

Physical and phytochemical profile of wild and domesticated carob (*Ceratonia siliqua* L.) genotypes

H. Gubbuk^{1*}, E. Kafkas², D. Guven³ and E. Gunes¹

¹ Department of Horticulture, Faculty of Agriculture, University of Akdeniz, 07058 Antalya, Turkey

² University of Çukurova, Biotechnology Research and Experimental Center, 01330 Balcali (Adana), Turkey

³ Bati Akdeniz Agricultural Research Institute, Antalya, Turkey

Abstract

One wild and two domesticated ('Etli' and 'Sisam') carob genotypes grown in Antalya, Turkey were assessed for their physical and chemical properties in a comparative study. In most physical pod traits, the domesticated genotypes had higher values than those found in the wild genotype. On the other hand, physical seed traits such as average number of seed and seed to husk ratio had higher values in the wild genotype compared with the domesticated genotypes. Soluble solid content and the content of fructose, glucose and sucrose of the domesticated genotypes were higher than those found in the wild genotype. The levels of sucrose were found to be the highest compared with all the other sugars, in all genotypes. The N and K concentrations in the husk of the wild genotype were higher than that found in domesticated genotypes. The content of macro and micro nutrients in the seeds were quite similar among all the genotypes. The most abundant fatty acids were the methyl-esters of oleic acid (C18:1), linoleic acid (C18:2n6), palmitic acid (C16:0) and stearic acid (C18:0). The husk of the wild genotype contained higher concentrations of mono and polyunsaturated fatty acids compared with that of the domesticated genotypes. Fat concentration and fatty acid composition are the first reported in this paper. Our results suggest that 'Etli' and 'Sisam' are advantageous over the wild type regarding pod properties, while the wild genotype was found to be better regarding seed properties.

Additional key words: Brix; fatty acids; mineral composition; pod and seed features; sugars.

Resumen

Perfil físico y fitoquímico de genotipos de algarrobo (*Ceratonia siliqua* L.) silvestres y cultivados

Se evaluaron comparativamente las propiedades físicas y químicas de un algarrobo silvestre y dos cultivados ('Etli' y 'Sisam') en Antalya, Turquía. En la mayoría de los caracteres físicos de las vainas, los genotipos cultivados tenían valores más altos que los del genotipo silvestre. En cambio, los caracteres físicos de las semillas, como el número promedio de semillas y la ratio cáscara/semilla presentaron mayores valores en el genotipo silvestre en comparación con los cultivados. El contenido de sólidos solubles y de fructosa, glucosa y sacarosa de los genotipos cultivados fue superior al del genotipo silvestre. En todos los genotipos, los niveles de sacarosa resultaron ser los más altos de todos los azúcares. Las concentraciones de N y de K en la cáscara del genotipo silvestre fueron superiores que la encontrada en los genotipos cultivados. El contenido de macro y micronutrientes en las semillas fueron muy similares en todos los genotipos. Los ácidos grasos más abundantes fueron los metil-ésteres de ácido oleico (C18:1), el ácido linoleico (C18:2n6), el ácido palmítico (C16:0) y el ácido esteárico (C18:0). La cáscara del genotipo silvestre contenía concentraciones más altas de los ácidos grasos mono y poliinsaturados en comparación con la de los genotipos cultivados. Este trabajo es el primer reporte sobre la concentración de grasa y la composición de ácidos grasos. Nuestros resultados sugieren que 'Etli' y 'Sisam' presentan mayores ventajas en las propiedades de la vaina, mientras que el tipo silvestre presenta mejores propiedades en la semilla.

Palabras clave adicionales: ácidos grasos; azúcares Brix; características de la vaina y la semilla; composición mineral.

* Corresponding author: gubbuk@akdeniz.edu.tr

Received: 21-10-09; Accepted: 21-07-10.

Abbreviations used: AOAC (Association of Official Analytical Chemists), CPCP (commercially prepared carob powder), GC (gas chromatography), HPCP (home prepared carob powder), HPLC (high performance liquid chromatography), LBG (locust bean gum), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid), SFA (saturated fatty acid), SSC (soluble solids content), TA (tritatable acid), UV (ultraviolet).

Introduction

The carob tree (*Ceratonia siliqua* L.) is an evergreen widely grown in Mediterranean countries. The tree is considered to be an important component of the vegetation and is economically important (Batlle and Tous, 1997). Carob trees are drought resistant; require little maintenance and produce a range of products from the seed and the pod (Fletcher, 1997). Carobs have been cultivated throughout the Mediterranean region for approximately 4,000 years. Portugal and Spain have approximately 100,000 ha of carob trees and process approximately half of the world's commercial carob supply. Current world production of carob pod has been estimated at about 310,000 tonnes per year harvested from an area of about 200,000 ha (Makris and Kefalas, 2004). Yields are highly variable depending on the cultivar, region, and farming practices (Makris and Kefalas, 2004).

The carob pod consists mainly of pulp (90%) and it is rich in sugars (48-56%, mainly sucrose) and contains 16-20% of condensed tannins (Batlle and Tous, 1997). Carob powder and carob chips are used as ingredient in cakes and cookies. Carob is also used as a substitute for chocolate. Carob has very low protein content (Marakis, 1996). The sugar contents of the husk depend on the cultivar (Marakis, 1996) and tillage of the trees (Tous *et al.*, 1996). It has been reported that ungrafted cultivars have lower amounts of total-soluble sugars than grafted ones (Marakis *et al.*, 1988; Marakis, 1996). This may be due to the higher amount of tannin-lignocellulose materials in ungrafted cultivars (Marakis *et al.*, 1993, 1997; Galtis *et al.*, 1994). In addition, the carob pod has antioxidant properties related to its polyphenolic composition (Kumuzawa *et al.*, 2002). The endosperm is extracted from the seed to produce a galactomannan, which forms locust bean gum (LBG) a valuable natural food additive. The pod is used for high-energy stock feed (although its high tannin content can limit the amount consumed) and in the human food industry for cocoa products and syrups. Recent studies have indicated that reducing the dietary ratio of n-6 to n-3 fatty acids might play a role in decreasing the risk of heart disease and cancer (Iso *et al.*, 2002). Dietary recommendations for healthy eating include the consumption of fruit juices (Williams, 1995). The beneficial effects are attributed, in part, to ascorbic acid, a natural antioxidant which may inhibit the development of major clinical conditions including heart disease and certain cancers (Diplock, 1994). Carob flour has proven effective in relieving diarrhea in infants (Fortier

et al., 1953). To our knowledge, very limited data are available concerning fatty acid and mineral composition of carob husks and seeds.

Both wild and cultivated forms of carobs have been exclusively grown in the Mediterranean and Aegean regions of Turkey. Most of carob trees in Turkey are located on southern slopes of rocky sites and calcareous soil. All carob trees are highly regarded as forest trees in Turkey (Baktir, 1988). Nevertheless, good size carob orchards have not been established. However, the amount of carob products has continuously increased due to the improvement of food industries and consumer demand. Two common domesticated genotypes ('Etili' and 'Sisam') (Vardar *et al.*, 1980) and also various wild genotypes have been grown in Turkey. Carob pods have multifunctional uses; wild genotypes give high quality seeds, whereas cultivars are important for their pulp. Therefore both are important for food and industrial purposes.

The aim of the study was to determine some physical and phytochemical features of domesticated and wild genotypes of carobs grown in Antalya province of Turkey.

Material and methods

Carob pods were randomly harvested from two domesticated genotypes ('Etili', 'Sisam') and a wild genotype from Antalya province of Turkey in 2006. The physical features, including pod weight, husk weight (deseeded carob pod), pod length, pod width and pod thickness, and husk percentage were measured. In addition, the number of seeds/pod, seed weight, length, width, thickness and seed percentage were recorded. Soluble solids (SSC) were measured on the same homogenate used for determination of sugars. Soluble solids (diluted with distilled water (1:9 v/v) were measured using a hand-held refractometer (ATAGO N1). Results were reported as «Brix» at room temperature after 30 min. Total titratable acidity (TA) was determined on this same mixture by titration with 0.1 M NaOH up to 8.1 pH, (AOAC 1980). Vitamin C content of carob genotypes was analyzed according to Bozan *et al.* (1997). From the same homogenate 1 g sample was weighed and powdered with liquid nitrogen in a mortar and mixed with 20 mL of 3% aqueous meta-phosphoric acid at room temperature for 30 min using a shaker. This mixture was filtered and made up to 25 mL with the same solvent, then used for HPLC analysis. The liquid chromatographic apparatus (Agilent 1100 Series) consisted

of an in-line degasser, pump, and controller coupled to an ultraviolet (UV) detector (Agilent 1100 Series) equipped with an automatic injector (20 μL injection volume) interfaced to a computer running Class chromatography manager software (Shimadzu, Japan). Separations were performed on a 250 mm \times 4.6 mm i.d., 5 μm reversed-phase Ultrasphere ODS (Silica-Based Columns) analytical column (Beckman) operating at room temperature with a flow rate of 1 mL min^{-1} . Detection was carried out with a sensitivity of 242 nm wavelengths. Elution was isocratic with 0.5% aqueous meta-phosphoric acid. L-ascorbic acid was identified by comparing its retention time with authentic standards under analysis conditions and UV spectra. A 10 min equilibrium time was allowed between injections. Samples were prepared by removing the seeds and grinding and powdering them in a mill. Mineral composition of husk and seed were analyzed. The content of the following minerals was determined: N (g kg^{-1}), P (g kg^{-1}), K (g kg^{-1}), Ca (g kg^{-1}), Mg (g kg^{-1}), Cu (mg kg^{-1}), Fe (mg kg^{-1}), Zn (mg kg^{-1}), and Mn (mg kg^{-1}). All values (except for N) were calculated per dry weight (USDA, 2004) using a Perkin-Elmer 2100 ICP with wavelengths from 190 nm to 900 nm.

For sugar analysis, approximately 50 g of each sample was used and each replicate was analyzed separately. From this homogenized material 1 g of sample was weighed then ground with liquid nitrogen in a mortar and 10 mL of aqueous ethanol (80%, v/v) and was placed in a screw-cap eppendorf tube. The reaction mixture was sonicated for 15 min at 80°C then filtered. The extraction procedure was repeated three times. All the filtered extracts were combined and evaporated to dryness in a boiling water bath. The residue was dissolved in 2 mL of distilled water and filtered. Sugar analysis was carried out using HPLC (Agilent 1100 series) with refractive index detector and separations were performed on a 150 mm \times 4.6 mm i.d., reverse-phase Nucleosil NH_2 5 μm analytical column (Shimadzu, Japan) operating at room temperature with a flow rate of 1 mL min^{-1} . Elution was done using an isocratic elution of the solvent, 75% aqueous acetonitrile (Miron and Schaffer, 1991). Components were identified by comparison of their retention times with those of authentic standards. A 15 min equilibrium time was allowed between injections. All the samples were directly injected into the reverse phase chromatography column. For the stock solution, the sugar standards (glucose, fructose and sucrose) were dissolved in water at 50 mg mL^{-1} .

Lipid extraction was carried out according to the method described by Bligh and Dyer (1959). Boron trifluoride/methanol was used for preparation of fatty acid methyl esters (AOAC, 1990). The fatty acid composition was analyzed by GC Clarus 500 with auto-sampler (Perkin Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m \times 0.32 mm, i.d. \times 0.25 μm , BP20 0.25 UM, USA). The oven temperature was 140°C, for 5 min, then rose to 200°C at a rate of 4°C min^{-1} and to 220°C at a rate of 1°C min^{-1} , while the injector was set at 220°C and the detector temperature at 220°C and 280°C, respectively. The sample size was 1 μL and the carrier gas was controlled at 16 ps. The split used was 1:100. Fatty acids were identified by comparing the retention times of FAME with a standard 37 component FAME mixture (Supelco).

Triplicate gas chromatography (GC) analyses were performed and the results were expressed in the percentage of GC area as a mean value \pm standard deviation. The experiment was laid out in a completely randomized design with three replicates per treatment with 15 pods for each replicate (ripe pods were randomly selected from 15 trees). The data were analyzed using the analysis of variance (ANOVA) method to assess differences with the SAS (1999) software and significance between means was tested by LSD using a multiple range test at a probability of 0.05.

Results and discussion

The highest pod weight, husk weight, pod length, pod width, pod thickness and husk percentage were found in the domesticated genotype 'Etli' (23.30 g, 20.51 g, 17.90 cm, 2.05 cm, 0.83 mm and 88.03%, respectively) (Table 1). The greatest pod thickness was detected in the domesticated genotype 'Sisam' (1.31 mm). The physical properties of seed in the wild and two cultivated carob genotypes are given in Table 1. Seed number was higher in the wild genotype than in domesticated genotypes, however, domesticated genotypes had higher seed weight. Seed length, width and thickness of 'Etli' and 'Sisam' genotypes were similar to each other, except for seed width, but different from the wild genotype. Seed percentage of the wild genotype (18.60%) was significantly greater ($p < 0.05$) than that of 'Sisam' (11.10%) and 'Etli' (11.97%). The proportion of pod and husk varied among genotypes.

Table 1. Pod and seed physical properties of carob genotypes. Means followed by the same letter are not significantly different according to LSD, $p < 0.05$

	Genotypes			5% LSD
	Wild type	'Etlí'	'Sisam'	
<i>Pod properties</i>				
Pod weight (g)	13.60 ^c	23.30 ^a	19.92 ^b	1.50
Husk weight (g)	11.10 ^c	20.51 ^a	17.30 ^b	1.40
Pod length (cm)	15.20 ^b	17.90 ^a	15.80 ^b	1.10
Pod width (cm)	1.50	2.05	2.03	NS ¹
Pod thickness (mm)	0.44 ^c	0.83 ^b	1.31 ^a	0.31
Husk percentage	81.40 ^b	88.03 ^a	88.90 ^a	1.50
<i>Seed properties</i>				
Number	10.18 ^a	7.10 ^c	7.43 ^b	0.14
Weight (g)	0.35 ^b	0.39 ^a	0.25 ^c	0.013
Length (mm)	10.41 ^a	10.00 ^a	8.07 ^b	0.831
Width (mm)	7.57 ^a	7.42 ^a	6.15 ^c	0.831
Thickness (mm)	3.40 ^c	4.14 ^a	3.66 ^b	0.060
Percentage	18.60 ^a	11.97 ^b	11.10 ^b	0.065

¹ NS: no significant.

Albanell *et al.* (1991) evaluated 182 common carob trees from different areas of Spain and assessed the average weight, length, width, and kernel yield. Their highest values were 14.88 g, 15.83 cm, 2.11 cm and 12.11% respectively. In this study, similar results were obtained based on average pod weight; length and width that ranged from 13.60-23.30 g, 15.20-17.90 cm and 1.50-2.05 cm, respectively. These authors reported that most of the variables measured showed significant differences according to their geographical origin. The physical properties of the pods of *C. siliqua* and *C. macrocarpa* carob varieties have been investigated by Shawakfeh and Ereifej (2005) who found that the pods varied significantly in all physical properties except for specific gravity. Tous *et al.* (2009) compared two carob cultivars in terms of yield and some pod characteristics. Significant differences were found on yield and pod characteristics. For instance, Batlle and Tous (1997) reported that pod consisted of about 90% pulp and 10% seeds.

Some physicochemical variables such as SSC, TA and vitamin C in the husk of wild and two domesticated carob genotypes are given in Table 2. These values were found to be lower in the wild genotype than in the domesticated genotypes. Marakis *et al.* (1988) found that the SSC were between 32% and 60% according to countries.

Among the measured macro and micro nutrients, K was the most abundant in the husk followed by N and

P (Table 2), whereas N was the highest in the seeds (Table 3). Potassium concentration was higher in the wild genotype than in 'Sisam' and 'Etlí' (Table 2), whereas N was the highest in both domesticated genotypes in the seeds (Table 3). Among the micro nutrients in both the pods and the seeds, Fe had the highest concentration (Table 2 and 3). The N concentration of seeds was approximately two fold greater than that of the husk. Albanell *et al.* (1991) reported that the most abundant element was K (9.70 mg g⁻¹ dry weight). These authors also found that the pods were richer in Ca than in K or Mg. The mineral content varied in the husk and in the seeds. Shawakfeh and Ereifej (2005) reported that *C. siliqua* pods had higher Na, K, Ca, and P concentrations than *C. macrocarpa* pods. *C. macrocarpa* seeds demonstrated higher concentrations of Na, K, Ca, and P. The variation in mineral content of *C. siliqua* and *C. macrocarpa* pods and seeds in carob trees may be due to differences in cultivars and to environmental effects.

The sugar content varied significantly among the genotypes (Table 2). The main sugars detected were sucrose and glucose. All sugar concentrations were significantly different from each other. The highest sugar content was in 'Sisam', while the lowest was in the wild genotype (Table 2). The glucose concentration in carobs ranged from 108.80 to 174.83 mg g⁻¹. Fructose was the lowest concentrations of all sugars among the carob genotypes. The carob sugar profiles reported

Table 2. Physicochemical variables, macro-micro nutrient content, fructose, glucose, sucrose and total sugar content in the husk of carob genotypes. Means followed by the same letter are not significantly different according to LSD, $p < 0.05$

	Genotypes			5% LSD
	Wild type	'Etli'	'Sisam'	
<i>Physicochemical variables</i>				
Soluble solid content (%)	50.00 ^b	70.00 ^a	70.13 ^a	2.39
Titratable acidity (%)	2.52	2.79	2.72	NS* ¹
Vitamin C (mg/100 g)	8.07 ^c	10.41 ^a	10.26 ^b	0.049
<i>Macronutrients (g kg⁻¹)</i>				
Nitrogen (g kg ⁻¹)	8.20 ^a	5.10 ^c	6.60 ^b	0.113
Phosphorus (g kg ⁻¹)	7.30	8.00	7.30	NS*
Potassium (g kg ⁻¹)	12.30 ^a	10.90 ^b	8.90 ^c	0.146
Calcium (g kg ⁻¹)	2.80 ^b	2.10 ^c	2.90 ^a	0.067
Magnesium (g kg ⁻¹)	0.73 ^a	0.50 ^c	0.56 ^b	0.009
<i>Micronutrients (mg kg⁻¹)</i>				
Copper (mg kg ⁻¹)	2.16 ^b	3.76 ^a	3.81 ^a	0.979
Iron (mg kg ⁻¹)	13.46 ^a	10.96 ^b	13.12 ^a	1.888
Manganese (mg kg ⁻¹)	4.45 ^{ab}	4.32 ^b	5.16 ^a	0.800
Zinc (mg kg ⁻¹)	6.81	7.40	7.66	NS*
Fructose (mg g ⁻¹)	5.45 ^c	10.08 ^b	14.18 ^a	2.565
Glucose (mg g ⁻¹)	108.80 ^c	137.66 ^b	174.83 ^a	12.781
Sucrose (mg g ⁻¹)	277.58 ^c	392.22 ^b	438.47 ^a	21.594
Total sugars (mg g ⁻¹)	391.83 ^c	539.96 ^b	627.48 ^a	50.515

¹ NS: no significant.

by several researchers demonstrated significant variations according to country of origin and variety (Marakis, 1996). Sucrose has been determined as the major sugar

in several studies (Marakis, 1996; Avallone *et al.*, 1997; Marakis *et al.*, 1997; Ayaz *et al.*, 2007, 2009; Biner *et al.*, 2007). For instance, Ayaz *et al.* (2007) detected

Table 3. Macro and micronutrient concentrations in the seed of carob genotypes. Means followed by the same letter are not significantly different according to LSD, $p < 0.05$

	Genotypes			5% LSD
	Wild type	'Etli'	'Sisam'	
<i>Macronutrients</i>				
Nitrogen (g kg ⁻¹)	19.80	22.90	20.10	NS* ¹
Phosphorus (g kg ⁻¹)	2.80	2.20	2.90	NS*
Potassium (g kg ⁻¹)	9.80	8.10	9.90	NS*
Calcium (g kg ⁻¹)	4.80	3.50	4.60	NS*
Magnesium (g kg ⁻¹)	1.20 ^b	1.70 ^a	1.50 ^{ab}	0.050
<i>Micronutrients</i>				
Copper (mg kg ⁻¹)	11.66 ^a	4.25 ^b	12.11 ^a	3.045
Iron (mg kg ⁻¹)	42.18	37.85	51.49	NS*
Manganese (mg kg ⁻¹)	27.20	20.75	20.40	NS*
Zinc (mg kg ⁻¹)	26.9 ^a	19.80 ^b	30.98 ^a	6.790

¹ NS: no significant.

sucrose at higher concentrations (437.3 mg g⁻¹ dry weight) than glucose (395.8 mg g⁻¹ dry weight) and fructose (42.3 mg g⁻¹ dry weight) in the pods. Biner *et al.* (2007) evaluated wild and cultivated carob genotypes from the Mediterranean and Aegean region of Turkey in terms of sugar characteristics. They reported that sucrose was the most abundant sugar in the pods, followed by glucose and fructose. In addition, total sugar concentration was found to be higher in pods of grafted (531 ± 93 g kg⁻¹ dry weight) than in wild types (437 ± 77 g kg⁻¹). Ayaz *et al.* (2009) reported large amounts of sucrose (146 and 309 mg g⁻¹ dry wt) in home-prepared carob powder (HPCP) and commercially-prepared carob flour (CPCP), respectively, but small amounts of glucose and fructose. Yousif and Alghzaw (2000) reported 38.7% and Avallone *et al.* (1997) reported 45% total sugar carbohydrates in carob powder. Variation in carob sugar profile and concentrations could have resulted from environmental conditions, cultural practices or cultivar differences, and the extraction and determination of sugar as indicated by Marakis (1996).

The fat and fatty acid composition and lipid percentages in the husk of wild and domesticated carob genotypes are given in Table 4. Total fat content of carob genotypes ranged between 0.53% ('Sisam') and 0.98% ('Etli'). Yousif and Alghzaw (2000) reported total fat content of carobs to be 0.74%, which is in agreement with our results (0.53-0.98%). Ayaz *et al.* (2009) reported 4.23% and 4.44% in CPCP and HPCP samples respectively. Studies on the content of fatty acids on carob are lacking, therefore is very difficult to report on the differences in the fat content of carob genotypes.

The most abundant fatty acids were oleic acid (C18:1n9), linoleic acid (C18:2n6), palmitic acid (C16:0), stearic acid (C18:0), and linolenic acid (C18:3n3) (Table 4). The primary saturated fatty acid was palmitic (6.33-18.30%), followed by stearic acid (2.27-3.42%). The highest palmitic acid content was found in 'Sisam' (18.30%), followed by 'Etli' (15.55%); the lowest being in the wild genotype (6.33%).

Unsaturated fatty acids are classified as monounsaturated (MUFA) or polyunsaturated (PUFA) fatty acids. Oleic acid (C18:1n9) was the main monounsaturated fatty acid of all carob genotypes (Table 4). The oleic acid content was the lowest in 'Etli' (42.92%) genotype and highest in the wild genotype (44.94%). Ayaz *et al.* (2009) reported that oleic acid was also the most abundant fatty acid in both CPCP and HPCP carob flour samples. The highest total concentration of fatty acids

Table 4. Fat and fatty acid composition and lipid percentages in the husk of carob genotypes

Fatty acids	Wild type	'Etli'	'Sisam'
% lipid	0.81 ± 0.21	0.98 ± 0.15	0.53 ± 0.12
C6:0	0.05 ± 0.01	0.42 ± 0.05	0.34 ± 0.06
C8:0	0.10 ± 0.03	0.81 ± 0.09	1.71 ± 0.00
C10:0	0.08 ± 0.01	0.31 ± 0.03	0.82 ± 0.08
C11:0	0.01 ± 0.01	0.10 ± 0.04	0.08 ± 0.01
C12:0	0.10 ± 0.01	0.22 ± 0.04	0.32 ± 0.01
C14:0	0.30 ± 0.04	0.58 ± 0.02	0.83 ± 0.01
C15:0	0.15 ± 0.02	0.38 ± 0.04	0.49 ± 0.02
C16:0	6.33 ± 0.02	15.55 ± 0.10	18.30 ± 0.43
C17:0	nd ¹	0.05 ± 0.00	nd
C18:0	2.27 ± 0.07	3.04 ± 0.71	3.42 ± 0.02
C20:0	0.19 ± 0.02	nd	nd
Σ SFAs ²	9.58	21.46	26.31
C14:1	0.03 ± 0.01	nd	nd
C15:1	0.09 ± 0.01	nd	0.07 ± 0.01
C16:1	1.32 ± 0.04	1.62 ± 0.11	0.29 ± 0.03
C17:1	0.20 ± 0.02	0.49 ± 0.03	0.60 ± 0.03
C18:1n9	44.94 ± 0.49	42.92 ± 0.58	44.85 ± 0.49
C20:1	0.26 ± 0.04	0.65 ± 0.05	0.04 ± 0.00
C22:1n9	0.48 ± 0.06	nd	nd
C24:1	0.22 ± 0.06	nd	nd
Σ MUFAs ³	47.54	45.68	45.85
C18:2n6	28.68 ± 0.23	22.99 ± 0.29	15.53 ± 0.49
C18:3n3	1.23 ± 0.25	1.72 ± 0.28	1.68 ± 0.11
C20:2cis	0.17 ± 0.03	1.20 ± 0.92	1.04 ± 0.03
C20:3n6	0.84 ± 0.04	1.55 ± 0.15	2.94 ± 0.15
C20:5n3	0.10 ± 0.02	nd	nd
Σ PUFAs ⁴	31.02	27.46	21.19
Σ	88.14	94.60	93.35

¹ Not detected. ² SFAs: saturated fatty acids. ³ MUFA: monounsaturated fatty acids. ⁴ PUFAs: polyunsaturated fatty acids.

was found in 'Etli' (94.60%) (Table 4). Eleven SFAs (saturated fatty acids), eight MUFAs and five PUFAs were detected in the carob husk. Oleic acid methyl ester (C18:1n9) was the most abundant MUFA, accounting for 42.92-44.94%. C18:2n6 was the most abundant among the detected PUFAs. Stearic acid methyl ester (C18:0) was the primary saturated fatty acid. Palmitic acid methyl ester (C16:0) ranged from 6.33-18.30%.

The results showed that the pods of the wild and the domesticated genotypes grown in Antalya Turkey varied significantly in their physical properties, chemical composition, mineral, sugars, fat and fatty acid content. Pod weight, length and width of pods of domestic carob genotypes were greater than those of the wild genotype. However, seed number and seed percentage of the wild genotype were greater than those of both domesticated genotypes.

Macro and micronutrients detected in husk and seeds varied but the domesticated genotypes demonstrated greater concentrations than the wild genotype. Potassium was the most abundant mineral in husk whereas N was the most abundant in seeds. Iron was found to be the major micronutrient in both pod and seeds (Tables 2 and 3).

Conclusions

Carob husk is a cheap source of sugars and natural fatty acids, minerals and vitamins, the nature and importance of which is, as yet, inadequately investigated. The present study reports on the physical and phytochemical profile of wild and domesticated carob genotypes. Based on these results we recommend 'Etlı' and 'Sisam' as possible domesticated genotypes for pod properties and the wild genotype for seed properties.

Acknowledgements

This work was supported by the Scientific Research Projects Coordination Unit of Akdeniz University. The authors thank Dr. David Turner, University of Western Australia/Australia, Dr Joe Sempik, Loughborough University/England, Dr. Uri Lavi, ARO-Volcani Center, Israel and Dr. Barbara Reed, National Clonal Germplasm Repository, United States Department of Agriculture/USA for their critical review of this manuscript.

References

- ALBANELL E., CAJA G., PLAIXATS J., 1991. Characteristic of Spanish carob pods and nutritive value of carob kibbles. *Opt Mediterr-Series Seminars* 16, 135-136.
- AOAC, 1980. Official methods of analysis, 13th ed. Association of Official Analytical Chemists, Washington, DC, USA. Section 31.034.
- AOAC, 1990. Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC, USA.
- AVALLONE R., PLESSI M., BARLDI M., MONZANI A., 1997. Determination of chemical composition of carob (*Ceratonia siliqua*): protein, fat, carbohydrates, and tannins. *J Food Compos Anal* 10, 166-172.
- AYAZ F.A., TORUN H., AYAZ S., CORREIA P.J., ALAIZ M., SANZ C., GRÚZ J., STRNAD M., 2007. Determination of chemical composition of Anatolian carob pod (*Ceratonia siliqua* L.): sugars, amino and organic acids, minerals and phenolic compounds. *J Food Qual* 30, 1040-1055.
- AYAZ F.A., TORUN H., GLEW R.H., BAK Z.D., CHUANG L.T., PRESLEY J.M., ANDREWS R., 2009. Nutrient content of carob pod (*Ceratonia siliqua* L.) flour prepared commercially and domestically. *Plant Foods Human Nutr* 64, 286-292.
- BAKTIR I., 1988. Akdeniz florasi meyveleri I [Fruits of Mediterranean flora I]. *Akd Univ Zir Fak Dergisi* 1, 11-21. [In Turkish].
- BATLLE 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, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy. 92 pp.
- BINER B., GUBBUK H., KARHAN M., PEKMEZCI M., 2007. Sugar profiles of the pods of cultivated and wild types of carob bean (*Ceratonia siliqua* L.) in Turkey. *Food Chem* 100, 1453-1455.
- BLIGH E.G., DYER W. J., 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37, 911-917.
- BOZAN B., TUNALIER Z., KOŞAR M., ALTINTAS A., BAŞER K.H.C., 1996. Quantitative analysis of vitamin C in rose hip products collected from local markets in Turkey. *Proc XI Symposium Plant Originated Crude Drugs*, Ankara, 22-24 May. pp. 258-266.
- DIPLOCK A.T., 1994. Antioxidants and disease prevention. *Mol Aspects Med* 15, 293-376.
- FLETCHER R., 1997. XIII. Carob agroforestry in Portugal and Spain, The Australian New Crops Newsletter. Available in: <http://www.newcrops.uq.edu.au/public1.htm>.
- FORTIER D.L., LEBEL G., FRECHETTE A., 1953. Carob flour in the treatment of diarrhoeal conditions in infants. *Can Med Assoc J* 68, 557-61.
- GALTIS F., MARAKIS S., OIAMANTOGLU S., 1994. Carob varieties from Greek Island Lefkada. *Proc XVI Congress of Hellenic Society of Biological Science* (Manolis S.K., ed), Valos, Greece. pp 241-242.
- ISO H., HATO S., UMEMURA U., KUDO M., KOIKE K., KITAMURA A., IMANO H., OKAMURA T., NAITO Y., SHIMAMOTO T., 2002. Linoleic acid, other fatty acids, and the risk of stroke. *Stroke* 33, 2086-2093.
- KUMUZAWA S., TANGUCHI M., SUZIKI Y., SHIMURA M., KWON M.S., NAKAYAMA T., 2002. Antioxidant activity of polyphenols in carob pods. *J Agric Food Chem* 49, 373-377.
- MAKRIS D.P., KEFALAS P., 2004. Carob pods (*Ceratonia siliqua* L.) as a source of polyphenolic antioxidants. *Food Technol Biotechnol* 42, 105-108.
- MARAKIS S., KALAITZAKIS J., MITRAKOS K., 1988. Criteria for recognizing carob tree varieties. *Proc II International Carob Symposium*. Valencia, Spain. pp. 195-208.
- MARAKIS S., LAMBAKIS M., OIAMANTOGLU S., 1993. Tannin chemistry of nine Cretan carob varieties. *Chim Chron New Ser* 22, 213-224.
- MARAKIS S., 1996. Carob bean in food and feed: current status and future potentials-A critical appraisal. *J Food Sci Technol* 33, 365-383.

- MARAKIS S., MARAKIS G., LAMBRAKIM., 1997. Tannins of eight carob varieties from the island of Lefkada, Greece. *Chim Chron New Ser* 26, 57-66.
- MIRON D., SCHAEFFER A.A., 1991. Sucrose phosphate synthase, sucrose synthase and acid invertase in developing fruit of *Lycopersicon esculentum* Mill. and the sucrose accumulating *Lycopersicon hirsutum* Himb. and Bonpl. *Plant Physiol* 95, 623-627.
- SAS Institute Inc, 1999. SAS/STAT user's guide. Version 8.0. Vol 2. SAS Inst, Cary N.C.
- SHAWAKFEHK Q., EREIFEJ K.I., 2005. Pod characteristics of two *Ceratonia siliqua* L. varieties from Jordan. *Ital J Food Sci* 17, 187-194.
- TOUS J., ROMERO A., PLANA J., BATLLE I., 1996. Current situation of carob plant material (Martins Laucao M.A., Catorina F., eds). Proc III International Carob Symposium. Cabanas, Tavira, Portugal.
- 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, 465-470.
- USDA, 2004. Soil survey laboratory methods manual. Nat Resour Conserv Serv, Soil Survey Inv, Report No 42, Version 4.0, 700 pp.
- VARDAR Y., SEÇMEN O., ÖZTÜRK M., 1980. Some distributional problems and biological characteristics of *Ceratonia* in Turkey. *Portug Acta Biol (A)* XXI (1.4), 75-86.
- WILLIAMS C., 1995. Healthy eating: clarifying advice about fruit and vegetables. *British Med J* 310, 1453-1455.
- YOUSIF A.K., ALGHZAW H.M., 2000. Processing and characterization of carob powder. *Food Chem* 69, 283-287.