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Review article

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Sugars and phenols in carob tree fruits from different producing countries: A short review

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ABSTRACT

In the last two decades, important advances have been made in the chemical analysis of the fruit of carob tree. After harvesting, the fruits (also known as dry pods) are ground. The seeds can then be separated from the pulp, which represents 80–90 % of a pod's weight. The health benefits of carob pulp derivatives are well-recognized, and carob pulp-based food products are becoming increasingly available to consumers. The major carob-producing countries are in southern Europe and northern Africa, including the Mediterranean islands, and carob pulp products are normally prepared and consumed regionally. In this review, we compare the sugar and phenol profiles of carob pods harvested from different countries in the Mediterranean basin while accounting for the different cultivars and soil conditions in each sample area. We conclude that pod nutritional composition varies widely among countries, making it necessary for future, multi-year studies to more closely evaluate how climate and soil properties affect the phenol and sugar contents of fruits from the same trees or cultivars.

1. Introduction

The carob tree (*Ceratonia siliqua* L.) is an evergreen tree species that grows in xeric Mediterranean environments [1,2]. The fruit of the carob tree is an indehiscent, dark brown pod that may be elongated, straight, or curved [3]. The length, thickness, colour, and shape of the pods can vary with local environmental conditions, tree genetics, and agricultural practices. In the northern hemisphere, fruit maturation begins in late spring and lasts until the end of summer. The fruits are then harvested in August and September, and the harvested pods are usually transported to small industrial units that separate the seeds from the pulp. Previous authors have extensively reviewed carob fruit processing methods and nutritional properties [4–8]; based on these reports, all components of the carob fruit (including the pulp and seeds) have great potential in the food, nutraceutical, and pharmaceutical industries.

Although the seeds of the carob fruit represent only 10-25 % of the total weight of the pod, their derivatives are the most valuable products of the fruit. For example, locust bean gum is obtained from the seed endosperm. The purity of locust bean gum can vary depending on the efficiency with which the endosperm is separated from other components of the seed (i.e., the embryo and the coat) as well as the original viscosity of the endosperm [3]. Locust bean gum is now considered a potential ingredient for functional foods (e. g. [9]) and has a variety of non-food applications [10].

In addition to the well-recognized economic importance of carob seed derivatives, the pulp of the carob pod has also become highly valued in recent years. The chemical composition of carob pulp is the result of several factors during tree growth and fruit harvesting

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including the tree variety, production practices, soil quality, climate, and fruit ripening stage at the time of harvest. These factors not only create distinct chemical profiles in pods from different origins but can also create variation among pods grown in the same area [11,12]. However, these distinct chemical traits are lost when the pulp is industrially processed, as storage companies and processing facilities do not differentiate the origin or variety of the pods.

Despite the potential for significant variation in the quality and composition of carob fruit pulp, there is little information on how pulp quality varies with agricultural practices or among production locations. To address this knowledge gap, the objective of this short review is to provide basic data on the concentrations of total sugars and phenols in the pulp of carob fruits from different producing countries and, where possible, from different cultivars. This preliminary survey will help producers and the broader carob industry select and evaluate the distinct raw materials that are normally available on the international food market.

2. Producing countries and genetic material

The countries that produce the most carob fruit and derivatives are in southern Europe, northern Africa, and the Middle East, though production varies widely among these regions: countries in the European Union account for more than 70 % of the 400,000 tonnes of carob pods produced worldwide each year. The largest producers and exporters of carob in the EU are Spain, Italy, Portugal, Morocco, Greece, Cyprus, and Turkey. Other countries with significant carob production include Malta, Algeria, Tunisia, Egypt, Israel, and Lebanon. Outside the Mediterranean basin, Australia is becoming an increasingly important producer as they intensify their economic exploitation of this crop [13].

In all producing countries, carob pods are harvested either from wild, non-grafted trees or from selected cultivars or accesses produced by grafting onto wild rootstocks [Fig. 1(A-E)]. These cultivars or accesses represent the genetic material of each producing country. Although morphological traits, production rates, and pest resistance can vary among carob trees, isoenzyme and molecular

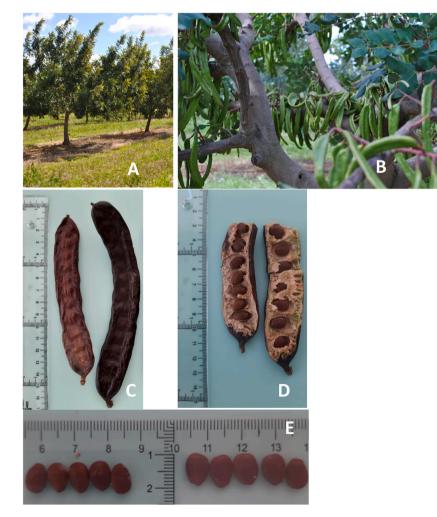


Fig. 1. (A) Germplasm collection in Tavira (Experimental Agriculture Center). (B) Unripe pods on trees. (C) Pods from a non-grafted tree (<u>left</u>) and from a 'Galhosa' cultivar (<u>right</u>). (D) and (E) show the pulp and seeds, respectively, of the same pods shown in (C).

analyses have revealed distinct polymorphisms characteristic of different regions [3,14]. Indeed, a bibliographic survey on carob tree cultivars reveals several female cultivars or accesses unique to each producing country.

- **Spain**: Battle and Tous [3] identified approximately 18 carob cultivars in Spain. Of these, 'Negra,' 'Matalafera,' 'Duraió,' and 'Rojal' are some of the most representative and economically important. Several newer cultivars are also being studied ('A-19,' 'Turis,' 'E-13 P,' 'SdC,' 'E3,' 'E4,' 'E-18,' 'E-19,' and 'E-26' [15]).
- Italy: Most carob fruit in Italy is produced in Sicily and Apulia, where the main cultivars are 'Albarcara', 'Racemosa', 'Saccarata', 'Amele di Bari', 'Sangimigniana', 'Ibla', 'Pasta', 'Tantillo', and 'Targia' [16,17].
- **Portugal:** Portuguese cultivars from the southern (Algarve) region of the country were characterized by Barracosa et al. [14] using 61 morphological descriptors. The most important of these cultivars are 'Mulata', 'Galhosa', 'Aida', 'Canela', 'Spargale' (also known as 'Mulata do Espargal'), and 'Lagoinha'. A more recent survey in the Algarve region (unpublished data) identified 22 new accessions that are now under study at the Experimental Agriculture Center of Tavira.
- Morocco: Gharnit and Ennabili [18] proposed intra-specific categories of the carob tree in Morocco, where the productive (female) varieties were identified as 'Lanta' and 'Dkar'. However, the genetic material used for biochemical, morphological, and yield assessment is normally identified according to its geographical origin [19–21].
- **Turkey:** The most important carob-producing region in Turkey is Antalya, on the south coast, where the main type cultivated is 'Sisam' [22,23].

3. Concentration of total sugars in the pulp

Carob pulp contains both reducing and non-reducing sugars. In general, the total sugar content of carob pulp can range from 45 to 58 % of the pulp dry weigh (DW) basis (for example: [6,7]) and the main sugars are sucrose, fructose, and glucose, representing 35–45 %, 6–7%, and 2–4%, respectively, of the pulp DW. Maltose, raffinose, and mannitol can be present in lower concentrations [24].

Table 1 shows the total sugar content in the pulp of carob pods obtained from different producing countries and regions. The values range from a minimum of 25 % in Portugal ('Mulata') to a maximum of 65 % in northern Algeria. Notably, many reports on the chemical composition of carob pulp do not indicate the cultivar that was sampled or sample only a small number of trees, limiting the ability to draw definitive conclusions about among-region variation in pulp sugar content.

Nevertheless, Kyratzis et al. [26] surveyed a large number of carob trees from several cultivars across nine agro-environmental zones in Cyprus in two consecutive years (N = 98 and N = 100). One cultivar in their sample, 'Tylliria,' can also be found in Greece and Israel. Overall, sugar content in their sample ranged from 41 to 49 % DW.

In Morocco, El Kahkahi and Diouri [29] analysed seven carob tree ecotypes (P1 to P7) selected from four locations at distinct altitudes: Fez, Meknes, Khemisset and Marrakech. The average total sugar content was 41.16 % DW, but the standard deviation among samples was high (Table 1) due to the distinct soil and climate of each region.

In Spain, Sánchez et al. [31] reported total sugar concentrations of 46–48 % DW in pods of cultivars grown in the semi-arid region

Table 1

Mean total sugar concentrations (g 100 g⁻¹ dry weight – DW) quantified in carob pulp from different countries. Where data was available, the mean is shown \pm standard deviation or standard error. ⁽¹⁾ N = 98 and N = 100 in 2018 and 2019, respectively; ⁽²⁾ Mean of N = 7 (ranging from 32.60 to 52.52); ⁽³⁾ Spanish origin.

Origin	Edapho-climatic conditions	Cultivar	Total sugars (g 100 g^{-1} DW)	Quantification methods	References
Algeria	Sub-humid and semi-arid climates	Not specified	65.0 ± 3.3 43.22 ± 0.53 to 50.11 ± 0.42	Colorimetry	Mahtout et al. [25] Boublenza et al. [19]
Cyprus	Several: mountains of inland zones; igneous or calcareous soils.	Several (including 'Tillyria')	$\begin{array}{l} 49.42 \pm 0.43 \\ 41.12 \pm 0.40 \ ^{(1)} \end{array}$	HPLC	Kyratzis et al. [26]
Greece	Crete; not specified	Wild Fleshy	35.7 43.3	HPLC	Vekiari et al. [27]
Italy	Sicily; not specified	Not specified	45.0	Colorimetry	Avallone et al. [28]
Morocco	Several altitudes	Seven accessions	$41.16 \pm 6.9 \ ^{(2)}$	Colorimetry	El Kahkahi and Diouri [29]
Portugal	Calcareous	Hermaphrodite ⁽³⁾ 'Mulata' 'Galhosa'	30.0 25.0 50.0	HPLC	Custódio [30]
Spain	Murcia-Alicante Mayorca	Mot specified 'Bugadera' 'Duraió' 'Matalafera' 'Rojal' 'Sayalonga' 'Orellona' 'Melera'	46 and 48 45.3 45.2 42.3 43.8 31.2 42.0 42.0	Not specified	Sánchez et al. [31] Tous and Franquet [32]
Turkey	Mediterranean region	Not specified Wild type	$\begin{array}{c} 53.1\pm9.3\\ 43.7\pm7.7\end{array}$	HPLC	Biner et al. [22]

of Murcia. More recently, Tous and Franquet [32] surveyed the fruits of 7 Spanish cultivars obtained in Mayorca and reported sugar contents ranging from 31.2 % to 45.3 % DW.

In Turkey, Biner et al. [22] found that pods from cultivated accessions have higher sugar contents (53.1 % DW) than wild, non-grafted trees (43.7 % DW). A more extensive Turkish survey by Tetik et al. [23] found that pod morphology differs significantly between cultivated and wild trees.

In Algeria, sugar concentrations ranged from 43.2 % to 50.3 % DW in samples of spontaneous or cultivated trees collected from ten distinct locations [19]. However, a separate study reported a total sugar content of 65 % DW in pods collected in the region of Tazmalt (north of Algeria [25]), though this study did not specify the number of pods harvested or the type of tree.

In Italy, Avallone et al. [28] reported the total sugar content of carob fruits sampled in several locations across Sicily, though they did not identify the cultivar(s) that were sampled.

On the Greek island of Crete, total sugar concentrations were 35.7 % and 43.3 % in wild-type and fleshy-type fruits, respectively [27].

The Portuguese cultivars 'Mulata' and 'Galhosa,' which are grown in the calcareous soils of Algarve, had average total sugar contents of 25 % and 50 %, respectively [30].

In addition to the three sugars discussed above, carob pulp also contains D-pinitol (3-*O*-methyl-D-chiro-inositol). D-pinitol has insulin-like and anti-inflammatory effects [23] and is found in several plants in the family Fabaceae, but the concentration of this bioactive compound is much higher in carob fruits than in other legumes such as soybean, chickpeas, or lentils [6]. In Turkey, Turhan [33] found that the concentration of D-pinitol in carob pods was positively correlated to the glucose concentration and consistently higher in wild trees (mean of 42.6 g kg⁻¹) than cultivated trees (mean of 37.8 g kg⁻¹). Among several Spanish cultivars, D-pinitol concentrations were highest (7.59 \pm 0.17 % DW) in the 'Roja' variety from the island of Mayorca [34].

4. Total phenols

Although sugars are the most important component of carob pulp, the pulp also contains significant concentrations of fatty acids, crude fibres, and polyphenols. Polyphenols are derivatives of flavones, isoflavones, flavanols, catechins, and phenolic acids; due to their intrinsic reducing capabilities, activation of endogenous defence systems [35], and metal-chelating potential [36], polyphenols have significant antioxidant properties. One of most important groups of phenolic compounds present in carob pulp are tannins, which can be either hydrolysable or condensed. Hydrolysable tannins form polymers of gallic or ellagic acid, whereas condensed tannins are flavonoid polymers that are particularly important in carob pulp (e.g., [6,12]). Overall, phenols play an important role in the health benefits of carob derivatives and should therefore be considered a chemical marker for the quality of carob pulp [5].

Table 2 reports the concentrations of total phenols in carob fruit samples taken from different producing countries. In all cases, the values reported represent the phenol concentration in unroasted, ripe pods. Because sample preparation and the phenol extraction method affect the total amount of phenol detected, Table 2 also reports the solvent used for sample preparation and extraction [37].

As with reports of total sugar content in carob fruit, most reports of total phenol content do not describe the edapho-climatic conditions where the trees selected for sampling were grown. Correia et al. [45] studied the Portuguese cultivar 'Mulata' grown in two soil types, calcareous soil and acid soil. 'Mulata' trees grown in acid soils had the highest total phenol content, expressed as mg of gallic acid equivalent (GAE) g^{-1} of DW pulp (33.2 mg GAE g^{-1}), whereas pods from calcareous soils had a phenol content of 28.1 mg

Table 2

Concentrations of total phenols quantified in carob pulp from different countries. Where data is available, the mean is shown \pm standard deviation or standard error. Total polyphenols are expressed as mg gallic acid equivalent (GAE) g⁻¹ of DW pulp. ⁽¹⁾N = 98; ⁽²⁾N = 7; ⁽³⁾N = 6; the cultivars were the following: 'Mulata', 'Galhosa', 'AIDA', 'Gasparinha', 'Costela-Canela', and 'Preta-de-Lagos.'

Origin	Cultivar	Edapho-climatic conditions	Total polyphenols	Extraction solvent	References
Cyprus	Several (36 ecotypes)	Multiple agri-environmental conditions (see Table 1)	$34.07 \pm 19\ ^{(1)}$	Methanol (and HCl)	Kyratzis et al. [26]
Cyprus and Greece (Crete)	Not specified	Not specified	7.2 and 7.7	57 % acetone	Ioannou et al. [5]
Greece	Not specified	Not specified	19.2 ± 0.3	Water	Kumazawa et al. [38]
Italy (Sicily)	Not specified	Not specified	19.3 ± 3.0	70 % acetone	Avalone et al. [28]
Marocco	Several accessions	Several	$4.95 \pm 0.05 \ ^{(2)}$	Acetone	El Kahkahi and Diouri [29]
	Not specified	Several	11.19	80 % acetone	El Bouzdoudi et al. [39]
Portugal	'Mulata'	Acid soil	33.2 ± 0.9	70 % acetone	Correia et al. [40]
-	'Mulata'	Calcareous soil	28.1 ± 1.9		
	Six cultivars (3)	Calcareous soil	18.0 ± 6.1	Methanol	Custódio et al. [11]
	Mixture of 'Mulata and	Not specified	20.4 ± 1.8	70 % acetone	Roseiro et al. [41]
	Galhosa'	*			
Spain	Hermaphrodites	Calcareous soil	41.3 ± 6.4	Methanol	Custódio et al. [11]
Tunisia	Sfax (?)	Calcareous soil	17.58	Water	Hadrich et al. [42]
	Not specified	Sandstone soil	13.19-23.11	80 % methanol	Richane et al. [43]
Turkey	Not specified	Not specified	13.51	80 % methanol	Ayaz et al. [44]

GAE g^{-1} . Nevertheless, these values were still higher than those reported by Custódio et al. [11], who considered a group of six female Portuguese cultivars, and those reported by Roseiro et al. [41], who used a mixture of two cultivars ('Mulata' and 'Galhosa'). However, the highest phenol content (41.3 mg GAE g^{-1}) in Portuguese carob fruits was reported from a hermaphrodite cultivar of Spanish origin that was grown in southern Portugal [11].

In carob fruit from southern parts of the Anatolian region of Turkey, the concentration of phenolic compounds was 13.51 mg GAE g^{-1} [44]. Ioannou et al. [5] reported phenol concentrations of 7.2 and 7.7 mg GAE g^{-1} in fruits from Cyrus and Greece, respectively. Pods from the Sfax region of Tunisia contained 17.6 mg GAE g^{-1} [42], and in Sicily, the mean phenol concentration was 19.3 mg GAE g^{-1} [28]. A recent study of fruits sampled from ten distinct locations in Tunisia suggest that total phenol concentrations and anti-oxidant activities are higher in pods from semi-arid and upper arid regions [43] than in pods from other type of region. In a large survey of almost 100 carob fruits in Cyrus, Kyratzis et al. [26] reported a maximum phenol concentration of 34.1 mg GAE g^{-1} among samples extracted with a mixture of methanol, water and HCl.

5. Constraints on the interpretation of fruit quality data

Because few studies report the nutrient content of fruits taken from the same trees for at least three consecutive years, it is difficult to robustly assess how environmental factors modulate the chemical profile of sugars and phenols in carob fruits. Nevertheless, some authors have attempted such a cross-environment comparison. In Cyprus, Kyratzis et al. [26] concluded that total phenolic content was modulated primarily by agro-environmental conditions in contrasting soil regions while total sugars were more related to plant genetics, though they noted that sucrose content can be affected by both factors. In a study of carob fruits from two different soil types in southern Portugal, tannin concentrations in ripe pods were positively correlated with the leaf concentrations of N, Zn and Mn, while the DPPH (2,2-diphenyl-1-picrylhydrazyl) concentration was inversely correlated to leaf Zn and Mn [40].

More broadly, the incomplete descriptions of soil types and climate in most the studies presented in Tables 1 and 2 is a major limitation of any large-scale analysis of carob fruit quality. An additional constraint is the lack of information on the genetic history of the carob fruits used in these studies, as most of the samples do not come from a region's germplasm core collection. The pods used for quality assessment are normally identified only as "wild" or "grafted," and the name of the material refers only to its geographical origin, resulting in a large number of fruit or cultivar designations that give little indication of how related or unrelated two cultivars might be. Although a germplasm characterization of carob tree cultivars and accessions from different countries lies outside the scope of this mini-review, data on this subject can be found in La Malfa et al. [46] and Di Guardo et al. [47].

6. Concluding remarks

The main objective of this short review was to summarize the sugar and phenol content of carob pulp from several carob-producing countries. As shown in Tables 1 and 2, sugar and phenol concentrations can vary substantively among regions, suggesting that environmental conditions can significantly affect the chemical composition of carob pulp. Furthermore, analytical procedures and especially nutrient extraction methods can influence the values obtained by different teams. Due to this variation in study methodology, it is not possible to establish a close relationship between nutrient composition and geographic origin, at least at the country level.

Even so, the results from this review point to key areas for future work. For example, Richane et al. [43] used information on local precipitation, temperature, and soil type to conclude that phenol content is higher in carob fruits grown under arid conditions, presumably because these compounds mediate the plant's response to abiotic stress. Future studies should similarly include soil and climate data, as this information can help contextualize the nutrient profiles obtained from different countries. Another bottleneck in the analysis is the lack of consolidated germplasm characterization in most countries, making it difficult to compare cultivars. To overcome this limitation, the establishment of core germplasm collections should be a priority, and this process should involve all the players who deal with this important Mediterranean crop.

Data availability statement

The authors confirm that the data supporting review paper are available within the article, in the cited references and are also available on request from the corresponding author, P.J. Correia.

CRediT authorship contribution statement

Pedro José Correia: Writing – review & editing, Writing – original draft, Validation, Methodology, Conceptualization. Maribela Pestana: Writing – review & editing, Writing – original draft, Validation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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