DETERMINATION OF CHEMICAL COMPOSITION OF ANATOLIAN CAROB POD (*CERATONIA SILIQUA* L.): SUGARS, AMINO AND ORGANIC ACIDS, MINERALS AND PHENOLIC COMPOUNDS

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ABSTRACT

*Carob pod is the fruit of the carob tree (*Ceratonia siliqua *L. Fabaceae). The fruit and its products, sold both in large stores and local markets, contribute strongly to the diet of people living in the Mediterranean areas of Europe and Turkey. This study reports the composition of carob pods sampled in West and South Anatolia. Sucrose (437.3 mg/g dry weight), glucose (395.8 mg/g dry weight) and fructose (42.3 mg/g dry weight) were the major sugars identified and quantified in the fruit. Total phenolics (13.51 mg gallic acid equivalents [GAE]/g dry weight), proanthocyanidin (0.36 mg GAE/g dry weight), gallotannins (0.41 catechin equivalents [CE]/g dry weight) and*

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Journal of Food Quality **30** (2007) 1040–1055.

flavanols (3.21 mg CE/g dry weight protein) content of the fruit were also determined. Gallic acid (3.27 mg/g dry weight) was the most abundant phenolic acid present in all three phenolic fractions (free, ester and glycoside) isolated from pods. Aspartic acid (18.25 mg/g dry weight protein) was the predominant amino acid in the pod protein fraction. Eight minerals were quantified in the fruit. Among the analyzed major minerals, K (9.70 mg/g dry weight) was the most abundant element present, and the pods were richer in Ca than in P and Mg. Levels of trace minerals were comparable to other plant species. The data are discussed in terms of the nutritional value of the carob pod.

PRACTICAL APPLICATION

The use of carob fruit and its food products in Turkey has been increasing in recent years. However, knowledge about the composition of carob fruit pod produced in Turkey as well as in Mediterranean countries is lacking. The present work describes a composition scale and the advantages to food technologists and consumers who use the fruit and its fruit products in their diets. The results of the study can also aid in the assessment of adequate compositional information for further studies.

INTRODUCTION

In many geographical regions, locally grown fruit and vegetables contribute substantially to local diet. The composition of such foods and their products is therefore a matter of considerable interest to nutritionists and food scientists.

The carob tree (*Ceratonia siliqua* L. Fabaceae) is a native evergreen plant of the Mediterranean area including West and South Anatolia (Turkey) (Chamberlain 1970; Batlle and Tous 1997). The nonfleshy and bean-like fruits, called "*carob pods*," are a traditional part of the diet in the Mediterranean region, and the plant has been cultivated in the region for centuries for its edible fruits. The pod is light to dark brown, oblong, flattened, straight or slightly curved, with a thickened margin, and ranges from 10 to 20 cm in length and 1.5–2 cm in width. The unripe pod is green, moist and very astringent, but the ripe pod is sweet. The broken pod has a characteristic odor caused by its 1.3% isobutyric acid content (Morton 1987). Current world production of carob pod has been estimated at about 310,000 tons per year, produced from about 200,000 hectares with very variable yields depending on the cultivar, region, and farming practices (Makris and Kefalas 2004).

Carob pod is widely used in the food industry to produce carob bean gum and locust bean gum, which are polysaccharides (galactomannans) (Morton 1987; Batlle and Tous 1997). Throughout the Mediterranean region including Turkey, gently milled carob pods are processed to a cocoa-like flour which is sold as a "*carob cocoa*" in big stores and local markets. The milled flour is often added to hot or cold milk for drinking (Morton 1987). The pod consists mainly of pulp (90%), which is rich in sugars (48–72%), but also may contain a large amount of condensed tannin (16–20%) (Würsch *et al.* 1984; Morton 1987; Saura-Calixto 1988; Bravo *et al.* 1994; Batlle and Tous 1997). Lower tannin values have been reported in some cases (Yousif and Alghzawi 2000).

Free sugars, organic acids and amino acids are natural constituents of many fruits and vegetables and play an important role in maintaining quality and determining nutrititive value (Ashoor and Knox 1982). The nature and the concentration of these constituents in fruits are also of interest because of their important organoleptic properties. Free sugars are one of the most important constituents of fruits and vegetables. Monosaccharides and disaccharides, such as fructose and glucose, are considered to be the major sugars in most fruits contributing to the flavor of fruits (Shaw 1988). Amino acids and their derivatives are important for human nutrition and affect the quality of foods including taste, aroma and color (Belitz and Grosch 1999). Among the different substances that make up fruit and vegetables, amino acids are becoming increasingly important and, for various reasons, their analytical determination is becoming more necessary. First, the concentration of amino acids in fruit varies significantly as a result of metabolic changes during growth, maturation and ripening (Gomis *et al.* 1990). Second, amino acid profiles vary from one species to another and among fruits of the same type but of different origin; they can therefore be used to characterize fruit products (Gomis *et al.* 1990, 1992).

Phenolic compounds, nonnutrient but biologically active secondary plant metabolites which can act as antioxidants, are widely distributed in the Plant Kingdom and are present in many foods and beverages of plant origin. The acceptability of fruit and vegetables for human consumption may be affected by their content of phenolics (Shahidi and Naczk 1995). Interest in the role of phenolic antioxidants in human health has prompted research into the separation and characterization of active phenolic components in various plant-derived foods (Häkkinen *et al.* 1999; Zuo *et al.* 2002; Ayaz *et al.* 2005).

Here, we report the results of chemical analyses carried out on carob tree fruit (carob pods) collected from various locations in West and South Anatolia (Turkey) where the plant is both cultivated and naturalized (Chamberlain 1970). To the best of our knowledge this is the first such study undertaken on pods of carob trees grown in Turkey.

MATERIALS AND METHODS

Plant Material

Carob pods (*Ceratonia siliqua* L.) were randomly harvested from various parts of several trees grown in different locations in western and southern parts of Anatolia in Turkey (250–300 m above sea level). The samples were collected in the morning from August to September in 2 consecutive years (2004, 2005). The carob pods were of the same physiological maturity (dark brown) and of uniform shape and size. Fruits (50 g per sample) collected from each natural habitat were combined to provide composite samples of 800 g. Samples were sun dried, seeds were removed and the residue was ground in a mill (0.08 mm). From these stocks, three or four samples of gently milled pods were used for subsequent analyses. The grounded and milled samples were stored at −20C for further extraction.

Protein Determination

Protein content was determined by elemental analysis using a LECO CHNS-932 analyzer (St. Joseph, MI), and was calculated as percentage nitrogen × 6.25.

Amino Acids

Samples containing 2 mg of protein were hydrolyzed using 6 mol/L HCl at 110C for 20 h under an inert nitrogen atmosphere and derivatized with diethyl ethoxymethylenemalonate. Amino acids were analyzed by reversed-phase high-performance liquid chromatography (HPLC) using d,l-α-aminobutyric acid as an internal standard following procedure of Alaiz *et al.* (1992). Tryptophan was analyzed by HPLC after basic hydrolysis according to Yust *et al.* (2004).

Sugar and Organic Acid Extraction

The finely grounded carob pods were defatted by repeated extraction (3 × 1 h) with a mixture of petroleum ether and chloroform (1:1, v/v) at room temperature. The defatted samples were dried *in vacuo* then re-extracted twice with 20 mL 95% ethanol for 5 min as previously described (Pérez *et al.* 1997). The homogenate was vacuum-filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England) and the residue washed three times with 80% ethanol. The filtrates were combined and evaporated to dryness using a rotary evaporator. The residue was redissolved in 80% ethanol and then centrifuged. The supernatant was collected, dried *in vacuo* and used for further analysis.

HPLC Analysis for Sugars and Organic Acids

Sugars and organic acids were analyzed by a Hewlet-Packard (1090) liquid chromatograph equipped with a photodiode array detector (PDA) and a Waters 410 differential refractometer (Milipore Corp., Milford, MA) connected in series. Data were processed using a Hewlet-Packard 85-B computing system and a Beckman Analogue Interface Module 406 with Gold V.711 software. Isocratic separation of the compounds was carried out at a flow rate of 0.4 mL/min on a stainless steel Ion-300 column (300 mm × 7.8 mm, 10 μm) containing a cation-exchange polymer in the ionic hydrogen form, combined with an IonGuard GC801 precolumn (Interaction, San Jose, CA). Filtered (0.22 μm nylon) and degassed 0.0085 mol/L H_2SO_4 solution was used as the mobile phase. Both columns were maintained at 23C. Samples were dissolved in mobile phase, filtered through a micro-filter (politetrafilvoretileno [PTFE] or Teflon, 4 mm, 0.22 μm) and 20 μL (50% of total sample volume before filtration) was injected. The post column effluent was introduced in sequence into the PDA detector (scanning range 210–300 nm; 1.2 nm resolution) and a refractive index detector (sensitivity setting 16×, [Pérez *et al.* 1997]).

Determination of Total Phenolics, Flavanols and Tannins

Total phenolics (Singleton and Rossi 1965) and flavanols (Arnous *et al.* 2001) were determined using Folin-Ciocalteu reagent and DMACA (4-[dimethylamino]cinnamaldehyde) (Sigma Chemical Co., St. Louis, Mo) with calibration curves for gallic acid and (+) catechin, respectively. The proanthocyanidins (Price *et al.* 1978) and gallotannins (Inoue and Hagerman 1988) were determined by using vanillin-HCl and rhodanine assays, respectively. Gallic acid was used to calibrate the quantification of total phenolics and gallotannins, and (±) catechin was used as a calibration standard to quantify total flavanols and proanthocyanidins. Data were expressed as mg gallic acid equivalents (GAEs) or mg catechin equivalents (CEs)/g dry weight.

Extraction of Phenolic Acids from Carob Pod

Phenolic acids, in particular phenolic fractions, were extracted and isolated according to Cvikrová *et al.* (1994). Triplicate 160-g fruit samples were treated with liquid N_2 and ground in 80% methanol containing an antioxidant 2,6-ditercbutyl-β-cresol in an electrical high-speed blender. The homogenate was boiled under reflux for 20 min, filtered and concentrated under vacuum in a rotary evaporator. The concentrate was acidified with 1 mol/L HCl to pH = 2 and then extracted four times with 100 mL diethyl ether. The organic phase was evaporated to dryness under vacuum at 40C, and the residue was dissolved in methanol, filtered using a 0.45-μm microfilter (Whatman No. 1) and then used for the analysis of the free phenolic acids.

After extraction, the aqueous phase was divided into two parts. The first half was hydrolyzed with 2-mol/L NaOH for 4.5 h under a nitrogen atmosphere at room temperature, then acidified with 6-mol/L HCl to pH = 2 and processed as described above for free acids (FAs). This fraction contained methanol-soluble phenolic esters (MSPEs).

One mole per liter HCl was added to the second half of the aqueous phase, and the concentrate (pH = 2) was placed under a nitrogen atmosphere and hydrolyzed for 1 h at 100C. The hydrolysate, containing methanol-soluble phenolic glycosides, was processed as described for the previous fractions.

Determination of Phenolic Acids

Phenolic acids were analyzed by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) as described earlier (Ayaz *et al.* 2005). Briefly, internal standards of deuterium-labeled salicylic and *p*-hydroxybenzoic acids were added to all the extracted and filtered sample solutions to a final concentration of 10^{-5} mol/L. Ten microliters of the sample solutions were injected on a reversed phase column (Luna Phenyl-Hexyl, 5 µm, 250 × 2 mm; Phenomenex, Torrance, CA). HPLC-MS analyses were performed on an Alliance 2690 Separations Module (Waters, Milford, MA) linked simultaneously to a PDA 996 (Waters) and a ZSpray Mass Detector (ZMD) mass (2000) single quadrupole mass spectrometer equipped with an electrospray interface (Micromass, Manchester, U.K.). Data were processed by MassLynx software (Data Handling System for Windows, version 4.0, Micromass, Altrincham, U.K.). The quantification was based on the ratio of peak area of the analyte to the average peak area of the internal standards. Deuterium labeled internal standards of (2,3,5,6-$^{2}H_4$) *p*-hydroxybenzoic and (3,4,5,6-$^{2}H_4$) salicylic acids were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA).

Mineral Analysis

Fruits were washed with tap water, followed by distilled water containing a nonionic detergent, and finally three rinses of distilled water, and then were oven-dried at 60C for 48 h. The pods were milled, ground, ashed at 450C, and digested in 10 mL 1 mol/L HCl. Among major minerals, K was measured by flame photometry and P colorimetrically by the molybdo-vanadate method (Kitson and Mellon 1944). Ca, Mg and trace minerals (Fe, Mn and Zn) were measured by atomic absorption spectrometry (AA680, Shimadzu, Japan). Standardized procedures for measuring the mineral element concentrations followed the guidelines of the AOAC (1990). The concentrations are expressed on a dry matter basis.

RESULTS AND DISCUSSION

Amino Acids

The protein content of carob pods collected from southern and western Anatolia and assayed by elemental analysis, was found to be 4.45 ± 0.40 g protein/100 g dry weight, closely similar to the value of 4.5 g/100 g dry weight protein for carob pod reported by Morton (1987). In eight pods of Sicilian origin, a variation in protein content ranging between 1–5% of dry weight (mean 3 ± 2%) was reported by Avallone *et al.* (1997).

Subsequently, amino acids in the fruit were identified and quantified in acid hydrolysates (6-mol/L HCl). Eighteen amino acids were detected in pods sampled in Anatolia (Table 1). Aspartic (aspartic acid + asparagine), alanine, glutamic acid (glutamic acid + glutamine), leucine and valine together comprised ca. 57% of the total amino acid content of the pods. At a concentration of 18.25 g/100 g dry weight protein was the predominant amino acid present. The level of four remaining amino acids, alanine, glutamic acid, leucine and valine, ranged between 9.1–10.6 g/100 g dry weight protein. Cysteine was the amino acid showing the lowest concentration (0.80 g/100 g dry weight protein).

Numerous studies have demonstrated that each fruit has its own characteristic amino acid pattern, which can vary between fruit type and their processed products depending on species variety, geographical origin, stage of maturation and cultural practices. For instance, asparagine, aspartic acid, glutamic acid and serine were found as the major free amino acids in apple fruit (Gomis *et al.* 1990, 1992), comprising about 80% of their total free amino acid content. Medlar (*Mespilus germanica* L.) fruit, which belongs to the same family (Rosaceae) as apple and pear, also contained an abundance of aspartate and glutamate (1.1 and 1.2 g/kg dry weight, respectively) (Glew *et al.* 2003a). In quince pulp and peel, the major amino acids found were aspartic acid (189.9 μg/kg) and glycine (217.6 μg/kg) (Silva *et al.* 2004). The abundance of γ-aminobutyric, asparagine and alanine in Arabian dates showed varietal differences in terms of their amino acids profiles (Booij *et al.* 1993). In contrast, we did not find any detectable levels of asparagine and glutamine in the Anatolian carob pod studied here and in medlar fruit reported previously (Glew *et al.* 2003a), while higher levels of glutamine, arginine and asparagine were determined in Italian kiwi fruits (Castaldo *et al.* 1992) (Table 1).

Sugars and Organic Acids

The ethanolic extracts of carob pod, obtained from carob trees (*C. siliqua* L.) sampled in Anatolia, contained three major sugars: sucrose (437.3 mg/g dry weight), glucose (395.3 mg/g dry weight) and fructose (42.3 mg/g dry

TABLE 1.
AMINO ACID COMPOSITION OF CAROB POD (*CERATONIA SILIQUA* L.) AND OTHER FRUITS

Compound	Carob pod*	Medlar*§	Apple¶	Banana**	Kiwifruit††
Aspartic acid†	18.25 ± 0.21	0.11	111	1.2	200
Glutamic acid‡	9.65 ± 0.35	0.12	78	1.4	144
Asparagine	–	–	613	56.5	709
Glutamine	–	–	68	32	956
Serine	6.80 ± 0.57	0.05	27	12.9	51
Histidine	2.80 ± 0.00	0.01	11.3	42.5	24
Glycine	3.55 ± 0.07	0.04	3.5	4.3	6.6
Threonine	5.10 ± 0.14	0.05	9.7	5.0	36
Arginine	3.20 ± 0.42	0.04	1.9	–	898
Alanine	10.55 ± 0.07	0.04	10.3	2.8	103
Proline	5.80 ± 0.42	0.06	–	7.5	–
Tyrosine	1.70 ± 0.00	0.02	–	–	–
Valine	9.05 ± 0.35	0.06	25.4	7.8	38
Methionine	1.40 ± 0.28	0.02	–	–	–
Cysteine	0.80 ± 0.28	0.005	–	–	–
Isoleucine	3.80 ± 0.14	0.07	–	–	–
Leucine	9.30 ± 0.00	–	19.9	14.8	19
Phenylalanine	3.10 ± 0.14	0.05	–	–	–
Lysine	4.20 ± 0.00	0.05	–	3.1	18
Tryptophan	0.95 ± 0.07	0.02	–	–	–
γ-Aminobutyric acid	–	–	5.1	8.5	156

* Data expressed as g/100 g protein are the mean ± SDS of two analyses.
† Aspartic acid + asparagine.
‡ Glutamic acid + glutamine.
§ Glew *et al.* (2003a) (data were converted into g/100 g dry wt).
¶ Gomis *et al.* (1990) data are µg/fresh wt.
** Steward (1960) data are µg/fresh wt.
†† Castaldo *et al.* (1992) data are µg/fresh wt.

weight). Together these three sugars accounted for 87.54% of the total dry weight of the extracts, with sucrose predominating.

Malic acid (2.4 mg/g dry weight) was detected in the pods, but citric and ascorbic acids were not present in detectable amounts (Table 2).

The sugar content of fruit can vary considerably according to species, variety, physiological maturity, harvest season, climate and storage conditions. For comparison with the Anatolian carob pod studied here, Table 1 includes the content of soluble sugars and organic acids of several other common fruit species. Compared to our observations on carob, high levels of fructose were found in an apple variety (Ackerman *et al.* 1992), in medlar (Romero-Rodriguez *et al.* 2000; Glew *et al.* 2003a,b) and in some varieties of persimmon (*Diospyros kaki* L.) (Senter *et al.* 1991) (Table 2). Elsewhere, the

TABLE 2.
SUGAR AND ORGANIC ACID COMPOSITIONS OF CAROB PODS (mg/g DRY WT) AND OTHER FRUITS*

Compounds		Fruits		
Sugars	Carob pod	Persimmon§	Pomegranate¶	Medlar**
Sucrose	437.3 ± 10.6	161.08	0.10	12.40
Glucose	395.8 ± 2.9	212.44	61.40	212.30
Fructose	42.3 ± 2.8	191.98	65.80	347.10
Glucose/Fructose	0.2	1.1	0.9	0.6
ΣSugar†	875.4	565.50	126.20	571.80
Acids				
Malic acid	2.4 ± 0.1	5.64	1.39	25.30
Citric acid	n.d.	1.56	2.82	n.d.
Ascorbic acid	n.d.	n.d.	n.d	n.d.
ΣAcid‡	2.4	7.20	4.74	25.30

* Values, means of three independent extractions and determinations ($n = 3$).
† ΣSugar is the sum of sucrose, glucose and fructose.
‡ ΣAcid is the sum of malic, citric and ascorbic acids.
§ Senter *et al.* (1991) data of average of five common cultivar (data were converted into mg/g dry wt).
¶ Melgarejo *et al.* (2000) data of average of sweet, sour sweet and sour varieties (data were converted into mg/g dry wt).
** Romero-Rodriguez *et al.* (2000) data of harvest season of the fruit (data were converted into mg/g dry wt).
n.d., not detected.

presence of sucrose, glucose, fructose and maltose were also reported in carob pod (Morton 1987). Almost 80.7 g carbohydrate per 100 g dry weight was reported by Morton (1987). Avallone *et al.* (1997) determined ~34 ± 3.6% sucrose, ~4 ± 1% glucose and ~6 ± 2% fructose in eight different carob pods from *C. siliqua* grown in Sicily (Italy). As found here, sucrose was the predominant sugar and fructose was present at relatively low concentrations in pods of Anatolian carob tree. Generally, however, levels of sucrose, glucose and fructose in carob pod reported in the literature showed geographical variation (Würsch *et al.* 1984; Saura-Calixto 1988; Avallone *et al.* 1997).

Phenolic Acids

Three phenolic acids (gallic, syringic and sinapic acids) were identified by HPLC-MS in the carob pod extracts (Table 3). With the exception of syringic acid, which was not detected in the MSPE fraction, the acids were found in all the fractions extracted, namely as the FAs, as MSPEs and as methanol soluble phenolic glycosides (MSPGs). Gallic acid was the most abundant phenolic acid in carob pod and dominated in all fractions isolated

TABLE 3.
PHENOLIC ACID COMPOSITION (μg/g DRY WEIGHT) OF PARTICULAR PHENOLIC FRACTIONS OF CAROB PODS*

Phenolic fractions				
Compounds	Free	Esters§	Glycosides¶	Total†
Gallic acid	1,249.5 ± 206.9	1,550.5 ± 119.8	468.3 ± 40.3	3,268.4
Syringic acid	3.6 ± 0.7	n.d.	4.4 ± 0.6	8.0
Sinapic acid	1.8 ± 0.1	2.0 ± 0.2	0.7 ± 0.2	4.5
$\Sigma_{benzoics}$	1,253.1	1,551	472.7	3,276.8
$\Sigma_{cinnamic}$	1.8	2.0	0.7	4.5
$\Sigma_{benzoics}$ (%)	99.9	99.9	99.9	99.9
$\Sigma_{cinnamic}$ (%)	0.14	0.13	0.15	0.14
Total‡	1,254.9	1,552.5	473.4	3,280.9

* Values, means of three independent extractions and determinations ($n = 3$).
† Total is sum of each phenolic acid of four phenolic fraction.
‡ Total is sum of individual phenolic acids identified in each phenolic fraction.
§ MSPEs, methanol soluble phenolic esters.
¶ MSPGs, methanol soluble phenolic glycosides.

(Table 3). The amount of gallic acid reached 1,550.5 μg/g dry weight in MSPEs, 1,249.5 μg/g dry weight in FAs and 468.3 μg/g dry weight in MSPG fraction. Total phenolics expressed as the sum of individual phenolic acids were estimated high for the MSPEs and free fractions, respectively (Table 3). The comparative analyses of phenolic acids in the three phenolic fractions revealed that amounts of MSPGs were generally lower. The carob pod estimated in the present study contained two hydroxybenzoic acid derivatives (HBAs) represented by gallic and syringic acids, and one hydroxycinnamic acid derivative (HCAs) represented by sinapic acid. The total HBAs were greater than the total HCAs (overall, about 99.9% and ~0.1% respectively; Table 3). A high level of gallic acid in carob pods was also reported by Nachtomi and Alumot (1963). The sum of individual phenolic acids (Table 3) was about 24.3% of total phenolic content determined by Folin-Ciocalteu reagent (see below). These findings could be explained by the presence of other phenolic compounds, like flavonoids, lignin derivatives and condensed polyphenols that were not monitored by the HPLC-MS method used in this study.

An apparent variation in phenolic contents has been found between Sicily and Anatolian carob pods. Exceptionally, in the present study, we found the concentration of total phenolic compounds to be 13.51 mg GAE/g dry weight. In Sicilian carob pods, content of total polyphenols was found at level of 1.9 mg/g dry weight (Avallone *et al.* 1997). Another study of carob pods showed their contents in total 19.2 g/100 g dry weight (Glew *et al.* 2003a). Furthermore carob pods were reported to contain 6.1% of total polyphenols,

and chemical degradation of tannins produced flavanols including catechin, epicatechin, epigallocatechin, epigallocatechin gallate, and epicatechin gallate along with simpler phenolics such as phloroglucinol, pyrogallol, catechol and gallic acid (Lambraki and Karagouni 1998). With regard to the content of other polyphenols and tannic fractions, Avallone *et al.* (1997) reported almost equal amount of proanthocyanidins (2.75 mg/g) and gallotannins (0.44 mg/g) in eight different carob pod samples collected in Sicily (Italy). In the present study, the analysis continued on Anatolian carob pods have revealed almost equal amount of total flavonoids, comprising 0.41 mg CE/g dry weight, when compared with Crete (Greece) origins (0.48 mg CE/g dry weight). Similarly, almost an equal amount of proanthocyanidins (3.21 mg CE/g dry weight) and gallotannins (0.36 mg GAE/g dry weight) in Anatolian carob pods were also reported in Sicily origins, 2.75 ± 0.75 mg CE/g and 0.44 ± 0.12 mg/g dry weight, respectively (Avallone *et al.* 1997).

Variations within and among carob pod origins in polyphenol and tannic fraction content can be probably due to the geographical, variety, cultural conditions or degree of maturation in origins. This relation was well documented in Sicilian carob pods by Avallone *et al.* (Avallone *et al.* 1997; Glew *et al.* 2003a) who found large variations in the content of total polyphenols (15.8–24.4 mg/g), proanthocyanidins (2.1–3.9 mg CE/g dry weight), ellagitannins (0.3–7 mg HHDG/g dry weight) and gallotannins (0.2–6 mg GAE/g dry weight) collected from eight different locations in one geographical region.

Mineral Nutrients

We determined the levels of four major (Ca, P, K and Mg) and four trace (Fe, Cu, Mn and Zn) minerals in Anatolian carob pod samples. Results are presented in Table 4, together with the published results of similar analyses conducted on other fruit species for comparison. Among the major minerals measured in carob, K predominated, but high levels of Ca were also found. Levels of P and Mg were considerably lower, but within the general range of concentrations found for these minerals in other fruit species (Table 4). Potassium was also determined as the major mineral in other fruits, including kiwi (Castaldo *et al.* 1992), apple, quince, loquat, fig and date (Souci *et al.* 1994), medlar (Glew *et al.* 2003c), apricot (Lo Voi *et al.* 1995) and persimmon (Gorinstein *et al.* 2001) (Table 4). Ca and P concentrations (300 and 71 mg/g dry weight, respectively) were very similar to those previously reported from carob pod by Morton (1987) (352 and 81 mg/g dry weight, respectively). Although the general trends in concentration of major minerals were similar in carob and in other fruit species (Table 4), substantial variation in the levels of individual elements occurred. In the absence of information on such factors as soil type and seasonal climate, more detailed comparison is not justified.

TABLE 4.
CONCENTRATIONS (mg/100 g DRY WEIGHT) OF SEVERAL MINERALS IN CAROB PODS AND OTHER FRUITS

	Major minerals					Date†	Medlar‡
	Carob pod*	Apple†	Quince†	Loquat†	Fig†		
K	970 ± 0	979.6	1,189.4	2,023.1	1,212.1	814.5	737.0
P	71 ± 1	81.6		176.9	161.6	71.4	10.80
Ca	300 ± 0	48.3	61.0	146.2	272.7	79.0	178.0
Mg	60 ± 0	43.5	48.5	76.9	101.0	62.7	66.1
	Trace minerals						
Fe	1.88 ± 0.07	3.3	3.6	2.3	3.0	2.4	1.34
Mn	1.29 ± 0.02	0.33	0.12		0.22	0.19	1.02
Zn	0.75 ± 0.05	0.7	1.18		0.12	0.50	0.1
Cu	0.85 ± 0.06	0.36	0.60		0.41	0.41	0.36

* Values, means of three independent extractions and determinations ($n = 3$).
† Souci *et al.* (1994).
‡ Glew *et al.* (2003c)

Among the trace minerals measured in carob pod, Fe and Mn predominated, although substantial amounts of Zn and Cu were also present. Iron is also the most abundant trace mineral present in the other fruits included in Table 4, although levels varied considerably between species, with medlar fruit and carob pod containing the lowest amounts. In common with quince and apple, carob pod also contained substantial amounts of Zn; amounts in fig, date and medlar fruits, however, were lower (Table 4).

CONCLUSION

The knowledge of the exact qualitative and quantitative distribution of the amino, organic and phenolic acids, sugars, and minerals, characteristic in the carob pod, is of vital importance for the evaluation of its food quality. In recent years, the sale and consumption of food products manufactured from carob pods, including "carob cocoa," finer flour, jam, pekmez (molasses) or other food products, has been increased. The fruit have a potential sugar content of around 48–72%. Thus the pulp of carob pods may be a significant source of sugars for industry, and its phenolics can be evaluated for its antioxidant capacity. This importance was previously reported (Makris and Kefalas 2004) in Greek carob pods. Carob pod is a cheap source of sugars and natural polyphenolics, the nature and importance of which is, as yet, poorly investi-

gated. Carob pods also contain nutritionally important amino acids (aspartic and glutamic acids, alanine, valine, etc.) and minerals (K and Ca) that play a significant role in human health. The present study reports the first data on the chemical composition of the Anatolian carob pod. This type of information could provide local and neighboring populations (where carob trees are native species in their geography) with a basis for food choices. Also, the present results could have a value of contribution to explain the effects of geographical, variety, cultural conditions or degree of maturation on the accumulation of phenolics in the fruit.

ACKNOWLEDGMENTS

We would like to thank the Ministry of Education of the Czech Republic for financial support (Grant No. MSM 198959216). Part of this work was also financially supported by the Fund of Karadeniz Technical University (Project No. 2004.111.004.6 and 2005.111.004.02) and Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) (Project No. TBAG-2341 [103T152]).

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