Environmental aspects of carob tree (*Ceratonia siliqua L.*)

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Abstract

Volatile organic compounds (VOCs) are of the specific interest of scientists for their environmental and health impacts. On the other hand, VOCs emitted from the plant kingdom play a vital role in nature. The latter are called biogenic VOCs and they contribute in the growth, breeding and defense of the plant. They also help to interconnect the plant with its various parts, to communicate between other plants and between insects. Floral scents may function as long- and/or short-distance attractants, not only to pollinators, but also to key insect pests. Since floral scent can be crucial in pollination, and therefore in reserving seed or fruit set, the presence or absence of an attractive scent to the locally available insect pollinators may conflict the yield of agronomically essential crops, such as that of carobs. Carob tree (*Ceratonia siliqua L.*) is an evergreen long-lived tree that belongs to the family *Leguminosae* and is cultivated mostly in the Mediterranean coast. Apart from the VOCs emitted from carob flowers and fruit, carob is also of great interest to food industry, not only for its health benefits, but also due to its characteristic aroma which can be maintained even after processing. SPME/GC-MS analyses of carob flowers, fruit and powder (commercial samples) were performed and the emitted VOCs are presented and discussed. The uniqueness of carob benefits is well-known in the agriculture, pharmaceutical and food sector and is closely related to the economy and long history of eastern Mediterranean countries (e.g. Cyprus, Greece, Egypt, Turkey).

Keywords: Biogenic VOCs, *Ceratonia siliqua L*. SPME/GC-MS

Introduction

Carob tree is of limited soil requirements as it thrives on various types of soil such as rocky, dry, sloping, as long as it is lightly fertile, and can be penetrated by the root system. The flowering season begins in the mid-September and is completed in November. Carob flowers are small and during the flowering season have been observed to attract a great number of insect visitors, which later help to the pollination [1][2]. Some people like the scent of carob flowers, which is mostly caused by the male flowers, whereas others not.

Carob has been used as human or animal food for many years. It is mostly cultivated for its edible pods, as well as, a decorative tree in fields and streets. Carob pods are also well known for their health benefits [3], as well as, for their characteristic strong persistent aroma. In addition, carobs can potentially be used as a substitute for cocoa in cakes, sweets and beverages.

A number of studies were performed in order to decode the aroma profile of carob powder and flower. These are presented in detail in Table 1.

Table 1: Analytical methods for the analysis of carob powder and flowers.

Analytical method	Sampling	Results	Literature
SPME/GC-MS • Quadropole • EI:70eV • Scan range: m/z 40-500	SPME: 50μm/30μm Divinyl benzene/ Carboxen/ Polydimethylsiloxane (DVB-CAR-PDMS)	31 VOCs (carob powder)	[4]
GC-MS • Quadropole • EI:70eV • Scan range: m/z 40-350	Solvent extraction (pentane-dichloromethane 2:1) followed by liquid injection	137 VOCs (carob powder)	[5]
In-tube extraction (ITEX)/GC-MS	ITEX	12 VOCs (carob powder)	[6]

GC-MS • EI:70eV • Spilt 25:1	Solvent extraction (2-methylbutane) followed by liquid injection	169 VOCs (carob powder)	[7]
GC-MS • Quadropole • EI:70eV • Scan range: m/z 40-350 e-nose: Aroma Scanner A32 S (AromScan, Crewe, UK)	SPME with a Hamilton 7000 series syringe	Acids, alcohols, aldehydes, furans, esters, ketones, pyrroles, pyrans, thiazoles and sulfur compounds (carob powder)	[8]
SPME/GC-MS • Quadropole • EI:70eV • Scan range: m/z 40-400	SPME: Polydimethylsiloxane (PDMS) 100μm	25 VOCs (carob flower)	[1]

In the present study, Volatile Organic Compounds (VOCs) emitted from different parts of the carob tree such as flowers, pods and commercial samples of carob powder, were analysed using the Solid Phase Microextraction/Gas Chromatography-Mass Spectrometry (SPME/GC-MS) method. SPME is a quick and powerful extraction green technique (solvents are not used), which enables the enrichment of VOCs from the headspace of the sample to the stationary coating phase of a fused silica fiber [4]. In this way, the sample is pre-concentrated. Then, by using high temperature at the inlet of the GC, the analytes are thermally desorbed, inserted to the GC for separation and subsequently on the MS for chemical identification.

Carob tree is widespread almost all around the world (e.g. Spain, Italy, Morocco, Greece, Cyprus, Australia, South Africa, California and Arizona). A variety of cultivars exist; only in Spanish there are five cultivars, whereas in Greece two and in Cyprus three [9]. According to the Food and Agriculture Organization of the United Nations (FAO), the countries with the largest production of carob in 2016 were Portugal (40385 tonnes), Italy (28925 tonnes), Spain (26185 tonnes), Morocco (22032 tonnes) and Turkey (13405 tonnes), followed by Greece (12150 tonnes), Cyprus (8280 tonnes) and Algeria (3257 tonnes) (FAO 2016).

According to our knowledge, the aroma characterization of Cyprus carob cultivars is missing from the literature.

Material and methods

SPME fiber 75μm Carboxen/ Polydimethylsiloxane (CAR/PDMS) was selected for the VOCs analysis (Supelco). Before each analysis, the SPME fiber was conditioned for 30 minutes at 300^{o}C in the GC injection port.

Initially, the commercial carob powder samples sieved through a 125μm molecular sieve. 5g of the grounded carob pod was then placed in a 20ml glass vial (Agilent) and sealed (Figure 1). The analysis of the sample was carried out the next day; the fiber was exposed into the headspace above the sample for 30 minutes at room temperature. A blank sample was also run as a control.

Carob pods (294g) were placed into 1L in house-made glass jars (Figure 2). The analysis of the samples was carried out at different time intervals: day 1, day 4, day 8, day 12, day 18, day 22, day 26, day 32 and day 64. SPME sampling procedure is described in detail above.

Carob flowers from three different trees were collected during mid-day (November 2017). 5g of the flowers were placed in a 20ml glass vial (Agilent) and sealed (Figure 3). The analysis was carried out the next day; SPME sampling is as described above.

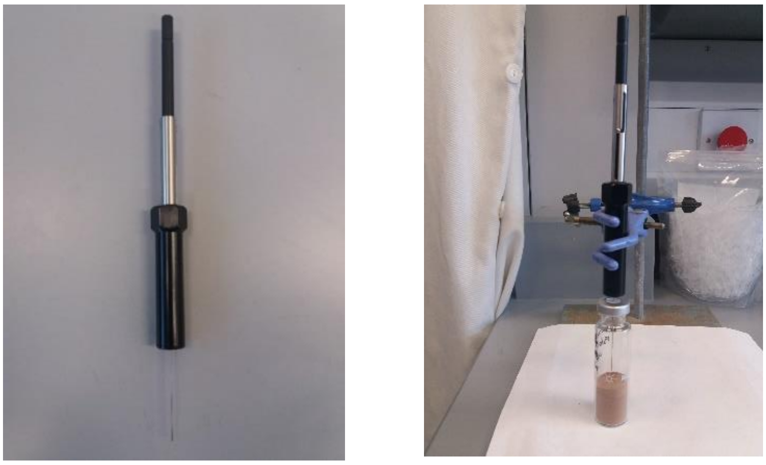

Figure 1: Left: SPME device for sample extraction and Right: Sampling VOCs of carob powder.

Figure 2: Sample preparation for carob fruit analysis (in-house made glass jars).

Figure 3: Carob flower sample preparation. The first two vials (1, 2) are male flowers from the same tree but at different stage of growth, whereas the other two vials (3, 4) are male flowers from another tree.

SPME fibers were thermally desorbed at 280ºC for 1 minute in the inlet of an Agilent GC 7890B/MS 5977B in split 10:1 mode for carob powder samples and splitless mode for the carob pods and flowers. VOCs were separated using SPB-624 capillary column (60m × 0.25mm × 1.4 film thickness, Supelco). The GC conditions that were applied are the following: inlet 280ºC, column oven 35ºC for 5 minutes then at a rate of 4ºC/min to 180ºC and kept for 20 min, He carrier gas at 1,7 ml/min. The MS source, transfer line and quadrupole temperature were 230ºC, 250ºC and 150ºC, respectively. The MS was operated in the electron ionization (EI) mode at 70eV and the scan range was set at 35-350 m/z. Volatile components were identified by retention times (Rt) relative to analytical standard EPA 524 VOC Mix A (Supelco) and mass spectrum matching to library database. The library used for the chemical identification was NIST11.

Results and discussion

The aroma profile of carob powder, carob fruits and flowers was decoded using SPME/GC-MS analysis. Each component emits a complex mixture of VOCs.

Figure 4 shows a representative chromatogram of carob powder. The most abundant VOCs emitted from the carob powder is presented in Table 2. In carob powder, propanoic acid, 2-methyl, butanoic acid, pentanoic acid, hexanoic acid, furfural and heptanoic acid have been previously detected with SPME/GC-MS analysis [4] and with ITEX coupled with GC-MS [6]. Isobutyric acid (propanoic acid, 2-methyl), which is the most abundant compound in the aroma profile of carob powder, emits a sweet and buttery flavour, while the fruity, floral, woody and almond flavour is a result of the presence of furfural.

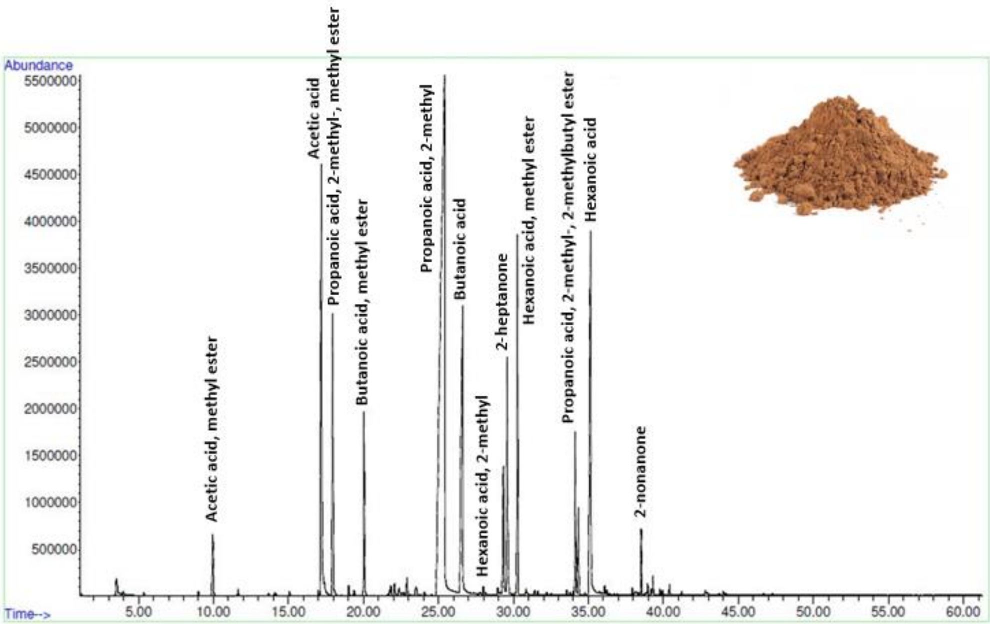

Figure 4: SPME/GC-MS chromatogram of carob powder.

Table 2: Indicative VOCs detected in commercial carob powder.

Retention Time (R_t)	VOCs	Literature
17.154	Acetic acid	[5][7]
22.065	Propanoic acid	[5][7]
25.040	Propanoic acid, 2-methyl (isobutyric acid)	[4][6]
26.373	Butanoic acid	[4][5][6][7]
27.998	Furfural	[4][5][6]
29.576	2-heptanone	[5][6]
35.042	Hexanoic acid	[4][5][7]

An indicative SPME/GC-MS chromatogram of carob flower is presented in Figure 5. The most common VOCs emitted from carob flower are summarised in Table 3. Linalool, linalool oxide, D-limonene, a-pinene and beta-pinene identified in the headspace of carob flowers, have been also detected with SPME/GC-MS analysis [1].

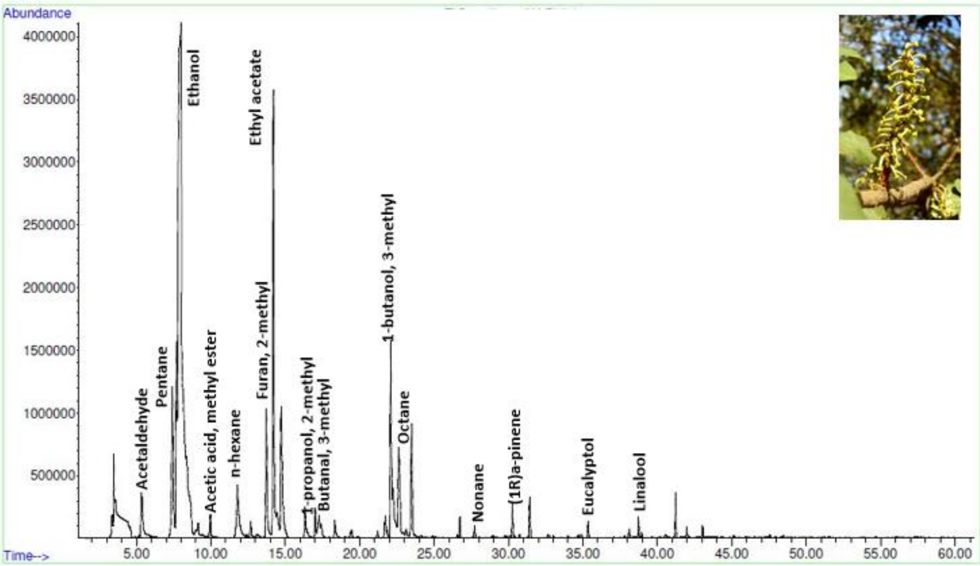

Figure 5: SPME/GC-MS chromatogram of carob flower.

Table 3: VOCs identified in carob flower.

R_t	VOCs	Literature
7.878	Ethanol	
14.203	Ethyl acetate	
25.040	beta-pinene	[1]
30.262	a-pinene	[1]
34.753	D-limonene	[1]
38.700	Linalool	[1]
42.769	Linalool oxide	[1]

The most abundant VOCs emitted from the carob fruit are shown in Table 4, while in Figure 6, a representative SPME/GC-MS chromatogram is presented.

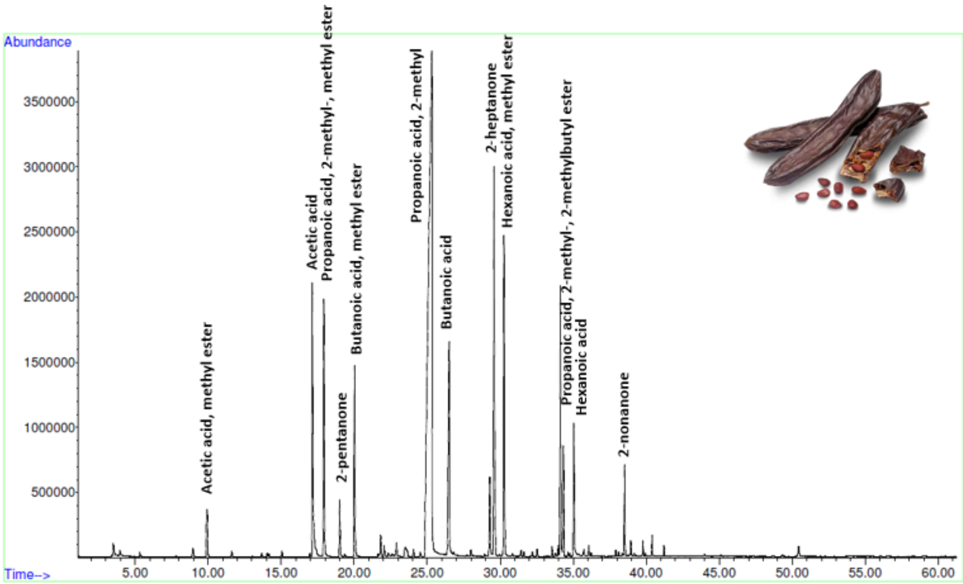

Figure 6: SPME/GC-MS chromatogram of carob fruit.

Table 4: VOCs found in carob fruit.

R_t	VOCs
17.154	Acetic acid
17.927	Propanoic acid, 2-methyl-, methyl ester
25.112	Propanoic acid, 2-methyl
26.451	Butanoic acid
29.582	2-heptanone
30.248	Hexanoic acid, methyl ester
35.047	Hexanoic acid

Conclusions

VOCs evolved from plants play a very important role, serving a wide array of processes in nature. *Ceratonia Siliqua L.* is being examined as a case study because of its direct link with the history, agriculture and food culture of Mediterranean countries. The VOCs emitted from the carob tree (i.e. pods, flower, powder) were determined serving as a link between the socioeconomic and cultural background (tradition), as well as the modern food and agriculture trends.

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