0.5% acetic acid)	HPLC-UV	Sakakibara et al. (2003)
		(−)-Gallocatechin gallate	Solid-liquid extraction (1.water, 30 °C, 2.water, 100 °C)	CZE-UV	Almanasrah et al. (2015)
			Decoction (water, 98.5 °C)	CZE-UV	Roseiro, Tavares et al. (2013)
	Flavanols	(−)-Epicatechin	Solid-liquid extraction (1.water, 30 °C, 2.water, 100 °C)	CZE-UV	Almanasrah et al. (2015)
			Decoction (water, 98.5 °C)	CZE-UV	Roseiro, Tavares et al. (2013)
		(−)-Epigallocatechin	Infusion (water, 100 °C)	HPLC-UV	Corsi et al. (2002)
		(+)-Catechin	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
			Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)
			Infusion (water, 100 °C)	HPLC-UV	Corsi et al. (2002)
			Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011a)
			Ultrasound assisted extraction (90% methanol/0.5% acetic acid)	HPLC-UV	Sakakibara et al. (2003)
	Flavanone glycosides	Eriodictyol glucoside Naringenin glucoside Naringeninhexoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
			Ultrasound assisted extraction (ethanol:water 80:20)	HPLC-MS	Rached et al. (2016)
	Flavanones	Eriodictyol Liquiritigenin Naringenin Taxifolin	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
	Flavone glycosides	Methoxy Genkwanin glucoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
	Flavones	3,4,7-Trihydroxyflavone 7,4′-dihydroxyflavone Apigenin Chrysoeriol Dihydoxyflavone Methoxy Genkwanin Tricetin Tricetin-3′,5′-dimethyl ether Trihydroxy trimethoxy flavone	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
	Flavonol glycosides	Kaempferol desoxyhexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Kaempferol rhamnosise	Soxhlet (1.hexane, ,ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Kampferide hexoside	Ultrasound assisted extraction (ethanol:water 80:20)	HPLC-MS	Rached et al. (2016)
		Myricetin desoxyhexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Myricetin glucoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Myricetin hexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
			Ultrasound assisted extraction (ethanol:water 80:20)	HPLC-MS	Rached et al. (2016)
		Myricetin rhamnoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Myricetin pentoside	Ultrasound assisted extraction (ethanol:water 80:20)	HPLC-MS	Rached et al. (2016)

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Table 2 (*continued*)

Plant parts	Polyphenol class	Polyphenol	Method of extraction (conditions)	Method of analysis	References
		Quercetin arabinoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Quercetin glucoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
			Ultrasound assisted extraction (90% methanol/0.5% acetic acid)	HPLC-UV	Sakakibara et al. (2003)
		Quercetin hexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Quercetin rhamnoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
			Ultrasound assisted extraction (ethanol:water 80:20)	HPLC-MS	Rached et al. (2016)
		Quercetin glucoside	Ultrasound assisted extraction (ethanol:water 80:20)	HPLC-MS	Rached et al. (2016)
	Flavonols	Isorhamnetin	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Myricetin	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011a)
		Quercetin	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
			Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
	Gallate deivatives	Digallate Ethylgallate Methylgallate Tetragallate Trigallate Gallate glucoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
	Isoflavone glycosides	Genistein glucoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
	Isoflavones	Genistein Genistein dimethylether Genistein-4,7-dimethyl ether	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
	Phenolic acids	3,4-Dihydroxybenzoic acid 4-Hydroxybenzoic acid	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Caffeic acid	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011a)
			Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)
		Cinnamic acid	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Coumaric acid	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Ellagic acid	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
			Ultrasound assisted extraction (ethanol:water 80:20)	HPLC-MS	Rached et al. (2016)
			Ultrasound assisted extraction (90% methanol/0.5% acetic acid)	HPLC-UV	Sakakibara et al. (2003)
		Ferulic acid	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
			Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)
		Gallic acid	Solid-liquid extraction (1.water, 30 °C, 2.water, 100 °C)	CZE-UV	Almanasrah et al. (2015)
			Solid-liquid extraction (80% methanol, reflux)	HPLC-MS	Ayaz et al. (2007)
			Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
			Infusion (water, 100 °C)	HPLC-UV	Corsi et al. (2002)
			Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011a)
			Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
			Decoction (water, 98.5 °C)	CZE-UV	Roseiro, Tavares et al. (2013)
			Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)
			Ultrasound assisted extraction (90% methanol/0.5% acetic acid)	HPLC-UV	Sakakibara et al. (2003)
		Gallic acid diglucoside Gallic acid glucoside	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Gentisic acid	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011a)
		Sinapic Acid		HPLC-MS	Ayaz et al. (2007)

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Table 2 (*continued*)

Plant parts	Polyphenol class	Polyphenol	Method of extraction (conditions)	Method of analysis	References
			Solid-liquid extraction (80% methanol, reflux)		
		Syringic acid	Solid-liquid extraction (80% methanol, reflux)	HPLC-MS	Ayaz et al. (2007)
			Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
		Vanillic acid	Soxhlet (1.hexane, 2.ethanol)	HPLC-MS & GC–MS	Rakib et al. (2010)
	Tannins	Digalloyl-glucose Digalloyl-glucose derivative Galloyl-glucose derivative Pentagalloyl-glucose	Ultrasound assisted extraction (ethanol:water 80:20)	HPLC-MS	Rached et al. (2016)
		Prodelphinidin trimer	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Tannic acid	Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)
		Tetragalloyl-glucose Trigalloyl-glucose	Ultrasound assisted extraction (ethanol:water 80:20)	HPLC-MS	Rached et al. (2016)
Carob fiber	Flavanones	Eriodictyol	Supercritical fluid extraction (CO_2/ethanol:water)	HPLC-UV	Bernardo-Gil et al. (2011)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
		Naringenin	Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
	Flavanol galloyl esters	Epigallocatechin gallate	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
	Flavanols	Epigallocatechin	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
	Flavones	Apigenin	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
		Chrysoeriol	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
		Luteolin	Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water)	HPLC-MS	Roseiro, Duarte et al. (2013)
		Tricetin 3′,5′ dimethyl ether	Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
				HPLC-MS	Roseiro, Duarte et al. (2013)
			Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
	Flavonol glycosides	Kaempferol desoxyhexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Kaempherol rhamnoside	Soxhlet (1.hexane, 2.methanol)	HP LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
		Myricetin desoxyhexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Myricetin glucoside		HPLC-UV	

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Table 2 (*continued*)

Plant parts	Polyphenol class	Polyphenol	Method of extraction (conditions)	Method of analysis	References
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)		Bernardo-Gil et al. (2011)
			Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
		Myricetin hexoside Myricetin pentoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Myricetin rhamnoside Quercetin arabinoside	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
		Quercetin desoxyhexoside Quercetin hexoside Quercetin pentoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Quercetin rhamnoside	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
		Quercetin rhamnoside	Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
	Flavonols	Isorhamnetin	Soxhlet (1.hexane, 2.methanol)	LC -MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
				HPLC-MS	Roseiro, Duarte et al. (2013)
		Kaempferol	Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
			Ultrasound assisted extraction (H_2O or 70% acetone)	HPLC-UV	Roseiro, Duarte et al. (2013)
		Kaempferol derivative	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Methoxykaempferol derivative	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Myricetin Quercetin	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
		Quercetin derivative	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
	Gallate derivatives	Methyl gallate	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
	Isoflavones	Genistein	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
	Phenolic acids	Caffeic acid	Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
		Cinnamic acid	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
		Coumaric acid	Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
		Ferulic acid	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)

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Table 2 (*continued*)

Plant parts	Polyphenol class	Polyphenol	Method of extraction (conditions)	Method of analysis	References
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
		Gallic acid	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-UV	Bernardo-Gil et al. (2011)
			Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
			Ultrasound assisted extraction (H_2O or 70% acetone)	HPLC-UV	Roseiro, Duarte et al. (2013)
			Solid-liquid extraction (H_2O or 70% acetone)	HPLC-UV	Roseiro, Duarte et al. (2013)
		Syringic acid	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
			Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
		4-hydroxybenzoic acid	Supercritical fluid extraction (CO_2/ethanol:water 80:20)	HPLC-MS	Roseiro, Duarte et al. (2013)
	Tannins	1,2,3,6-Tetra-*O*-galloyl-*β*-D-glucose 1,2,6-Tri-*O*-galloyl-*β*-D-glucose	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003)
		1,6-Di-*O*-galloyl-*β*-D-glucose	Soxhlet (1.hexane, 2.methanol)	LC-MS, GC–MS. ESI-MS-MS, ^{1}H- & ^{13}C NMR	Owen, Haubner, Hull et al. (2003))
		Procyanidin dimer Procyanidin trimer Prodelphinidin dimer Prodelphinidin trimer	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
Carob flour	Flavanols	(+)-Catechin	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
		Epigallocatechin	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
	Flavanones	Naringenin	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
	Flavone glycosides	Apigenin glucose	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
	Flavones	Apigenin Luteolin	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
	Flavonol glycosides	Kaempferol desoxyhexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Kampferol rhamnoside Myricetin glucose Myricetin rhamnoside	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
		Myricetin desoxyhexoside Myricetin hexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Quercetin arabinoside Quercetin glucose Quercetin rhamnoside	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
		Quercetin desoxyhexoside Quercetin hexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
	Flavonols	Kampferol Myricetin Quercetin	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
	Gallate derivatives	Methyl gallate	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
	Isoflavones	Genistein	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
	Phenolic acids	Cinamic acid	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
		Ferulic acid	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
		Gallic acid	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
			Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
			Solid-liquid extraction (80% methanol)	HPLC-MS	Torun et al. (2013)
		Gentisic acid	Solid-liquid extraction (80% methanol)	HPLC-MS	Torun et al. (2013)

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Plant parts	Polyphenol class	Polyphenol	Method of extraction (conditions)	Method of analysis	References
		Coumaric acid	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
			Solid-liquid extraction (80% methanol)	HPLC-MS	Torun et al. (2013)
		Protocatechuic acid Sinapic acid	Solid-liquid extraction (80% methanol)	HPLC-MS	Torun et al. (2013)
		Syringic acid	Solid-liquid extraction (1.hexane, 2.70% acetone-water)	UPLC-MS/MS	Ortega et al. (2009)
			Solid-liquid extraction (80% methanol)	HPLC-MS	Torun et al. (2013)
	Tannins	Procyanidin dimer	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
Carob syrup	Flavanol galloyl esters	Catechin gallate Epigallocatechin gallate	Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
	Flavanols	(+)-Catechin Epigallocatechin	Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
	Flavonol glycosides	Myricetin 3-glycoside	Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
		Myricetin desoxyhexoside Quercetin desoxyhexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Quercetin glucoside	Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
	Flavonols	Kaempferol	Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
	Phenolic acids	Caffeic acid	Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
			Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Cinnamic acid	Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
			Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Coumaric acid	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
			Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
		Ferulic acid	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Gallic acid	Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
			Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
		Syringic acid	Ultrasound assisted extraction (water)	HPLC-MS	Dhaouadi et al. (2014)
Germ flour	Benzaldehydes	Vanillin	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011c)
	Flavanols	(+)-Catechin	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011c)
	Flavonols	Myricetin Quercetin	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011c)
	Gallate derivatives	Methyl gallate	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011c)
	Phenolic acids	Chlorogenic acid Ferulic acid Gallic acid Gentisic acid Syringic acid	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011c)
LBG	Flavanols	(+)-Catechin	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
	Flavonol glycosides	Kaempferol desoxyhexoside Kaempferol dihexoside Myricetin hexoside Myricetin pentoside Quercetin desoxyhexoside Quercetin hexoside	Pressurized liquid extraction (acetone:water 50:50)	HPLC-MS/MS	Papagiannopoulos et al. (2004)
Leaves	Benzaldehydes	Vanillin	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011b)
	Flavanol galloyl esters	(−)-Epicatechingallate (−)-Epigallocatechingallate	Infusion (water, 100 °C)	HPLC-UV	Corsi et al. (2002)
		(−)-Epigallocatechingallate	Ultrasound assisted extraction (methanol)	HPLC-UV, HPLC-MS/MS	Aissani et al. (2012)
	Flavanols	(−)-Epicatechin (+)-Catechin	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011b)
		(+)-Catechin	Ultrasound assisted extraction (methanol)	HPLC-UV, HPLC-MS/MS	Aissani et al. (2012)

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Plant parts	Polyphenol class	Polyphenol	Method of extraction (conditions)	Method of analysis	References
			Soxhlet (methanol)	HPLC-UV	Uysal et al. (2016)
			Solid-liquid extraction (water)	HPLC-UV	Uysal et al. (2016)
	Flavanones	Naringenin	Solid-liquid extraction (70% ethanol)	HPLC-MS	Vaya and Mahmood (2006)
	Flavanonols	Taxifolin	Solid-liquid extraction (70% ethanol)	HPLC-MS	Vaya and Mahmood (2006)
	Flavones	7,3′,4′ trihydroxy Flavone	Solid-liquid extraction (70% ethanol)	HPLC-MS	Vaya and Mahmood (2006)
		Apigenin	Soxhlet (methanol)	HPLC-UV	Uysal et al. (2016)
			Solid-liquid extraction (water)	HPLC-UV	Uysal et al. (2016)
		Luteolin	Solid-liquid extraction (70% ethanol)	HPLC-MS	Vaya and Mahmood (2006)
	Flavonol glycosides	Isoquercetin	Ultrasound assisted extraction (methanol)	HPLC-UV, HPLC-MS/MS	Aissani et al. (2012)
		Myricetin glucoside Myricetin rhamnoside	Maceration (ethanol-water 80%)	HPLC-MS	Hsouna et al. (2011)
		Myricitrin	Ultrasound assisted extraction (methanol)	HPLC-UV, HPLC-MS/MS	Aissani et al. (2012)
		Rutin	Soxhlet (methanol)	HPLC-UV	Uysal et al. (2016)
	Flavonols	Kaempferol	Soxhlet (methanol)	HPLC-UV	Uysal et al. (2016)
			Solid-liquid extraction (70% ethanol)	HPLC-UV -MS	Vaya and Mahmood (2006)
		Myricetin	Solid-liquid extraction (70% ethanol)	HPLC-UV -MS	Vaya and Mahmood (2006)
		Quercetin	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011b)
			Soxhlet (methanol)	HPLC-UV	Uysal et al. (2016)
			Solid-liquid extraction (70% ethanol)	HPLC-UV -MS	Vaya and Mahmood (2006)
	Isoflavones	Biochanina Genistein	Solid-liquid extraction (70% ethanol)	HPLC-UV -MS	Vaya and Mahmood (2006)
	Phenolic acids	Chlorogenic acid	Ultrasound assisted extraction (methanol)	HPLC-UV, HPLC-MS/MS	Aissani et al. (2012)
			Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011b)
			Solid-liquid extraction (water)	HPLC-UV	Uysal et al. (2016)
		Coumaric acid	Solid-liquid extraction (water)	HPLC-UV	Uysal et al. (2016)
		Ferulic acid	Soxhlet (methanol)	HPLC-UV	Uysal et al. (2016)
			Solid-liquid extraction (water)	HPLC-UV	Uysal et al. (2016)
		Gallic acid	Ultrasound assisted extraction (methanol)	HPLC-UV, HPLC-MS/MS	Aissani et al. (2012)
			Infusion (water, 100 °C)	HPLC-UV	Corsi et al. (2002)
			Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011b)
			Soxhlet (methanol)	HPLC-UV	Uysal et al. (2016)
			Solid-liquid extraction (water)		
		Gentisic acid	Soxhlet (1.hexane, 2.methanol)	HPLC-UV	Custódio et al. (2011b)
		p-Hydroxybenzoic acid	Soxhlet (methanol)	HPLC-UV	Uysal et al. (2016)
		Syringic acid	Maceration (ethanol-water 80%)	HPLC-MS	Hsouna et al. (2011)
	Tannins	1,2,3,6-Tetra-O-galloyl-glucose 1,2,6-Tri-O-galloyl-glucose 1,6-Di-O-galloyl-glucose	Maceration (ethanol-water 80%)	HPLC-MS	Hsouna et al. (2011)
Seeds	Flavanols	(+)-Catechin	Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)
	Phenolic acids	Gallic acid	Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)
		Vanillic acid	Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)

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Plant parts	Polyphenol class	Polyphenol	Method of extraction (conditions)	Method of analysis	References
	Tannins	Tannic acid	Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)
	Trihydroxybenzene	Pyrogallol	Solid-liquid extraction (water)	HPLC-UV	Rtibi et al. (2015a)
Sapwood	Benzaldehydes	Vanillin	Solid liquid extraction (water:methanol)	GC–MS	Mualla, 2004)
			Soxhlet (1.Hexane, 2.Methanol)	HPLC-UV	Custódio et al. (2013)
	Flavanols	(+)-Catechin	Solid liquid extraction (water:methanol)	GC–MS	Mualla, 2004)
			Soxhlet (1.Hexane, 2.Methanol)	HPLC-UV	Custódio et al. (2013)
	Flavanone glycosides	Rutin	Soxhlet (1.Hexane, 2.Methanol)	HPLC-UV	Custódio et al. (2013)
	Flavanones	(−)-Epicatechin	Soxhlet (1.Hexane, 2.Methanol)	HPLC-UV	Custódio et al. (2013)
	Flavones	Trihydroxyflavone Dihydoxyflavone	Solid liquid extraction (water:methanol)	GC–MS	Mualla, 2004)
	Flavonols	Kaempferol	Soxhlet (1.Hexane, 2.Methanol)	HPLC-UV	Custódio et al. (2013)
	Gallate deivatives	Methylgallate	Solid liquid extraction (water:methanol)	GC–MS	Mualla (2004)
	Phenolic acids	Ellagic acid Vanillic acid Gallic acid	Solid liquid extraction (water:methanol)	GC–MS	Mualla (2004)
		Gallic acid Chlorogenic acid Gentisic acid Syringic acid	Soxhlet (1.Hexane, 2.Methanol)	HPLC-UV	Custódio et al. (2013)

Huang, Xue, Niu, Jia, & Wang, 2009). Another novel method which is called high hydrostatic pressure (HHP), demonstrated high mass transport phenomena and higher extraction yields from several matrixes (Briones-Labarca, Plaza-Morales, Giovagnoli-Vicuña, & Jamett, 2015; Jiménez-Martínez et al., 2017). Supercritical fluid extraction (SFE) is an environmentally friendly alternative to the conventional organic solvent extraction because it uses very small amounts of toxic solvents. Particularly, SFE is based on the fact that, closed to the critical point, the solvent changes its properties rapidly with only slight variations of pressure. Moreover, due to the absence of light and air during the process, the degradation that usually occurs with the conventional techniques is eliminated.

The above-mentioned conventional and modern extraction techniques have, over the years, been widely used for the extraction of polyphenols from carob fruit and leaves. The results obtained regarding extraction yield and efficiency seem to be in agreement with those previously described. Briefly, solvent polarity and temperature are the two most important parameters that affect both the polyphenol extraction yield and the selectivity. Ultrasound-assisted extraction and SFE proved to be the most efficient techniques. The differences on the extraction results along with the parameters that affect the polyphenolic profile and antioxidant capacity of the carob extracts are further described below.

The characterization of the polyphenolic profile of the extracts, is performed by using several analytical methods. One strategy that is usually followed involves the estimation of the total polyphenolic content and then the identification and quantification of polyphenolic compounds. A possible correlation between the polyphenolic content and the antioxidant capacity and/or cytotoxicity is additionally examined. Several spectroscopic methods are used for the determination of total polyphenols. Total phenolic content (TPC) is determined by Folin-Ciocalteu colorimetric-based methods, total flavonoid content (TFC) by the aluminium chloride method and condensed tannins content (TTC) by vanillin and *p*-dimethylaminocinnamaldehyde methods. Chromatographic techniques are used for the identification and quantitation of polyphenolic compounds. High-performance liquid chromatography (HPLC) and gas chromatography (GC) are the two most frequently applied separation techniques, while capillary electrophoresis (CE) is another promising technique (Martí, Valcárcel, Herrero-Martínez, Cebolla-Cornejo, & Roselló, 2017; Nicolaou & Kapnissi-Christodoulou, 2010). HPLC and CE are usually coupled with ultraviolet detection, electrochemical detection, mass spectrometry (MS), while GC is coupled with MS. However, GC is not preferred due to the low volatility of the phenolic compounds. The hyphenation of chromatographic techniques, such as HPLC-MS and HPLC-MS/MS, provide not only higher sensitivity (lower limits of detection and quantitation) but also more accurate information about the structural features of the compounds.

Several methods can be used for the assessment of the antioxidant capacity of the extract. The scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical is mostly applied to the phenolic compounds in order to evaluate the free radical scavenging capacity and the total antioxidant ability. The antiradical activities of polyphenols are determined by using the DPPH. Briefly, DPPH has a characteristic absorption at 515 nm, which decreases proportionally when an amount of antioxidant is added. Although this is the most common method, other methods, such as 2,21-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), reduction of superoxide anion and inhibition of lipid peroxidation are also used for the assessment of the antioxidant capacity of different extracts.

4. Polyphenolic contents and antioxidant capacity of carobs

The total content and the identification and quantitation of the polyphenolic compounds have, over the years, been estimated in carob fruit, leaves and even in tree barks. The process that is followed prior to analysis involves the drying of the carob samples, removal of the seeds, grinding in a mill and storage in a dry, cool or even at −20 °C place until the extraction. The extracts that are obtained after the extraction process are analysed using HPLC, GC or CE coupled with UV or MS detectors.

It is important here to clarify the terms that are usually used by researchers and sometimes are misleading. As mentioned above, the carob fruit, which is sometimes wrongly referred as carob pod, constitutes the pulp and the seeds. The seedless carob fruit is called “carob pod” or “pulp”, and when it is crashed, the crashed pieces are called “kibbles”. Therefore, in this review, the terms carob pods, pulp and

kibbles are referred to the seedless part of the carob fruit. In some cases, the carbohydrates (sugars) are extracted from the pulp and then the remaining solid residue is mentioned as carob fiber or carob pulp preparation. The seeds, also referred as kernels, consist of germ and gum. Polyphenolic compounds are found in carob fruit as free, bound or soluble forms. Based on the carob tree variety and gender, the extraction method and/or the process conditions that are used, different polyphenolic profiles can be observed. The antioxidant capacity is often correlated with the phenolic content of the carob extracts. Therefore, the assessment of the antioxidant capacity is performed along with the estimation of the TPC, TFC and TTC.

The main categories of phenolic compounds found in carob fruit are phenolic acids, gallotannins and flavonoids. Polyphenols can be found in the carob fruit and more specifically in pulp, seeds and germ. Although most of the research has, so far, been performed on the estimation of polyphenols in carob fruits, some studies have demonstrated the presence of polyphenols in carob leaves and carob tree bark, as well. Table 2 summarizes the most common polyphenols that have, over the years, been detected in parts of carob tree. The polyphenolic composition in carob fruit (kibbles, seeds and germ) and other parts (leaves, sapwood and barks) is described below.

In the following chapters, the content of polyphenols of carobs, the antioxidant capacity and their health impact are summarized and discussed.

4.1. Polyphenols in carob fruit

A lot of research work focused on the identification and quantification of polyphenols in the carob fruit. Basically, the seedless carob pod (kibbles or pulp) contains a higher amount of polyphenols in comparison to seeds or germ. The concentration of total polyphenols in carob fruits depends strongly on genetic, environmental and extraction methods and ranges between 45 and 5376 mg gallic acid equivalents per 100 g of dry extract.

4.1.1. Polyphenol content in carob pulp

The most important and abundant polyphenols that have been identified in carob pulp are shown in Table 2. In 1997, Avallone, Plessi, Baraldi, & Monzani (1997) reported one of the first studies that proved the presence of tannins among total polyphenols in carob kibbles from eight different carob trees grown in eastern parts of Sicily. Briefly, in this work, the total polyphenols were determined by using the Folin-Ciocalteu method, the total proanthocyanidins by using vanillin assay, and ellagitannins, gallotannins and non-extracted tannins were determined. Among the eight different carob samples, the carob pod of Modica and Ispica areas consisted of the highest polyphenol levels in comparison to the other samples. A higher level of condensed tannins was detected (proanthocyanidins) than the hydrolyzable ones, such as ellagitannins and gallotannins.

The effect of the solvent on the extraction selectivity was examined by using acetone, methanol and water. Briefly, when 70% of aqueous acetone was used, a significant higher amount of total polyphenols and proanthocyanidins were determined in contrast to 100% acetone. Moreover, 70% acetone was a more efficient solvent than 70% methanol. It was therefore concluded that the solvent polarity affects the solubility, and in turn, the extraction of some tannins (condensed and hydrolyzable). On the other hand, condensed tannins, such as proanthocyanidins (4.6 mg cyaniding / g meal), were not able to be completely extracted, and they were detected in the residues. The latter suggests that this method excludes the extraction of a significant amount of proanthocyanidins. This observation is of great importance when polyphenol extracts are going to be used as therapeutic agents, since high amount of proanthocyanidins should be avoided due to their excessive astringency.

A lot of similar studies followed, which focused on the estimation of TPC, TTC, TFC and the identification and quantification of polyphenolic compounds in the seedless carob pods (Almanasrah et al., 2015; Corsi et al., 2002; Roseiro, Tavares, Roseiro, & Rauter, 2013; Sakakibara, Honda, Nakagawa, Ashida, & Kanazawa, 2003; Youssef, El-Manfaloty, & Ali, 2013). Corsi et al. (2002) demonstrated the presence of gallic acid, (−)-epigallocatechin, (+)-catechin, (−)-epigallocatechingallate and (−)-epicatechingallate, with gallic acid to be the dominant compound in the extract. Kumazawa et al. (2002) used a similar extraction method after the removal of sugars from the carob kibbles. The residue, which is also commercially known as carob fibre, contained high TPC (19.2 g gallic acid equivalents/100 g dry extract), while the condensed tannins were found at lower levels. However, the value of TPC cannot be compared with the values reported elseware obtained from kibbles, because the polyphenol extract from the carob fiber does not contain sugars. Therefore, the relative polyphenol content, which is expressed as g per g of extract, is condensed in comparison to that of the conventional carob kibble extract.

A different study by Sakakibara et al. (2003) reported the use of sonication as an alternative method of extraction by using 90% methanol and 0.5% acetic acid as the extraction solvents. HPLC coupled with UV and MS detectors revealed that dry carob contains quercetin glycosides, (+)-catechin, (−)-epicatechingallate, (−)-epigallocatechingallate and the phenolic acids of gallic acid and ellagic acid. Gallic acid was found again at the highest concentration, indicating that it is the most abundant phenolic compound in carob pods. It can again be concluded that the extraction solvent is of great importance. Particularly, with water, gallic acid, (−)-epigallocatechin, (+)-catechin, (−)-epicatechingallate and (−)-epigallocatechingallate can be extracted (Almanasrah et al., 2015; Corsi et al., 2002; Roseiro, Tavares et al., 2013), while when organic solvents were used, such as 90% methanol (Sakakibara et al., 2003) or 70% acetone (Avallone et al., 1997), some flavonoids (e.g. quercetin glucosides) and tannins were able to be additionally extracted.

Phenolic acids, (+)-catechin and (−)-epicatechin are the simplest forms of phenolic compounds. They are also structural units of other compounds, such as flavanols and tannins. Degradation of the latter, results in the formation of the free phenolic monomers. This is why, in most cases, gallic acid, (+)-catechin and (−)-epicatechin are found to be the most abundant compounds. The characterization though of the polyphenolic profile could be misleading. In order to further investigate the structure of the phenolic compounds in carobs, Owen, Haubner, Hull et al. (2003), utilized HPLC-MS, GC–MS along with NMR spectroscopy. In this study, the structure elucidation provided more accurate information about the phenolic composition in carob fiber samples (de-sugared kibbles). The results demonstrated that carob fiber has higher phenolic content in comparison to olives (Owen, Haubner, Mier et al., 2003). Particularly, it was found that carob fiber contained several classes of polyphenolic compounds, such as simple phenols (0.079 g/Kg), polyphenols (1.688 g/Kg), free flavones and flavanones (0.132 g/Kg), glycosylated flavonols (0.879 g/Kg), isoflavones (0.0005 g/Kg), flavonones (0.019 g/Kg) and gallotannins (1.15 g/Kg). Gallic acid was again the most abundant compound (1.65 g/Kg) among the 24 identified polyphenols, while the isoflavone genistein was found at the lowest concentration. In comparison to the studies described previously (Corsi et al., 2002; Sakakibara et al., 2003), this study demonstrated much higher yield and variation in the phenolic compounds that were detected and identified.

Two important factors that contribute to this difference are the extraction processes and the analytical techniques that were used. In the former case (Corsi et al., 2002), this difference can be attributed to the extraction of the carob pod instead of the carob fiber, while in the latter case (Sakakibara et al., 2003), it is probably due to the extremely short extraction time (1 min) used during the sonication. Owen, Haubner, Hull et al. (2003) noted that their results were considered superior when hexane, followed by methanol, were used for soxhlet extraction, in comparison to the extraction performed with hexane and water. Water, at its boiling point, was also used as the extraction solvent by Corsi et al. (2002). Their results demonstrated lower polyphenol yield and variation. It is well known that water may affect the dissolution of some phenolic compounds.

Another study performed by Spigno et al. (2007) reported the effect of temperature, solvent and extraction time on the concentration of grape pomace phenolics. It was demonstrated that phenolic concentrations of extracts decreased when the amount of water in the aqueous ethanolic solution was above 50%, suggesting that lower amounts, and not different compounds, were recovered (Spigno et al., 2007). In addition, it was indicated that thermal treatment during the drying step or the extraction may cause degradation of polyphenols, and, it may release bound phenolic compositions in several matrixes, leading thus, to different variations (Carrasco-Pancorbo et al., 2007; Kim et al., 2006; Xu & Chang, 2009). Based on the above, it is concluded that the processes used during the extraction are of great importance for a more accurate characterization of the phenolic profile.

The methods of analysis that are used (HPLC-UV, CE-UV) may sometimes not provide satisfactory information about the individual polyphenol components. For example, HPLC-MS, GC–MS and NMR studies were utilized by Owen, Haubner, Hull et al. (2003) for the successful elucidation of the structure of phenolic compounds in carob fiber, for the first time. A similar study by Papagiannopoulos, Wollseifen, Mellenthin, Haber, and Galensa, (2004) reported the identification and examination of the phenolic compound distribution in kibbles, carob fiber, syrup and LBG (from the seeds). As for the identification of polyphenols in carob fiber, the results were similar to those obtained earlier by Owen, Haubner, Hull et al. (2003). However, they detected 41 individual phenolic compounds in contrast to Owen et al., who were able to detect 21 compounds. This observation may be attributed to the fact that Pressurized-Liquid Extraction (PLE) and Solid-Phase Extraction (SPE) were used for the extraction. The carob variety does not seem to be the reason because the carob fiber was provided by the same company.

Briefly, in this study (Papagiannopoulos et al., 2004), the carob pods contained gallic acid, hydrolysable and condensed tannins, flavonol-glycosides and traces of isoflavonoids. In particular, they estimated the amounts of polyphenols in kibbles, carob fiber and syrup and they stated the distribution and the fate of the compounds in carob fruit. During the preparation of carob fiber (de-sugared kibbles) the polyphenols are distributed between the insoluble solid residue (fiber) and the cold-water extract (syrup), which is rich in sweet carbohydrates. Different polyphenolic compositions were observed when these processed products were analysed. Briefly, carob fiber contained the highest amounts of polyphenolic compounds, with quercetin- and myricetin- derivatives, procyanidins galloyl esters and gallic acid to be the most dominant. On the other hand, syrup mainly consisted of gallic acid and some polar hydrolysated tannins. This suggests that gallic acid and hydrolysated tannins are co-extracted with the carbohydrates during fiber preparation. In the same work, the research group also examined the effect of roasting on the polyphenolic pattern of some commercially available carob flours (seedless carob pod powder). They demonstrated that roasting plays an important role on the hydrolysable tannin and gallic acid content. It was observed that hydrolysable tannin concentration decreased, while the gallic acid increased, due to hydrolysation processes. These results are in agreement with those obtained from other studies (Ortega et al., 2009; Torun, Ayaz, Colak, Grúz, & Strnad, 2013).

Briefly, Ortega et al. (2009) observed the presence of phenolic acids, flavonoid aglycones and glycosides in carob flour (seedless carob pod powder), while no tannins where found due to hydrolysis. It is worth here to mention that, although the majority of literature reports mention that no caffeine or theobromine is found in carobs (Srour et al., 2016), in this research work, approximately 38.5 μg of caffeine and 24.8 μg of theobromine per gram of carob flour, were determined. However, as they noted, these concentrations are around 50 times lower than in cocoa derivatives, and therefore, carob powder can be used as a substitute of cocoa and wheat derived products (Youssef, Ali, & El-Manfaloty, 2013; Youssef, El-Manfaloty et al., 2013).

Through the years, a number of researchers made attempts to examine the correlation between the phenolic composition of carob pods and the different varieties and regions of the trees. For more accurate results, the comparison must be carried out only when the same processes (extraction, isolation and analysis methods) are used. For example, Ayaz et al. (2007) reported that the amount of TPC in Anatolian carob pods was 13.51 mg gallic acid equivalents/g dry weight, while, Avallone et al. (1997), determined 1.9 mg/g in Sicilian carob pods. The extraction solvents though were different; so, a comparison between these two reports would be misleading and inaccurate. Recently, Rakib et al. (2010) utilized HPLC-MS and GC–MS techniques in order to identify and quantify the phenolic contents of carob pod ethanolic extracts, derived from six regions in Morocco (Essaouira, Béni-Mellal, Tafraout, Nador, Fès and Taza). A total of fifty-two (Rakib et al., 2010) compounds were identified and the predominant phenolic compounds, in all cases, were gallic acid, gallate glucoside and gallic acid glucoside. Among all the ethanolic extracts, Essaouira, Béni-Mellal and Taza samples exhibited higher phenolic content than the others. Several classes of polyphenols were identified, such as simple phenols, gallic acid glucoside, gallate derivatives and flavonoids. In the case of flavonoids, some flavonol-glycosides (myricetin rhamnoside and quercetin rhamnoside) were predominantly contained in Essaouira and Taza samples.

It was clearly demonstrated from this study that the geographical origin and the nature of the cultivar are factors that have a significant impact on the polyphenolic composition. A lot of other studies were performed to enhance this statement (Custódio et al., 2011a; Custódio, Fernandes, & Romano et al., 2007; Sigge, Lipumbu, & Britz, 2011; Vekiari, Ouzounidou, Ozturk, & Görk, 2011). It is concluded that, apart from variety and cultivar differences, gender and growing stage affect significantly the polyphenolic profile of carob pods. In particular, extracts from hermaphrodite have a higher content of polyphenols than the females, while TPC is reduced as the growing stage increases. It has also been recently found that wild carob trees have different phenolic content than the domesticated trees (El Bouzdoudi et al., 2016). It has generally been demonstrated that carob pod powders from wild trees are richer in polyphenols than those from domesticated trees. Nevertheless, the latter has sometimes the tendency to reach the content of the former.

During the last years, more advanced methods have been developed for the extraction of polyphenols from carob pods. The classical techniques are often time-consuming, they require relatively large quantities of solvents and the active compounds sometimes undergo hydrolysis. Alternatively, SFE has been recently utilised for the extraction of polyphenols from de-sugared pulp residue (carob fiber) (Bernardo-Gil et al., 2011). Compressed carbon dioxide was used as the solvent and 80% aqueous ethanol as the co-solvent. Several parameters were examined and optimized (pressure, temperature, particle diameter, co-solvent percentage and flow rate), in regard to the yield of the extraction. The characterization of the de-sugared carob residue revealed similarities with the commercially available Carobmax fiber (Owen, Haubner, Hull et al., 2003; Papagiannopoulos et al., 2004), and it was proposed as a dietary fiber containing polyphenols. Roseiro, Duarte et al. (2013) examined the effect on the phenolic profile of SFE, ultrasound and conventional extracts from carob kibbles. Interestingly, SFE extract demonstrated a diversity of phenolic compounds at high concentrations, while ultrasound and conventional extracts contained mainly gallic acid. However, when SFE was utilized, the carob kibbles were de-sugared and the extraction was applied to the insoluble carob fiber residue. As a result, some water soluble phenolics, such as gallic acid, probably co-extracted during the sugar removal. Additionally, the effect of the extraction solvent with ultrasound and conventional extraction methods demonstrated, once again, that a 70%-aqueous acetone was a more efficient solvent than water (Avallone et al., 1997; Corsi et al., 2002). A comparison between the ultrasound and conventional extraction methods demonstrated that the former is more rapid and efficient than the latter for the particular solvent system used.

Therefore, a pre-treatment of carob kibbles by ultrasound assisted extraction prior to SFE with water at low temperatures could enable the removal and isolation of the sugars, which could be used in other applications, e.g. syrup preparation. Then, the SFE can provide a more selective extract, which can further be utilized for the characterization of phenolic composition, and for testing antioxidant and cytotoxic activities.

4.1.2. Polyphenol content in carob seeds

As mentioned earlier, LBG is mainly used in industry as a stabilizer (Barak & Mudgil, 2014; Calixto & Cañellas, 1982; Rizzo, Tomaselli, Gentile, La Malfa, & Maccarone, 2004), while germ flour has recently been used for the preparation of dough products (Tsatsaragkou, Gounaropoulos et al., 2014; Tsatsaragkou et al., 2012; Tsatsaragkou, Yiannopoulos et al., 2014). Therefore, their chemical composition attracted the interest of researchers. The main phenolic composition is summarized in Table 2. In seeds, TPC and TTC are approximately 0.66–24.28 mg gallic acid equivalents/g dry extract and 0.19 mg C equivalents/g dry extract, respectively (Avallone et al., 1997; Durazzo et al., 2014). In germ, TPC and TTC were found to be about 19.25–40.8 mg gallic acid equivalents/g dry extract and 15.4–16.2 mg (+)-catechin equivalents/g dry extract, respectively (Avallone et al., 1997; Custódio et al., 2011a; Durazzo et al., 2014). Custódio et al. (2011a) analysed germ-flour extracts of carob tree from different origins, and they identified a number of polyphenolic compounds by use of HPLC. It was observed that, as in carob pulp, the region, the variety and certain genotypes within a plant species, resulted in a significant variation in the phenolic profile. Along the samples from different female cultivars, the Galhosa samples exhibited the highest content of the three classes of phenolic compounds (TPC, TFC and TTC). HPLC revealed that gallic acid was present in all samples, while other compounds were only detected in some cultivars. Particularly, (+)-catechin and gentisic acid were identified only in Galhosa, and chlorogenic, ferulic and vanillin only in Mulata. Other polyphenols that were detected in some of the samples were myricetin, methyl gallate, quercetin and syringic acid. Apart from the regional and genetic diversity between cultivars, another factor responsible for these composition differences was the particular HPLC method (UV detection), which may not have been able to detect some polyphenols at very low concentrations. Another parameter that was not mentioned by the authors, and may also have affected the chemical composition, is the treatment for the preparation of germ flour. Carob seed germ can be obtained by acid treatment or boiling water treatment of the whole seeds. It has previously been reported that the carob germ meal composition could be affected by the isolation procedures (Dakia et al., 2007).

Papagiannopoulos et al. (2004) identified and quantified polyphenols in locust bean powder (confusingly also referred as locust bean gum in the same article). The unclear definition of the examined sample may refer to the seed powder (flour) or the LBG powder. However, LBG consists mainly of high-molecular-weight hydrocolloidal polysaccharides. Therefore, the report probably refers to seed powder (seed flour). In this study, quercetin—desoxyhexoside (44.7 mg/Kg), quercetin-hexoside (4.8 mg/Kg), (+)-catechin (23.8 mg/Kg), kaempferol-dihexoside (7.0 mg/Kg) and kaempferol-desoxyhexoside (1.2 mg/Kg) were the only phenolic compounds detected. These compounds, as they stated, may be restricted to the outer, dark brown seed-coat. Interestingly, in contrast to Custódio et al. (2011a), no gallic acid was detected in locust bean powder. In a recent study (Durazzo et al., 2014), the TPC of wholegrain seed flour, germ flour and LBG powder (E410) was determined. It was observed that the former two demonstrated higher TPC than LBG powder. In this study, the lignans isolariciresinol, laricireSinol, secoisolariciresinol, pinoresinol were also reported. Particularly, it was found that the wholegrain seed flour contains the highest amount of total lignans, and it was within the range 160.22–1732.21 μg/100 g dry weight.

4.2. Polyphenols in other carob-tree parts

Apart from the carob fruit, other tree parts, such as leaves and carob tree bark, were examined for their phenolic composition. The TPC and TFC of carob leaf extract were found to be about 91.2–680 mg gallic acid equivalents/g dry weight and 193.3 mg quercetin equivalents/g dry weight (or 16–70 mg rutin equivalents/g dry weight), respectively (Custódio et al., 2007; Hajaji et al., 2010; Hsouna et al., 2011; Uysal, Zengin, Aktumsek, & Karatas, 2016). A comparison of TPC, TTC and TFC levels between the three genders revealed that in the leaves of male and hermaphrodite trees the total phenol and flavonoid amounts were higher than in the female ones (Custódio et al., 2007). As far as the condensed tannin content is concerned, different results were obtained, according to the method used. Briefly, the vannilin and *p*-dimethylaminocinnamaldehyde methods were used for the estimation of TTC. When vannilin method was utilized, female tree leaves consisted of the highest level of TTC (9.7 g (+)-catechin equivalents/100 g dry weight). On the contrary, when *p*-dimethylaminocinnamaldehyde method was applied, hermaphrodite tree leaves demonstrated the highest amount (20.3 g (+)-catechin equivalent/100 g dry weight). It was also demonstrated that carob leaves contain significantly higher amounts of total phenols, condensed tannins and flavonoids than the pulp (Corsi et al., 2002; Custódio et al., 2007). This suggests that leaves could be considered as a more potential source of phenolic compounds than the pulp, and thus, more extensive studies on their applications should be performed.

In a different study, TPC was estimated to be higher in leaves from grafted female carob trees than from spontaneous female and male carob trees. The main phenolic compounds that have, so far, been detected in carob leaves are the following: gallic acid, *p*-hydroxybenzoic acid, chlorogenic acid, coumaric acid, ferulic acid, syringic acid, gentisic acid, (+)-catechin, (−)-epicatechin, (−)-epigallocatechingallate, myricetin, rutin, quercetin, kaempherol, apigenin, isoquercetin, myricetin glucoside, myricetin rhamnoside, 1,6-di-O-galloyl-gucose, 1,2,6-tri-O-galloyl-gucose and 1,2,3,6-tetra-O-galloyl-gucose (Table 2) (Aissani, Coroneo, Fattouch, & Caboni, 2012; Corsi et al., 2002; Custódio et al., 2011b; Hajaji et al., 2010; Hsouna et al., 2011; Uysal et al., 2016). Diversity and different phenolic profiles are observed between different studies due to the reasons that have already been described (gender, origin-variety, methods). For example, methanol and ethyl acetate proved to be more efficient extraction solvents than boiling water, and the techniques HPLC-MS and HPLC-MS/MS could detect more compounds than HPLC-UV (Aissani et al., 2012; Corsi et al., 2002; Custódio et al., 2011b; Hajaji et al., 2010; Hsouna et al., 2011; Uysal et al., 2016).

Another part of carob tree that has recently attracted interest for its phenolic composition was the bark from three genders of carob trees growing in Morocco (Hajaji et al., 2011). The study demonstrated that the barks contain high amounts of polyphenolic compounds and that their composition is affected by the variety of the tree. The presence of flavonoids and tannins was reported, as well as the estimation of the TPC. TPC from ethyl acetate and methanolic extracts of three varieties of Ceratonia siliqua L. barks varied from 0.46 to 0.76 (g/L gallic acid equivalents). In this study, the methanolic extract had the highest phenolic content than ethyl acetate extract. It was found that the methanolic extract from the spontaneous female contained 0.54 (g/L gallic acid equivalents), from the spontaneous male contained 0.76 (g/L gallic acid equivalents) and from the grafted female 0.62 (g/L gallic acid equivalents). In a similar study, the sapwood of carob tree was investigated as a potential source of bioactive compounds (Custódio et al., 2013). It was found that methanol- and hot water-extracts of carob tree sapwood were rich in phenolic compounds, with the main compounds identified by HPLC/DAD as gentisic acid and (−)-epicatechin. Briefly, the researchers reported that the sapwood has a higher TPC than leaves, pulps and germ flour, but lower concentrations of TTC and TFC. The

HPLC/DAD results revealed that gentisic acid was the major compound, followed by (−)-epicatechin, gallic acid, (+)-catechin and chlorogenic acid. On the contrary, syringic acid, vannilin, rutin and kaempferol were detected in minor amounts. Some years earlier, in 2004, in another similar study the phenolic composition of methanol and water extracts of heartwood and sapwood was investigated by GC/MS (Mualla, 2004). More specific, aqueous methanolic extracts of carob heartwood and sapwood were fractionated using organic solvents of increasing polarity, and GC–MS analyses were performed before and after hydrolysis. Prior to hydrolysis, gallic acid, (+)-catechin and its derivatives, methyl inositol and chalcone were determined as the major compounds found in the free form. Aqueous fractions of both wood types were hydrolysed with hydrochloric acid in methanol and extracted with organic solvents and water. These fractions were rich in methyl inositol, gallic acid, glucose and other monosaccharides. The results show that carob wood contains predominantly gallotannins and proanthocyanidins. However, the results were slightly different from the study obtained by Custódio et al. (2013), since gentisic acid was referred to be the main compound in carob tree sapwood.

4.3. Antioxidant capacity, cytotoxic effect and health impact of carob extracts

Another important parameter that is widely examined is the antioxidant capacity of carob extracts and its correlation with the polyphenolic content. The presence of the latter in a matrix is highly correlated with the antioxidant capacity. Therefore, it is suggested that carob may be used for the prevention of free radical-related diseases as a dietary natural antioxidant supplement.

4.3.1. Antioxidant capacity and cytotoxic effect

Over the years, the free-radical scavenging ability, antioxidant and cytotoxic activities of carob extracts (from pulp, seeds, gum, germ, leaves and tree bark) have widely been examined (Aissani et al., 2012; Custódio et al., 2011a, 2011b; Dhaouadi et al., 2014; Durazzo et al., 2014; Hajaji et al., 2010, 2011; Rached et al., 2016; Roseiro, Duarte et al., 2013; Şahin, Topuz, Pischetsrieder, & Özdemir, 2009; Torun et al., 2013; Uysal et al., 2016). The DPPH and ABTS are the major methods used for such purposes. In most cases, carob-pulp extracts exhibited relatively high DPPH free radical scavenging ability. However, it was reported that a higher concentration of polyphenols in carob extracts is required for the free radical scavenging in comparison to those of some individually examined polyphenols, such as (+)-catechin and (−)-epicatechin (Kumazawa et al., 2002). In this study, the radical scavenging capacity of the extracts at 5 μg/mL was 13%, and it was not as strong as those of other polyphenols. Nevertheless, the capacity of extracts at 25 μg/mL was stronger than those of (+)-catechin and (−)-epicatechin. It was also demonstrated, by the use of rabbit erythrocyte membrane ghost system, that carob extracts inhibited about 25% and 50% of peroxidation at concentrations of 100 and 500 μg/mL, respectively. The β-carotene-linoleic acid system was also used in this study for the determination of the antioxidant capacity. 10 μg/mL of carob extract demonstrated an equivalent capacity to those of (−)-epicatechingallate and (−)-epigallocatechingallate and quercetin.

Custódio et al. (2011a) noticed that carob pulp extracts from hermaphrodite trees exhibited higher antioxidant capacity and cytotoxic effects than those from female cultivars, which were correlated with their higher phenolic content. Among female trees, Aida cultivar had the highest radical scavenging activity, Mulata had the highest capacity to inhibit lipid oxidation and Gasparinha demonstrated the strongest cytotoxic activity on human cervical cancer cells (HeLa). Briefly, the exposure to the extract from female Mulata for 24 h resulted in HeLa cell retraction and rounding. It was, therefore, concluded that the gender and cultivar influence polyphenolic content, and antioxidant and cytotoxic activities. In a similar study performed by the same research group (Custódio et al., 2011c), the antioxidant capacity of carob germ flour extract was evaluated. The methanol extracts, which were rich in phenolic compounds, had considerable antioxidant capacity, and reduced viability of HeLa cancer cells. The activities were likewise connected with the phenolic content and were affected by the gender and cultivar. Variation on the phenolic content and in turn, on the antioxidant capacity, was observed when different extraction methods were used.

Recently, Uysal et al. (2016) compared the antioxidant capacity of nine fruit tree leaves by using methanol and water as the extraction solvents. Briefly, a higher antioxidant and enzyme inhibitory activity was observed in pomegranate and carob extract in comparison to other leaves, while an alteration on the biological abilities of the extracts was observed due to the different extraction solvent (water and methanol). It was concluded that the leaves demonstrate higher antioxidant capacity than the fruit. This is in accordance with the results obtained from similar studies, and it is correlated with the high polyphenolic content found in carob leaves (Aissani et al., 2012). The idea that carobs, and particularly carob leaves, could be a promising source of natural antioxidants, was enhanced by Hussein, Shedeed, Abdel-Kalek, & Shams El-Din (2011). They demonstrated strong DPPH radical scavenging activity of tea infusions that contained carob and other leaves. The relatively high antioxidant capacity of carob leaves was also exhibited in a report published in 2010 (Hajaji et al., 2010). The carob-leaf extract showed significant activities in all antioxidant assays, in comparison to the reference antioxidant butylated hydroxytoluene and ascorbic acid in a dose-depended manner. Among several extraction solvents (e.g. dichloromethane, diethylether), ethyl acetate was the most efficient solvent that provided the highest antioxidant capacity. In another similar study, ethyl acetate carob leaf extract demonstrated the highest free radical scavenging activity in comparison to hexane, dichloromethane and water (Hsouna et al., 2011). The *in vivo* results showed that oral administration (in rats) of CCl_4 enhanced levels of hepatic and renal markers in the serum of the experimental animals, which resulted in increased levels of the lipid peroxidation. The pretreatment of experimental rats with ethyl acetate extracts for 8 days, prevented CCl_4 induced disorders in the levels of hepatic and kidney markers, suggesting a marked hepatoprotective and nephroprotective effect of the extract.

As mentioned earlier, the extraction technique, not only affects the polyphenol composition, but the biological activities ashe well. For example, when SFE, ultrasound assisted and conventional extractions were compared, the former proved to be a more efficient technique (Roseiro, Duarte et al., 2013). Briefly, a much higher antiproliferative effect in tumour cells (HeLa, MCF-7 breast cancer and rat N1E-115 neuroblastoma cells) was observed, indicating their greater potential as natural antitumor compounds. SFE is probably the most suitable technique due to its selectivity and efficiency on polyphenol extraction, which, in turn, may lead to greater antioxidant activities (Bernardo-Gil et al., 2011; Roseiro, Duarte et al., 2013). The polyphenolic content is directly related to the antioxidant activities and reducing power. In a lot of studies, the high antioxidant capacity was explained by the observed high polyphenolic content. Roasting, a process that leads to the degradation of polyphenolic compounds, demonstrated an increase in antioxidant capacity. This is probably attributed to the Maillard Reaction Products (MRPs) that are formed during roasting. The formed MRPs have phenolic-type structures. Therefore, despite the fact that roasting degrades polyphenolic compounds, the antioxidant capacity is relatively high due to polyphenols and MRPs. Nevertheless, prolonged or high temperature roasting results in a decreased antioxidant capacity due to further degradation of MRPs and phenolics into products without reducing properties (Şahin et al., 2009; Torun et al., 2013). The biological activity of carob syrup is affected, along with its chemical characteristics, by the sucrose supplementation during the syrup processing (Dhaouadi et al., 2014). The radical scavenging assays demonstrated that the sugar supplemented syrup exhibited lower

antiradical potential than the non-supplemented syrup. Similarly, the cytotoxic effect was stronger in tumorigenic SH-SY5Y cells treated with non-supplemented syrup than in those treated with sugar supplemented syrup.

Moreover, Rtibi et al. (2015a) investigated the capacity of carob to inhibit the phosphorylation of p47phox-Ser-328. The phosphorylation was changed by the carob extracts at various doses, which induceδ a modulation of NADPH-oxidase activity and reduced the superoxide anion overproduction. In addition, it was reported that the carob aqueous extract protects the cells from disturbances provoked by lipid peroxidation caused by dextran sulphate sodium and ethanol

4.3.2. Health impact

Several studies have, over the years, demonstrated the health impact of carob extracts and carob products (Rtibi, Selmi, Grami, Amri et al., 2017). The effect of feeding pigs with carob pulp on meat quality has recently been investigated (Inserra et al., 2015). Feeding carob-containing diets reduced the concentration of saturated fatty acids, while the concentration of monounsaturated and n-3 polyunsaturated fatty acids increased. No effect on meat lipid oxidation and colour stability was found over aerobic refrigerated storage. The meat underwent slow oxidative deterioration over 9-day storage, and therefore, a possible sufficient antioxidant effect of phenolic compounds present in carob pulp against meat-oxidative deterioration was actually not observed. An interesting study on the effects of condensed tannins in carob pods on feed intake, digestive and metabolic responses of kids was carried out (Silanikove et al., 2006). The nutritional experiment highlighted that, when mammals consumed carob-pods rich diets, the following aspects were observed: tannins increased the variability in feed intake between days, lipid binding capacity of non-extractable condensed tannins in the digestive tract is related to hypocholesterolemic effect of the carob pods, and supplementing high levels of carob pods to animals with normal blood cholesterol level may induce hypocholesterolemia. The consumption of carob fiber (rich in condensed tannins), decreases postprandial responses of acetylated ghrelin (an orexigenic hormone that may affect substrate utilization in humans), triglycerides, and non-esterified fatty acids and alters respiratory quotient (a number used in calculations of basal metabolic rate), suggesting a change toward increased fatty-acid oxidation. Therefore, it is suggested that carob fiber could have beneficial effect on body weight as well.

It can be concluded that the presence of polyphenols in carobs have valuable effect on human health. It is suggested that they can prevent or protect gastric mucosa from acute gastric mucosal injury and promote the healing of chronic gastric ulcers thanks to their antioxidant capacity (Hamaishi, Kojima, & Ito, 2006; Rtibi et al., 2015b). Due to the presence of flavonoids, gallotannins and other associated polyphenols, carobs are proposed to be good sources of antidiabetic and antioxidant agents (Gupta, Singh, & Yadav, 2015; Hasan & Mohieldein, 2016). It has recently been established that the immature carob bean prevents intestinal glucose absorption by the inhibition by electrogenic sodium-depended glucose transport in mice by using a technique of Ussing chamber, which participates in the hypoglycaemic effect (Rtibi, Selmi, Grami, Saidani et al., 2017). Immature carob bean demonstrated a significant reduction in the blood glucose and biochemical profiles in the diabetic rats.

5. Conclusions and future perspectives

The carob tree parts (carob pulp, seeds germ, gum, leaves and tree barks) are significant sources of polyphenolic compounds. Their use as supplements in food or pharmaceutical products is of great importance due to the beneficial antioxidant properties of polyphenolic compounds. The main polyphenolic compounds that are found in carob parts are gallic acid, (+)-catechin, (−)-epicatechin, (−)-epicatechingallate, (−)-epigallocatechin, (−)-epigallocatechingallate, myricetin, quercetin and their derivatives, and tannin compounds. Different composition was observed in the different carob parts, with carob pulp and leaves containing the highest amount of phenolic compounds. However, the most important remark that is obtained by this review article is that there are no standardized extraction and analysis methods and this, sometimes provides misleading and not comparable results. In particular, the polyphenolic profile of an extract could vary, not only due to the gender, cultivar, and geographical factors but also to the process that is followed during the extraction.

Extraction solvent and temperature are some of the most important factors. Methanol and ethyl acetate demonstrated to be the most efficient solvents, while boiled water may lead to degradation of some polyphenolic compounds. This is not important if TPC is determined, since all the polyphenolic compounds are finally degraded to gallic acid. Though, if the characterization of the polyphenolic profile of a specific carob part is required, the polyphenol degradation should be excluded. SFE and ultrasound-assisted extraction do not use extreme conditions, and they demonstrate more representative results. Therefore, a validated standardised extraction method of polyphenols from carobs should be established. Apart from this, roasting of carob flour also affects the polyphenolic profile and TPC due to degradation.

The quantitation of polyphenols is also a challenging task. LC-MS/MS is probably the most suitable technique because low detection and quantitation limits are provided, and at the same time, useful information on the structures of more complicated compounds can be obtained. Moreover, NMR, along with MS, are remarkable tools for the structure elucidation of polyphenols. Although gallic acid is referred as the most abundant compound, this observation is not precisely true because gallic acid may be formed during the extraction processes. This is the reason why the concentration highly fluctuates, and sometimes, significant differences on the concentration levels are observed between different studies, even if the examined carob part is obtained from the same carob tree cultivar.

Nevertheless, carobs demonstrate significant antioxidant and cytotoxic activities. They are proposed as potential food or pharmaceutical supplements for diseases correlated with cancer, diabetes, gastric ulcer, gastric mucosal and chronic gastric ulcers. Their potential use for such purposes, increases the need for further development of optimum methods for extraction, analysis and isolation of polyphenols from carobs.

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References

Abascal, K., Ganora, L., & Yarnell, E. (2005). The effect of freeze-drying and its implications for botanical medicine: A review. *Phytotherapy Research, 19*(8), 655–660.

Aissani, N., Coroneo, V., Fattouch, S., & Caboni, P. (2012). Inhibitory effect of Carob (Ceratonia siliqua) leaves methanolic extract on listeria monocytogenes. *Journal of Agricultural and Food Chemistry, 60*(40), 9954–9958.

Almanasrah, M., Roseiro, L. B., Bogel-Lukasik, R., Carvalheiro, F., Brazinha, C., Crespo, J., ... Duarte, L. C. (2015). Selective recovery of phenolic compounds and carbohydrates from carob kibbles using water-based extraction. *Industrial Crops and Products, 70*, 443–450.

Alothman, M., Bhat, R., & Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry, 115*(3), 785–788.

Avallone, R., Plessi, M., Baraldi, M., & Monzani, A. (1997). Determination of chemical composition of carob (Ceratonia siliqua): protein, fat, carbohydrates, and tannins. *Journal of Food Composition and Analysis, 10*(2), 166–172.

Ayaz, F. A., Torun, H., Ayaz, S., Correia, P. J., Alaiz, M., Sanz, C., ... Strnad, M. (2007). Determination of chemical composition of anatolian carob pod (Ceratonia Siliqual.): sugars, amino and organic acids, minerals and phenolic compounds. *Journal of Food Quality, 30*(6), 1040–1055.

Aydın, S., & Özdemir, Y. (2017). Development and characterization of carob flour based functional spread for increasing use as nutritious snack for children. *Journal of Food Quality, 2017*, 1–7.

Barak, S., & Mudgil, D. (2014). Locust bean gum: Processing, properties and food applications—A review. *International Journal of Biological Macromolecules, 66*, 74–80.
Battle, I., & Tous, J. (1997). *Carob tree: Ceratonia siliqua L. – Promoting the conservation and use of underutilized and neglected crops. 17: Institute of Plant Genetics and Crop Plant Research*. Rome: Gatersleben/International Plant Genetic Resources Institute.
Bernardo-Gil, M. G., Roque, R., Roseiro, L. B., Duarte, L. C., Gírio, F., & Esteves, P. (2011). Supercritical extraction of carob kibbles (Ceratonia siliqua L.). *Journal of Supercritical Fluids, 59*, 36–42.
Biner, B., Gubbuk, H., Karhan, M., Aksu, M., & Pekmezci, M. (2007). Sugar profiles of the pods of cultivated and wild types of carob bean (Ceratonia siliqua L.) in Turkey. *Food Chemistry, 100*(4), 1453–1455.
Briones-Labarca, V., Plaza-Morales, M., Giovagnoli-Vicuña, C., & Jamett, F. (2015). High hydrostatic pressure and ultrasound extractions of antioxidant compounds, sulforaphane and fatty acids from Chilean papaya (Vasconcellea pubescens) seeds: Effects of extraction conditions and methods. *LWT – Food Science and Technology, 60*(1), 525–534.
Calixto, F. S., & Cañellas, J. (1982). Components of nutritional interest in carob pods (Ceratonia siliqua). *Journal of the Science of Food and Agriculture, 33*(12), 1319–1323.
Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Lercker, G., & Fernández-Gutiérrez, A. (2007). Evaluation of the influence of thermal oxidation on the phenolic composition and on the antioxidant activity of extra-virgin olive oils. *Journal of Agricultural and Food Chemistry, 55*(12), 4771–4780.
Coit, J. E. (1951). Carob or St. John's Bread. *Economic Botany, 5*(1), 82–96.
Corsi, L., Avallone, R., Cosenza, F., Farina, F., Baraldi, C., & Baraldi, M. (2002). Antiproliferative effects of Ceratonia siliqua L. on mouse hepatocellular carcinoma cell line. *Fitoterapia, 73*(7), 674–684.
Custódio, L., Escapa, A. L., Fernandes, E., Fajardo, A., Aligué, R., Alberício, F., ... Romano, A. (2011a). Antioxidant and cytotoxic activities of carob tree fruit pulps are strongly influenced by gender and cultivar. *Journal of Agricultural and Food Chemistry, 59*(13), 7005–7012.
Custódio, L., Escapa, A. L., Fernandes, E., Fajardo, A., Aligué, R., Alberício, F., ... Romano, A. (2011b). In vitro cytotoxic effects and apoptosis induction by a methanol leaf extract of carob tree (Ceratonia siliqua L.). *Journal of Medicinal Plant Research, 5*(10), 1987–1996.
Custódio, L., Escapa, A. L., Fernandes, E., Fajardo, A., Aligué, R., Alberício, F., ... Romano, A. (2011c). Phytochemical profile, antioxidant and cytotoxic activities of the carob tree (Ceratonia siliqua L.) germ flour extracts. *Plant Foods for Human Nutrition, 66*(1), 78–84.
Custódio, L., Escapa, A. L., Patarra, J., Aligué, R., Alberício, F., Nuno Rosa, N., ... Romano, A. (2013). Sapwood of carob tree (Ceratonia siliqua L.) as a potential source of bioactive compounds. *Records of Natural Products, 7*(3), 225–229.
Custódio, L., Fernandes, E., & Romano, A. (2007). Quantification of polyphenols in carob tree (Ceratonia siliqua L.) fruits and leaves in Portuguese cultivars. In II International Symposium on Human Health Effects of Fruits and Vegetables: FAVHEALTH 2007 841, (pp. 503–506).
Daglia, M., Papetti, A., Gregotti, C., Bertè, F., & Gazzani, G. (2000). In vitro antioxidant and ex vivo protective activities of green and roasted coffee. *Journal of Agricultural and Food Chemistry, 48*(5), 1449–1454.
Dakia, P. A., Wathelet, B., & Paquot, M. (2007). Isolation and chemical evaluation of carob (Ceratonia siliqua L.) seed germ. *Food Chemistry, 102*(4), 1368–1374.
Davies, W. N. L. (1970). The carob tree and its importance in the agricultural economy of Cyprus. *Economic Botany, 24*(4), 460–470.
Dhaouadi, K., Belkhir, M., Akinocho, I., Raboudi, F., Pamies, D., Barrajón, E., ... Fattouch, S. (2014). Sucrose supplementation during traditional carob syrup processing affected its chemical characteristics and biological activities. *LWT – Food Science and Technology, 57*(1), 1–8.
Diaz, C. S. (1997). Syrup of natural carob sugars and a process for its production. In: Google Patents.
Durazzo, A., Turfani, V., Narducci, V., Azzini, E., Maiani, G., & Carcea, M. (2014). Nutritional characterisation and bioactive components of commercial carobs flours. *Food Chemistry, 153*, 109–113.
El Bouzdoudi, B., El Ansari, Z. N., Mangalagiu, I., Mantu, D., Badoc, A., & Lamarti, A. (2016). Determination of polyphenols content in carob pulp from wild and domesticated moroccan trees. *Am. J. Plant. Sci. 07*(14), 1937–1951.
FAO. FAOSTAT database. (2017). http://www.fao.org/faostat/en/#data/QC Accessed 20.07.2017.
Fraga, C. G., Galleano, M., Verstraeten, S. V., & Oteiza, P. I. (2010). Basic biochemical mechanisms behind the health benefits of polyphenols. *Molecular Aspects of Medicine, 31*(6), 435–445.
Garcia-Castello, E. M., Rodriguez-Lopez, A. D., Mayor, L., Ballesteros, R., Conidi, C., & Cassano, A. (2015). Optimization of conventional and ultrasound assisted extraction of flavonoids from grapefruit (Citrus paradisi L.) solid wastes. *LWT – Food Science and Technology, 64*(2), 1114–1122.
Garrido, I., Monagas, M., Gómez-Cordovés, C., & Bartolomé, B. (2008). Polyphenols and antioxidant properties of almond skins: Influence of industrial processing. *Journal of Food Science, 73*(2), C106–C115.
Gharnit, N., & Ennabili, A. (2016). Categories of carob tree (Ceratonia siliqua L.) from morocco. *International Journal of Fruit Science, 16*(3), 259–274.
Gómez-Caravaca, A. M., Verardo, V., Segura-Carretero, A., Fernández-Gutiérrez, A., & Caboni, M. F. (2014). Chapter 3 – phenolic compounds and saponins in plants grown under different irrigation regimes A2 – watson, ronald ross. *Polyphenols in Plants* (pp. 37–52). San Diego: Academic Press.
Gupta, A., Singh, S., & Yadav, A. (2015). Pharmacological evaluation of antidiabetic activity of Urginea indica in laboratory animals. *International Journal of Nutrition, Pharmacology, Neurological Diseases, 5*(2), 63–68.
Hajaji, H. E., Lachkar, N., Alaoui, K., Cherrah, Y., Farah, A., Ennabili, A., ... Lachkar, M. (2010). Antioxidant properties and total phenolic content of three varieties of carob tree leaves from morocco. *Records of Natural Products, 4*(4), 193–204.
Hajaji, H. E., Lachkar, N., Alaoui, K., Cherrah, Y., Farah, A., Ennabili, A., ... Lachkar, M. (2011). Antioxidant activity, phytochemical screening, and total phenolic content of extracts from three genders of carob tree barks growing in Morocco. *Arabian Journal of Chemistry, 4*(3), 321–324.
Hamaishi, K., Kojima, R., & Ito, M. (2006). Anti-ulcer effect of tea catechin in rats. *Biological and Pharmaceutical Bulletin, 29*(11), 2206–2213.
Hasan, M., & Mohieldein, A. (2016). In vivo evaluation of anti diabetic, hypolipidemic, antioxidative activities of Saudi date seed extract on streptozotocin induced diabetic rats. *Journal of clinical and diagnostic research: JCDR, 10*(3), FF06.
Higdon, J. V., & Frei, B. (2003). Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Critical Reviews in Food Science and Nutrition, 43*(1), 89–143.
Hsouna, A. B., Saoudi, M., Trigui, M., Jamoussi, K., Boudawara, T., Jaoua, S., & Feki, A. E. (2011). Characterization of bioactive compounds and ameliorative effects of Ceratonia siliqua leaf extract against CCl4 induced hepatic oxidative damage and renal failure in rats. *Food and Chemical Toxicology, 49*(12), 3183–3191.
Huang, W., Xue, A., Niu, H., Jia, Z., & Wang, J. (2009). Optimised ultrasonic-assisted extraction of flavonoids from Folium eucommiae and evaluation of antioxidant activity in multi-test systems in vitro. *Food Chemistry, 114*(3), 1147–1154.
Hussein, A., Shedeed, N., Abdel-Kalek, H., & Shams El-Din, M. (2011). Antioxidative, antibacterial and antifungal activities of tea infusions from berry leaves, carob and doum. *Polish Journal of Food and Nutrition Sciences, 61*(3), 201–209.
Ignat, I., Volf, I., & Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry, 126*(4), 1821–1835.
Iipumbu, L. (2008). *Compositional analysis of locally cultivated carob (Ceratonia siliqua) cultivars and development of nutritional food products for a range of market sectors.* Stellenbosch: Stellenbosch University.
Inserra, L., Luciano, G., Bella, M., Scerra, M., Cilione, C., Basile, P., ... Priolo, A. (2015). Effect of including carob pulp in the diet of fattening pigs on the fatty acid composition and oxidative stability of pork. *Meat Science, 100*, 256–261.
Jiménez-Martínez, M. C., Montalvo-González, E., Sáyago-Ayerdi, S. G., Mercado-Mercado, G., Ramírez-de León, J. A., Paz-Gamboa, E., & Vivar-Vera, M. A. (2017). Effect of high hydrostatic pressure on the polyphenols and antioxidant activity of plantain pulp (Musa paradisiacaAAB). *Journal of the Science of Food and Agriculture, 97*(8), 2508–2515.
Kaity, S., Isaac, J., & Ghosh, A. (2013). Interpenetrating polymer network of locust bean gum-poly (vinyl alcohol) for controlled release drug delivery. *Carbohydrate Polymers, 94*(1), 456–467.
Kalia, K., Sharma, K., Singh, H. P., & Singh, B. (2008). Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of Potentilla atrosanguinea Lodd. and quantification of its phenolic constituents by RP-HPLC. *Journal of Agricultural and Food Chemistry, 56*(21), 10129–10134.
Khan, M. K., Abert-Vian, M., Fabiano-Tixier, A.-S., Dangles, O., & Chemat, F. (2010). Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (Citrus sinensis L.) peel. *Food Chemistry, 119*(2), 851–858.
Kim, H. S., Quon, M. J., & Kim, J. A. (2014). New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. *Redox Biol, 2*, 187–195.
Kim, S.-Y., Jeong, S.-M., Park, W.-P., Nam, K. C., Ahn, D. U., & Lee, S.-C. (2006). Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. *Food Chemistry, 97*(3), 472–479.
Kontogianni, V. G. (2014). Chapter 8 – novel techniques towards the identification of different classes of Polyphenols A2 – Watson, Ronald Ross. *Polyphenols in Plants* (pp. 159–185). San Diego: Academic Press.
Kotrotsios, N., Christaki, E., Bonos, E., & Florou-Paneri, P. (2012). Dietary carob pods on growth performance and meat quality of fattening pigs. *Asian-Australasian Journal of Animal Sciences, 25*(6), 880–885.
Kumazawa, S., Taniguchi, M., Suzuki, Y., Shimura, M., Kwon, M.-S., & Nakayama, T. (2002). Antioxidant activity of polyphenols in carob pods. *Journal of Agricultural and Food Chemistry, 50*(2), 373–377.
Lapornik, B., Prošek, M., & Golc Wondra, A. (2005). Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering, 71*(2), 214–222.
Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition, 79*(5), 727–747.
Marakis, S. (1996). Carob bean in food and feed: Current status and future potentials: A critical appraisal. *Journal of Food Science and Technology, 33*(5), 365–383.
Martí, R., Valcárcel, M., Herrero-Martínez, J. M., Cebolla-Cornejo, J., & Roselló, S. (2017). Simultaneous determination of main phenolic acids and flavonoids in tomato by micellar electrokinetic capillary electrophoresis. *Food Chemistry, 221*, 439–446.
Martin-Diana, A. B., Izquierdo, N., Albertos, I., Sanchez, M. S., Herrero, A., Sanz, M. A., & Rico, D. (2017). Valorization of Carob's germ and seed peel as natural antioxidant ingredients in gluten-free crackers. *Journal of Food Processing and Preservation, 41*(2), e12770.
Mualla, B. (2004). Identification of the main phenolic compounds in wood of Ceratonia siliqua by GC-MS. *Phytochemical Analysis, 15*(6), 385–388.
Naghmouchi, S., Khouja, M. L., Romero, A., Tous, J., & Boussaid, M. (2009). Tunisian carob (Ceratonia siliqua L.) populations: Morphological variability of pods and kernel. *Scientia Horticulturae, 121*(2), 125–130.
Nicolaou, I. N., & Kapnissi-Christodoulou, C. P. (2010). Analysis of polyphenols using capillary zone electrophoresis – Determination of the most effective wine sample pretreatment method. *Electrophoresis, 31*(23–24), 3895–3902.

Ortega, N., Macià, A., Romero, M.-P., Trullols, E., Morello, J.-R., Anglès, N., & Motilva, M.-J. (2009). Rapid determination of phenolic compounds and alkaloids of carob flour by improved liquid chromatography tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 57*(16), 7239–7244.

Owen, R. W., Haubner, R., Hull, W. E., Erben, G., Spiegelhalder, B., Bartsch, H., & Haber, B. (2003). Isolation and structure elucidation of the major individual polyphenols in carob fibre. *Food and Chemical Toxicology, 41*(12), 1727–1738.

Owen, R. W., Haubner, R., Mier, W., Giacosa, A., Hull, W. E., Spiegelhalder, B., & Bartsch, H. (2003). Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food and Chemical Toxicology, 41*(5), 703–717.

Papagiannopoulos, M., Wollseifen, H. R., Mellenthin, A., Haber, B., & Galensa, R. (2004). Identification and quantification of polyphenols in Carob fruits (Ceratonia siliqua L.) and derived products by HPLC-UV-ESI/MSn. *Journal of Agricultural and Food Chemistry, 52*(12), 3784–3791.

Rached, I., Barros, L., Fernandes, I. P., Santos-Buelga, C., Rodrigues, A. E., Ferchichi, A., ... Ferreira, I. C. F. R. (2016). Ceratonia siliqua L. hydroethanolic extract obtained by ultrasonication: Antioxidant activity, phenolic compounds profile and effects in yogurts functionalized with their free and microencapsulated forms. *Food and Function, 7*(3), 1319–1328.

Rakib, E. M., Chicha, H., Abouricha, S., Alaoui, M., Bouli, A. A., Hansali, M., & Owen, R. W. (2010). Determination of phenolic composition of carob pods grown in different regions of Morocco. *Journal of Natural Products,* 3.

RamÓN-Laca, L., & Mabberley, D. J. (2004). The ecological status of the carob-tree (Ceratonia siliqua, Leguminosae) in the Mediterranean. *Botanical Journal of the Linnean Society, 144*(4), 431–436.

Rizzo, V., Tomaselli, F., Gentile, A., La Malfa, S., & Maccarone, E. (2004). Rheological properties and sugar composition of locust bean gum from different Carob varieties (Ceratonia siliqua L.). *Journal of Agricultural and Food Chemistry, 52*(26), 7925–7930.

Roseiro, L. B., Duarte, L. C., Oliveira, D. L., Roque, R., Bernardo-Gil, M. G., Martins, A. I., ... Rauter, A. P. (2013). Supercritical, ultrasound and conventional extracts from carob (Ceratonia siliqua L.) biomass: Effect on the phenolic profile and anti-proliferative activity. *Industrial Crops and Products, 47*, 132–138.

Roseiro, L. B., Tavares, C. S., Roseiro, J. C., & Rauter, A. P. (2013). Antioxidants from aqueous decoction of carob pods biomass (Ceretonia siliqua L.): Optimisation using response surface methodology and phenolic profile by capillary electrophoresis. *Industrial Crops and Products, 44*, 119–126.

Rtibi, K., Jabri, M. A., Selmi, S., Souli, A., Sebai, H., El-Benna, J., ... Marzouki, L. (2015a). Carob pods (Ceratonia siliqua L.) inhibit human neutrophils myeloperoxidase and in vitro ROS-scavenging activity. *RSC Advances, 5*(102), 84207–84215.

Rtibi, K., Jabri, M. A., Selmi, S., Souli, A., Sebai, H., El-Benna, J., ... Marzouki, L. (2015b). Gastroprotective effect of carob (Ceratonia siliqua L.) against ethanol-induced oxidative stress in rat. *BMC Complementary and Alternative Medicine, 15*(1), 292.

Rtibi, K., Selmi, S., Grami, D., Amri, M., Eto, B., El-Benna, J., ... Marzouki, L. (2017). Chemical constituents and pharmacological actions of carob pods and leaves (Ceratonia siliqua L.) on the gastrointestinal tract: A review. *Biomedicine and Pharmacotherapy, 93*, 522–528.

Rtibi, K., Selmi, S., Grami, D., Saidani, K., Sebai, H., Amri, M., ... Marzouki, L. (2017). Ceratonia siliqua L. (immature carob bean) inhibits intestinal glucose absorption, improves glucose tolerance and protects against alloxan-induced diabetes in rat. *Journal of the Science of Food and Agriculture, 97*(8), 2664–2670.

Şahin, H., Topuz, A., Pischetsrieder, M., & Özdemir, F. (2009). Effect of roasting process on phenolic, antioxidant and browning properties of carob powder. *European Food Research and Technology, 230*(1), 155.

Sakakibara, H., Honda, Y., Nakagawa, S., Ashida, H., & Kanazawa, K. (2003). Simultaneous determination of all polyphenols in vegetables, fruits, and teas. *Journal of Agricultural and Food Chemistry, 51*(3), 571–581.

Sęczyk, Ł., Świeca, M., & Gawlik-Dziki, U. (2016). Effect of carob (Ceratonia siliqua L.) flour on the antioxidant potential, nutritional quality, and sensory characteristics of fortified durum wheat pasta. *Food Chemistry, 194*, 637–642.

Sigge, G. O., Lipumbu, L., & Britz, T. J. (2011). Proximate composition of carob cultivars growing in South Africa. *South African Journal of Plant and Soil, 28*(1), 17–22.

Silanikove, N., Landau, S., Or, D., Kababya, D., Bruckental, I., & Nitsan, Z. (2006). Analytical approach and effects of condensed tannins in carob pods (Ceratonia siliqua) on feed intake, digestive and metabolic responses of kids. *Livestock Science, 99*(1), 29–38.

Smith, B. M., Bean, S. R., Schober, T. J., Tilley, M., Herald, T. J., & Aramouni, F. (2010). Composition and molecular weight distribution of carob germ protein fractions. *Journal of Agricultural and Food Chemistry, 58*(13), 7794–7800.

Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering, 81*(1), 200–208.

Srour, N., Daroub, H., Toufeili, I., & Olabi, A. (2016). Developing a carob-based milk beverage using different varieties of carob pods and two roasting treatments and assessing their effect on quality characteristics. *Journal of the Science of Food and Agriculture, 96*(9), 3047–3057.

Torun, H., Ayaz, F. A., Colak, N., Grúz, J., & Strnad, M. (2013). Phenolic acid content and free radical-scavenging activity of two differently processed carob tree (Ceratonia siliqua L.) Pod. *Food and Nutrition Sciences, 04*(05), 547–553.

Tous, J., Romero, A., Hermoso, J. F., Ninot, A., Plana, J., & Batlle, I. (2009). Agronomic and commercial performance of four spanish carob cultivars. *HortTechnology, 19*(2), 465–470.

Tsatsaragkou, K., Gounaropoulos, G., & Mandala, I. (2014). Development of gluten free bread containing carob flour and resistant starch. *LWT – Food Science and Technology, 58*(1), 124–129.

Tsatsaragkou, K., Yiannopoulos, S., Kontogiorgi, A., Poulli, E., Krokida, M., & Mandala, I. (2012). Mathematical approach of structural and textural properties of gluten free bread enriched with carob flour. *Journal of Cereal Science, 56*(3), 603–609.

Tsatsaragkou, K., Yiannopoulos, S., Kontogiorgi, A., Poulli, E., Krokida, M., & Mandala, I. (2014). Effect of carob flour addition on the rheological properties of gluten-free breads. *Food and Bioprocess Technology, 7*(3), 868–876.

Uysal, S., Zengin, G., Aktumsek, A., & Karatas, S. (2016). Chemical and biological approaches on nine fruit tree leaves collected from the Mediterranean region of Turkey. *Journal of Functional Foods, 22*, 518–532.

Vaya, J., & Mahmood, S. (2006). Flavonoid content in leaf extracts of the fig (Ficus carica L.), carob (Ceratonia siliqua L.) and pistachio (Pistacia lentiscus L.). *BioFactors, 28*(3–4), 169–175.

Vekiari, S. A., Ouzounidou, G., Ozturk, M., & Görk, G. (2011). Variation of quality characteristics in Greek and Turkish carob pods during fruit development. *Procedia – Social and Behavioral Sciences, 19*, 750–755.

Xu, B., & Chang, S. K. C. (2009). Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (Phaseolus vulgaris L.) as affected by thermal processing. *Journal of Agricultural and Food Chemistry, 57*(11), 4754–4764.

Yao, L. H., Jiang, Y. M., Shi, J., Tomas-Barberan, F. A., Datta, N., Singanusong, R., & Chen, S. S. (2004). Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition, 59*(3), 113–122.

Yousif, A. K., & Alghzawi, H. M. (2000). Processing and characterization of carob powder. *Food Chemistry, 69*(3), 283–287.

Youssef, M. K. E., Ali, H. M., & El-Manfaloty, M. M. (2013). Nutritional assessment of wheat biscuits and fortified wheat biscuits with carob pod powder (Ceratonia siliqua L.). *Food and Public Health, 3*(6), 336–340.

Youssef, M. K. E., El-Manfaloty, M. M., & Ali, H. M. (2013). Assessment of proximate chemical composition, nutritional status, fatty acid composition and phenolic compounds of carob (Ceratonia siliqua L.). *Food and Public Health, 3*(6), 304–308.