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Nutrient Content of Carob Pod (*Ceratonia siliqua* L.) Flour Prepared Commercially and Domestically

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Abstract Although the fruit of the carob tree (*Ceratonia siliqua* L. Fabaceae) is nutritious and widely available in Turkey, especially in West and South Anatolia, much remains to be learned about its nutrient composition. The main goal of our study was to determine if there are differences in the content of certain nutrients in commercially-prepared carob flour (CPCP) and domestic or home-prepared carob powder (HPCP). Sucrose was the main sugar in CPCP and HPCP. Total protein was 40% lower in CPCP than HPCP due mainly to decreases in the content of several essential amino acids. However, except for lysine in CPCP, HPCP and CPCP compared favourably to a WHO protein standard. There were large differences in terms of their content of the two essential fatty acids, linoleic and α-linolenic acid, and the linoleic acid/α-linolenic acid ratio was 3.6 for CPCP, and 6.1 for HPCP. Manganese and iron were 2.5-fold higher in HPCP than CPCP. This study demonstrates that carob flour prepared in either the household or industrially is a good source of many, but not all essential nutrients, and that commercial processing of carob fruit into flour seems to affect its content of several important nutrients.

Keywords *Ceratonia siliqua* · Carob pod · Sugar · Minerals · Fatty acids · Amino acids

Abbreviations

AA	Ascorbic acid
HDL	High-density lipoprotein
HPCP	Home-prepared carob powder
HPLC	High-performance liquid chromatography
LDL	Low-density lipoprotein
MUSFA	Monounsaturated fatty acid
MVD	Micro-vacuum degasser
CPCP	Commercially-prepared carob powder
PUSFA	Polyunsaturated fatty acid
RID	Refractive index detector
TCC	Thermostated column compartment
WHO	World Health Organization

Introduction

The carob (*Ceratonia siliqua* L.) tree, also called locust, belongs to the family of Fabaceae and grows in many parts of the Mediterranean region. The non-fleshy, bean-like fruit of carob tree [1] which is called carob pod, is light to dark brown and straight or slightly curved. In Turkey, carob pod fruit is used either directly in a form designated "*home prepared flour*" or as a comercially-processed flour called

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"carob powder", which is sold in local markets and large stores. From a nutritional perspective, two parts of the pod can be distinguished: the kibble or "locust bean" and the seeds or "locust kernel gum". The kibble or locust bean is used directly after sun-drying and grinding to a fine powder in the home by the local population; this product is called "*household flour*". Alternatively, carob kibble is processed industrially and sold in large stores and local markets as "*carob* or *cacao powder* " [2–5]. According to recent data, carob-pod production world-wide amounts are nearly 400,000 tonnes per year from about 200,000 ha [3, 6, 7], Turkey being the least (5%) with 13,500 tonnes production per year from 354,000 trees [4], depending on the cultivar, region and cultivation practices [4, 6].

Our laboratory recently reported the nutrient contents of carob fruit [2]. We found that domestically produced carob flour contained 4.45% protein: aspartic (aspartic acid + asparagine), alanine, glutamic acid (glutamic acid + glutamine), leucine and valine; together comprised 57% of the total amino acid content of the pods. With regard to sugars, carob pod contained mostly sucrose and glucose with lesser amounts of fructose. The minerals that were especially abundant were potassium and calcium. As for trace elements, carob fruit contained significant quantities of iron, manganese, zinc, and copper. The main phenolic compound we found in carob pods was gallic acid [2].

This study was undertaken because we were aware of the considerable differences between the methods in which carob flour is prepared commercially versus how this food is prepared domestically by the local population. Commercial producers use mechanical milling devices and high-temperature drying ovens to turn the carob pods into flour. In contrast, small-scale domestic producers sun-dry the carob pods for several weeks in the open air, then grind the dried pods with stone or wooden mortar and pestle, and finally store the dry product in cloth bags, glass jars, ceramic bowls, or metal containers. Since these differences in terms of production and packaging could have very different effects on the nutrient content of the final carob product, we were interested in comparing the amino acid, mineral and trace element, and fatty acid compositions of carob flour commercially and domestically-prepared in Turkey.

Materials and Methods

Selection and Purchasing Carob Pod (Kibble) Samples

Six samples of home powdered carob kibble designated "home-prepared carob powder, HPCP" were provided by local villagers (30 different farmers, ~10 g each) living in towns of cities (Antalya, Mersin, Muğla and İzmir) in the southwestern region of Anatolia in Turkey. Four samples of industrially-produced carob flour designated "commercially-prepared carob powder, CPCP" were obtained from four different manufacturers with their permission. The two different kinds of carob flour were blended individually and re-powdered with the aid of a mortar and pestle to provide a uniformity in grain size. The blended samples were divided into three portions which were then analyzed. All extractions and determinations were made in triplicate and the results were expressed as dry weight basis (dry wt).

Soluble Sugar Analysis

Samples of the two kinds of carob flour (1 g dry wt) were extracted using the method previously described by Ayaz et al. [2]. Ethanol soluble sugars were analyzed by HPLC-RI. One mililiter of extract filtered through a 0.45 μm nylon filter (Whatman Inc., Springfield Mill, UK) and 10 μL were injected to a Agilent 1100 Series (Palo Alto, CA) instrument equipped with a Nucleosil C18 Carbohydrate analytical column (250×4.0 mm i.d., 10 μm particle size) and a RI Agilent 1100 dedector (Palo Alto, CA). The column temperature was 25 °C. The mobile phase was acetonitrile:water (79:21) for isocratic elution at a flow rate of 2 ml/min. Sugars (sucrose, glucose, fructose, maltose, and lactose) were identified by comparison of their retention times to those of authentic standards. Peak areas were quantified using HP ChemStation software.

Amino Acid Analysis

Five to 9 mg of each of two kinds of carob preparations were weighed and extracted according to Glew et al. [8], and hydrolysis of the samples was performed using the method of Cohen and Strydom [9]. Quantification of amino acids was performed using a Pierce Standard H amino acid calibration mixture (Rockford, IL) with gradient conditions described by Bidlingmeyer et al. [10].

Fatty Acid Analysis

Total lipid extraction of triplicate samples of each of the dried specimens were extracted according to Glew et al. [8], and their transmethylation was performed using the method of Morrison and Smith [11]. Fatty acids were separated and quantified using a Hewlett-Packard gas chromatograph (5890 Series II) equipped with a flame-ionization detector, and the running conditions were carried out according to previously published article [8]. Heptadecanoic acid (C17:0) as a internal standard and calibration standards (NuCheck, Elysian, MN) were used for quantitation of fatty acids in the lipid extracts. The fatty acid values we report represent the average of three determinations.

Mineral Analysis

The samples (approx. 0.2 g of each) were dried for five days in a desiccator. Digestion of the samples and ICP-OES analysis of trace metals were performed according to Glew et al. [8]. A 1:20 dilution was required for calcium analysis of all sample solutions. This is a modification of NMAM 7300 that is used for bulk samples. This digestion technique does not dissolve any siliceous material present in the samples.

The amino acid, fatty acid, and mineral and trace element contents are reported as mean values ± standard deviation.

Results and Discussion

Soluble Sugar Composition of HPCP and CPCP

Previous studies have demonstrated that carob pod is rich in sugars [2, 4, 12–14], a property that has allowed it to serve as a raw material in the manufacture of fermentation products such as citric acid [3, 15]. We found large amounts of sucrose (146 and 309 mg/g dry wt in HPCP and CPCP, respectively), but small amounts of glucose and fructose (Table 1). The finding of an abundance of sucrose agrees with our previous and the other studies of carob flour [2, 4, 12–14].

Amino Acid Composition of HPCP and CPCP

Seventeen amino acids were analyzed in acid hydrolysates of the two carob pod preparations. Except glycine, the content of the other amino acids was higher in HPCP than in CPCP, except glycine. Aspartic acid (aspartic acid plus asparagine) was the most abundant amino acid (4.13 mg/g dry wt) in HPCP whereas glutamic acid (glutamic acid + glutamine) was the most abundant amino acid (2.47 mg/g dry wt) in CPCP. The second most abundant amino acid in HPCP was alanine (2.76 mg/g dry wt) and in CPCP aspartic acid (2.19 mg/g dry wt) was the major amino acid (Table 2). Similar results were obtained in a recent study by Ayaz and colleagues [2] of the amino acid composition of raw carob pod harvested in southwestern Anatolia (Turkey). In the present both the present study of HPCP and our previous report of raw carob pod [2], we found that the cysteine + methionine pair was present in the lowest amount. In contrast, in the CPCP preparation, lysine (0.26 mg/g dry wt) rather then cysteine + methionine was the least abundant amino acid. The difference in the lysine content of domestically versus commercially-prepared carob flour could be the result of the higher temperatures used in the commercial process which could have resulted in more extensive destruction of lysine caused by the Maillard

Table 1 Fatty acid and sugar profiles of two preparations of carob tree (*Ceratonia siliqua* L.) pod[a]. Values represent the mean ± SD of three separate extractions and determinations of completely random experimental design. Duncan's Multiple Range Test was used to determine the statistical significance of differences among the means (SAS Institute Inc., Cary, NC, USA). Means were compared within each row of the data. For comparisons among the means analysis of variance was used. Values with the same letter are not significantly different at $P<0.05$

Compound	HPCP[b] (n=3)	CPCP[c] (n=3)
Fatty acids (μg/g dry wt)		
C14:0	5.9±0.5 a	n.d.[d]
C16:0	270±13 a	257±4 a
C16:1n-7	27.5±0.5 b	4.6±0.2 a
C18:0	38.5±2.8 a	47.1±1.4 b
C18:1n-9	562±32 a	730±18 b
C18:1n-7	11.5±0.3 a	12.8±0.4 b
C18:2n-6	245±16 b	208±8 a
C18:3n-3	40.1±3.9 a	57.3±2.4 b
C20:0	11.4±1.1 b	7.6±1.3 a
C22:0	9.8±0.5 a	9.9±1.3 a
C22:1	10.1±0.8 a	20.5±3.4 b
$\Sigma_{\text{total fat}}$[e]	1,230±71	1,350±40
$\Sigma_{\text{saturation}}$	335±18	322±8
% Saturation	27.21	23.74
$\Sigma_{\text{unsaturation}}$	897±53.1	1,030±32
% Unsaturation	72.8	76.3
$\Sigma_{\text{total lipid (\%)}}$[f]	4.44	4.23
MUSFA[g]	611	768
PUSFA[h]	285	265
USA/SA[i]	2.7	3.2
Sugars (mg/g dry wt)		
Fructose	69.6±0.8 a	73.9±5.5 a
Glucose	29.7±1.1 a	53.4±2.8 b
Sucrose	146±3 a	309±5 b
G/F[j]	0.4	0.7
ΣSugar[k]	245	436

[a] The following fatty acids were not detected: C14:1, C15:0, C18:3n-6, C20:1, C20:2n-6, C20:3n-6, C20:4n-6, C20:3n-3, C20:5n-3, C22:2, C22:4n-6, C22:5n-6, C22:5n-3, C24:0, C22:6n-3 and C24:1

[b] *HPCP* home-prepared carob powder

[c] *CPCP* commercially processed carob powder (purchased from the manufacturers with their permission)

[d] *n.d.* not detected

[e] $\Sigma_{\text{total fat}}$ total fat is the sum of the individual fatty acids

[f] $\Sigma_{\text{total lipid}}$ total extractable lipid with chloroform:methanol (2:1, *v*/*v*)

[g] *MUSFA* monounsaturated fatty acids

[h] *PUSFA* polyunsaturated fatty acids

[i] *USA/SA* unsaturation/saturation

[j] *G/F* glucose/fructose

[k] ΣSugar total sugar is the sum of the individual sugars

Table 2 Amino acid composition (mg/g dry wt) of two preparations of carob tree (*Ceratonia siliqua* L.) pod and comparison of the proportions (mg/g dry wt) of essential amino acids in two preparations of carob tree (*Ceratonia siliqua* L.) pod versus the WHO standard protein. Values represent the mean ± SD of three separate extractions and determinations of completely random experimental design. Duncan's Multiple Range Test was used to determine the statistical significance of differences among the means (SAS Institute Inc., Cary, NC, USA). Means were compared within each row of the data. For comparisons among the means analysis of variance was used. Values with the same letter are not significantly different at $P<0.05$

Amino acid	HPCP[a] (n=3)	CPCP[b] (n=3)
Aspartic, Asp	4.13±0.18 b[c]	2.19±0.10 a
Threonine, Thr	1.46±0.14 b	1.00±0.05 a
Serine, Ser	1.47±0.18 b	1.06±0.05 a
Glutamic, Glu	2.50±0.27 a	2.47±0.11 a
Glycine, Gly	0.86±0.11 a	0.95±0.04 a
Alanine, Ala	2.76±0.06 b	1.19±0.04 a
Valine, Val	2.39±0.13 b	1.21±0.07 a
Isoleucine, Ile	1.19±0.04 b	0.86±0.05 a
Leucine, Leu	2.41±0.15 b	1.41±0.08 a
Tyrosine, Tyr	0.71±0.06 a	0.61±0.06 a
Phenylalanine, Phe	0.82±0.11 a	0.75±0.05 a
Histidine, His	0.74±0.07 b	0.56±0.02 a
Lysine, Lys	1.06±0.15 b	0.26±0.02 a
Arginine, Arg	0.81±0.12 b	0.32±0.01 a
Cysteine, Cys	0.52±0.03 b	0.41±0.01 a
Proline, Pro	2.08±0.12 b	1.05±0.05 a
Methionine, Met	0.62±0.09 b	0.33±0.02 a
Tryptophan, Trp	n.d.[d]	n.d.
$\Sigma_{protein}$[e]	26.5±2.01	16.6±0.83
$\Sigma_{protein}$%	2.64	1.67
Comparison of essential amino acids with WHO[f] standard protein		
Cysteine + methionine	123	126
Threonine	138	150
Valine	181	145
Isoleucine	90	129
Leucine	130	121
Tyrosine + phenylalanine	97	136
Lysine	73	28
Tryptophan	n.d.	n.d.

[a] *HPCP* home-prepared carob powder

[b] *CPCP* commercially processed carob powder (purchased from the manufacturers with their permission)

[c] Values are the means of three separate extractions and determination

[d] *n.d.* not determined

[e] $\Sigma_{protein}$ total protein is the sum of the individual amino acids

[f] A score of 100 means the amino acid is present at a level (i.e., proportion or percentage) equivalent to that in the WHO standard protein. The only amino acid that fall significantly below the WHO standard was lysine

(browning) reaction which involves the reaction of the epsilon amino group of lysine residues in proteins with carbohydrates in the carob flour [16, 17].

The protein content, estimated by the sum of individual amino acids, was 26.5 mg/g dry wt for HPCP and 16.6 mg/g dry wt for CPCP (Table 2). We did not test for tryptophan in the present study; however, in our previous study of Anatolian carob pod, tryptophan was present at approximately 1 mg/g dry wt [2]. The amino acid content of carob pod we report in the present study agrees with values reported by other investigators [12, 18].

To assess the relative nutritional quality of the protein component of the two carob preparations, we compared their proportions of essential amino acids versus the percentages of the various essential amino acids in a World Health Organization (WHO) standard protein [19]. As shown in Table 2, the protein in the two carob pod preparations compared reasonably well with the WHO standard protein: for HPCP and CPCP, only one essential amino acid, namely lysine, had a score that fell much below 100% (Table 2). The results of the present study indicate that, in terms of both absolute amounts and proportions of the essential amino acids, commercially prepared carob pod (trade name "carob powder", "carob cacao" or "Keçi boynuzu tozu" in Turkish), appears to be inferior to the domestically-prepared powder (HPCP) (Table 2).

Fatty Acid Composition of HPCP and CPCP

Analysis of fatty acid methyl esters by gas-liquid chromatography revealed the presence of 11 fatty acids in home-prepared (HPCP), and ten fatty acids in commercially processed (CPCP) carob pod. Oleic acid (C18:1n-9) was the most abundant fatty acid in both samples, accounting for 562±32 (45%) and 730±18 (54%) μg/g of the dry wt in HPCP and CPCP, respectively, and these differences were significant (Table 1). The cardiovascular protective effects of oleic acid are widely recognized [20]. Oleic acid has also been shown to slow the progression of adrenoleucodystrophy (ALD), a fatal disease that affects the brain and adrenal glands [21]. A small amount of myristic acid (C14:0) was present in HPCP (5.9 μg/g of dry wt), but was not detected in the chloroform:methanol extract of CPCP. Palmitic acid (16:0) was the most abundant saturated fatty acid in both kinds of carob flour: it represented 22% and 19% of the fatty acid total in HPCP and CPCP, respectively. With regard to linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), both carob flour preparations contained nutritionally significant amounts of these two essential fatty acids; however, the linoleic acid/α-linolenic acid ratio was much higher in HPCP than in CPCP (6.1 versus 3.6). Since, a high linoleic acid/α-linolenic acid ratio is pro-inflammatory and widely regarded as a risk factor for

cardiovascular disease [22], it appears that in this regard at least CPCP flour is more advantageous nutritionally than HPCP flour. With regard to total saturated (TS) fatty acids, and total unsaturated (TUS) fatty acids, HPCP and CPCP contained similar amounts of TS fatty acid (335 μg/g dry wt and 322 μg/g dry wt, respectively). In contrast, there was a large difference in the amount of TUS fatty acids in the two kinds of carob flour: CPCP contained 1,030±32 μg/g dry wt TUS fatty acids compared with 897±53 μg/g dry wt TUS fatty acids in HPCP. The total lipid content of the two carob preparations was nearly the same (4.44% for HPCP and 4.23% for CPCP).

In general, plants are a good source of ω-3 and ω-6 fatty acids. The relatively large quantities of both C18:1n-9 and C18:2n-6 acids in carob flour, could provide beneficial amounts of these important fatty acids in the diets of people who live in the Mediterranean region and elsewhere in the world where *Ceratonia siliqua* grows, and for individuals who appreciate carob flour because of its relatively low content of saturated fatty acids and much higher content of unsaturated fatty acids. Diets containing α-linolenic acid and other ω-3 fatty acids are thought to lower the risk of various metabolic and cardiovascular diseases [23].

Mineral Composition in HPCP and CPCP

We analyzed 34 minerals and trace elements, but detected only those indicated in Table 3. Substantial and comparable quantification of the following elements that are essential in humans were found in the two preparations of carob flour: calcium, chromium, potassium, magnesium, phosphorus, and zinc. However, there were large differences in the content of iron and manganese. There was 2.5-fold more iron in CPCP than in HPCP, but only two-thirds as much manganese in CPCP as HPCP. All of the essential elements that we detected in HPCP were within the range of values reported for apple, loquat, date [24], and medlar [25]. Likewise, the small amount of lead in the domestically-prepared carob flour may have been originated from contact with lead-containing utensils or surfaces used during the processing of the carob fruit. Lead is toxic to humans in large measure because of its pathophysiologic effects on energy metabolism in the brain and its inhibition of heme

Table 3 Mineral and trace element content (μg/g dry wt) of two preparations of carob tree (*Ceratonia siliqua* L.) pod[a]. Values represent the mean ± SD of three separate extractions and determinations of completely random experimental design. Duncan's Multiple Range Test was used to determine the statistical significance of differences among the means (SAS Institute Inc., Cary, NC, USA). Means were compared within each row of the data (except Co and Pb contents). For comparisons among the means analysis of variance was used. Values with the same letter are not significantly different at $P<0.05$

Mineral	HPCP[b]	CPCP[c]	Apple[d]	Loquat[d]	Date[d]	Medlar[e]
Barium, Ba	14.3±2.6 b	1.2±0.0 a	–	–	–	19.7
Calcium, Ca	3,040±121a	2,910±44 a	483	1,462	790	1,780
Cobalt, Co	0.035±0.02	n.d.[f]	0.04	–	–	–
Chromium, Cr	0.4±0.0 a	0.6±0.2 a	–	–	–	–
Copper, Cu	5.2±1.3 a	3.4±0.1 a	3.6		4.1	3.6
Iron, Fe	15.1±2.8 a	38.5±7.3 b	33	23	24	13.4
Potassium, K	9,070±82 a	11,200±153 b	9,796	20,231	8,145	7,370
Magnesium, Mg	554±6 a	618±10 b	435	769	627	661
Manganese, Mn	10.4±0.8 b	3.7±0.4 a	3.3	–	1.9	10.2
Sodium, Na	113±42 a	140±26 a	–	–	–	183
Nickel, Ni	2.1±1.6 b	0.8±0.0 a	0.2	–	–	0.3
Phosphorus, P	703±16 a	803±17 a	816	1,769	714	1,080
Lead, Pb	0.21±0.02	n.d.	–	–	–	–
Strontium, Sr	3.0±0.1 a	7.9±0.1 b	–	–	–	16.3
Titanium, Ti	0.16±0.02 a	0.21±0.05 a	–	–	–	0.5
Zinc, Zn	6.6±0.7 a	7.0±0.3 a	7.0		5.0	7.1

[a] The following elements were not detected: molybdenum, selenium, tin and tungsten

[b] *HPCP* home-prepared carob powder

[c] *CPCP* commercially processed carob powder (purchased from the manufacturers with their permission)

[d] [24]

[e] [25]

[f] *n.d.* not detected

synthesis in reticulocytes which results in anemia. The developing fetus, infants and young children are especially vulnerable to even low-level lead exposure [26, 27]. If one were to consume in one day the equivalent of 10 g of HPCP that contained 0.21 μg Pb/g dry weight, this would account to a daily intake of 2.1 μg of lead, which corresponds to less than one percent of the toxic threshold (0.21–0.25 mg/day) [28]. The content of minerals and trace elements reported in this study for HPCP agrees well with data reported elsewhere for raw carob pod [2].

Since the domestically- and commercially-prepared carob flours analyzed in our study were not derived from the same batch of carob pod, we cannot say for certain that the differences observed in the content of particular nutrients (e.g., glucose, sucrose, lysine, iron, polyunsaturated fatty acids) between CPCP and HPCP were the result of some harsh and deleterious step, such as exposure to high temperature, which was involved in the commercial preparation of carob flour. We have planned a future study that is aimed to determine the amino acid, fatty acid, mineral and trace element composition of carob flour at each stage of the industrial process.

However, it is unlikely that the carob used by the commercial producers of carob flour and the carob plants used by the local populations to prepare "domestic" carob flour were very different. This is because the commercial and domestic producers of carob flour we analyzed purchased their raw carob from the same growers in towns located in the south and southwest regions of Turkey. The "domestic" carob pod flour we analyzed was purchased from 30 different farmers who were supplying carob pods to the commercial and domestic producers of carob flour. Thus, the differences we found in the content of certain nutrients between the commercial and HPCP carob flours are likely to be due to processing differences.

Conclusions

The main result of this study was that despite finding several differences in the content of essential nutrients between domestically and commercially prepared carob flours, both kinds of foods provide useful amounts of many critical factors such as protein, essential fatty acids and calcium and other minerals. Noteworthy differences between CPCP and HPCP included a higher content of iron, lower content of magnesium, less lysine and a more healthful linoleic/α-linolenic acid ratio in CPCP relative to HPCP. Future studies should asses the bioavailability of minerals and trace elements in care of flour.

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