CARBOHYDRATE CONTENT IN BULGARIAN AND TURKISH CAROB PODS AND THEIR PRODUCTS

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Abstract: Carob, *Ceratonia siliqua*, is cultivated for ornamental and industrial purposes in many Mediterranean countries. This study assessed carob pulp and syrup, a rich source of carbohydrates and sugars, by evaluating content of reducing sugars and total sugars in carob pulp before extraction of syrups. We identified the sugar content before and after treatment by using thin-layer (TLC) and high performance liquid chromatography with refractive index detection (HPLC-RID). It was established that total sugars increased with extraction and heat treatment. Sucrose (34.2 g/100 g dry weight; dw), glucose (11.1 g/100 g dw) and fructose (6.5 g/100 g dw) were the major sugars identified and quantified in pulp of the Turkish carob. *Ceratonia siliqua* pods of Turkish origin produced higher levels of total and of reducing sugars (fructose and sucrose) than did the pods from Bulgaria. The carbohydrate content in the syrup prepared from Turkish carob pods was highest, with the sucrose content especially reaching up to 45 g/100 g dw. The data are discussed in terms of nutritional and energy value of the carob pod. The carob and obtained products (flour or syrup) are identified as highly caloric and as a prospective energy source alternative to cocoa and its products.

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Introduction

Carob is mainly cultivated in the Mediterranean and Aegean regions of Turkey, while growing naturally in various parts of Bulgaria, including the coast of Black Sea, North-East Bulgaria, and the Balkan Mountains. The carob species, *Ceratonia siliqua*, belongs to the Fabaceae family, and grows well in varying climatic conditions, including high temperature and subtropical areas. This plant tolerates hot and humid coastal conditions and could adapt to regions with average 250 to 500 mm of rainfall per year (Santos, Rodrigus, & Teixeira, 2005). The deep rooted systems of this species can adapt to a wide variety of soil conditions. Turkey is considered one of the smallest producers of carob, with an annual production of 15 000 tons per year, while Bulgaria has no manufacturing related to this cultivar. Minimal cultivation management is required and the adaptability of this plant to various climatic and geographical conditions makes it a preferable cultivar (Hills, 1980).

The carob pod consists mainly of pulp (90%) and seeds (10%). Depending on the genotype, many species (wild genotypes) are important for their seeds (Gubbuk, Kafkas, Guven, & Gunes, 2010). The pulp is rich in sugars (48–56%; mainly sucrose) and consists of 16–20% condensed tannins (Battle & Tous, 1997; Sahin, Topuz, Pischetsrieder, & Ozdemir, 2009). It has low protein content, and according to the type of the cultivar (grafted or ungrafted), grafted trees will have high total-soluble sugar content (Marakis & Marakis, 1996). Regarding the antioxidant capacity (Kumazawa et al. 2002; Klaus, Pultz, Thone-Reineke, & Wolfram, 2005) related to its polyphenolic composition, carob kibbles reportedly contain 448 mg/kg extractable polyphenols, comprising gallic acid (174 mg/kg; Zunft, Luber, Harde, Graubaum, & Gruenwald, 2001; Zunft et al., 2003), hydrolysable tannins (26 mg) and condensed tannins (15 mg; Avallone, Plessi, Barldi, & Monzani, 1997; Makris & Kefalos, 2004), as well as derivatives of myricetin, quercetin, and kaempferol (Papagiannopoulos, Wollseifen, Mellenthin, Haber, & Galensa, 2004). The main phenolic compound in carob pods is gallic acid (Ayaz et al., 2007).

It has been reported that carobs contain high amounts of insoluble dietary fiber, as well as pinitol (Zunft et al., 2001). Fadel, et al. (2006) described the sensory qualities and flavor stability of low-priced cocoa substitute with high-quality characteristics. When carob samples (pulps, seeds, and flour) were compared for minerals, the seeds of the grafted samples generally contained higher mineral concentrations. Considering the mineral content of the carob fruit, calcium, potassium, magnesium, sodium, phosphorus, and iron were abundant. Magnesium was the mineral with the highest concentrations (between 265 and 859 mg/kg) in all samples. Among the micro minerals,

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iron had the highest concentration (between 16.8 and 82.0 mg/kg) in the grafted carob fruit seeds, which generally contained higher macro and micro minerals than the carob fruit pulp (Fidan & Sapundzhieva, 2015).

Ceratonia siliqua extract has shown antibiotic activity against the *Staphylococcus aureus* strains S6 and FRI 722, and inhibitory action against some pathogenic strains of *Listeria monocytogenes* and *Salmonella enteritidis* (Tassou, Drosinos, & Nychas, 1997).

Carob pods (pulp and seeds) have multiple uses, both in food and industrial purposes. Seeds are mainly used to produce the natural food additive, "locust bean" gum, valued for its galactomannan content, as a stabilizer of emulsions and dispersions, and ability to form viscous solutions (Macleod & Forcen, 1992; Sahin et al., 2009). Carob pods is used for manufacturing of citric acid (Roukas, 1988) and antiarrheic and antiemetic products, and in pastry baking (Calixto & Canellas, 1982). Some studies have shown that the sugars present in the carob pod extract can be used for ethanol production (Marakis et al., 1996; Hossein, Seyed, & Hasan, 2011; Turhan, Bialka, Demirchi, & Karhan, 2010).

Carob pulp is mainly used to produce human and animal food products. The pulp is used to produce carob powder, which is the main ingredient in bakery products, such as cookies and cakes. Because it is inexpensive, it is widely used as a substitute for cocoa and thus, has application in the making of chocolate. In different parts of Turkey, it has been produced commercially as carob powder and domestically, or home-prepared, as carob flour. The local population sun-dry the kibble and directly grind it to produce a fine powder, which is used in various local bakeries, whereas the commercially manufactured carob powder is sold in large stores and markets (Yousif & Alghzawi, 2000; Ayaz et al., 2007).

The pulp of *C. siliqua* is used to obtain concentrated syrup (Petit & Pinilla, 1995). The production of the Turkish concentrated syrup, "pekmez", is carried out at home or commercially. It has been reported that known and used extracts of fruits such as grapes (*Vitis vinifera* L.), figs (*Ficus carica* L.), mulberries (*Morus alba* L. and *M. nigra* L.), and carob trees (*C. siliqua* L.) can be used to produce pekmez. This concentrated syrup has high-carbohydrate content and can provide a suitable source of energy for people during winter months (Simsek & Artik, 2002). The carob plants grown in Bulgaria and products produced from their pods have not been investigated and hence, the specifics of their chemical composition remain unknown. This includes data about carbohydrate composition in the Bulgarian carob. Subsequently, this study aims to evaluate and compare the carbohydrate content in the carob pods and syrup products between the two countries, Bulgaria and Turkish.

Materials and Methods

Reagents and Samples

All reagents and solvents used in this study were high performance liquid chromatography (HPLC) grade chemicals with analytical grade purity. Carbohydrate standards for fructose, sucrose, 1-kestose, and nystose were obtained from Sigma-Aldrich (Steinheim, Germany). Fructooligosaccharides Frutafit®CLR (degree of polymerization, DP 7-9) and inulin Frutafit®TEX (DP =22) were supplied by Sensus (Roosendaal, the Netherlands).

Samples

Randomly chosen carob fruits (*C. siliqua* L.) were harvested from Bulgaria (Plovdiv region) and from Turkey (Mersin province) during summer 2015. They were dried and finley ground in a laboratory mixer and homogenized to coarse powder.

Carob Syrup Preparation

Turkish and Bulgarian carob cultivars were used in two methods of syrup preparation. First, a sample was an analogue of commercially produced carob extract. For the production of syrup, the samples were separated from the seeds and dried at 40 ^{0}C for one day. These were then extracted by suspending carob particles in water in a ratio of pulp to water of 1:2 for approximately 55 hours at 22 ^{0}C. The solids were separated. The obtained juice was concentrated under vacuum using a rotary evaporator to that of commercial levels (45^{0}C for 30 min). Second, the analogue of domestically produced carob extract involved the same steps as the commercially produced carob syrup except that the juice extracted from the carob extract was concentrated in a pot that was heated for approximately 3 hours at 65 ^{0}C. These samples were indicated as syrup after three days.

Moisture Content

The moisture content of the carob pods and their extracted syrups were analyzed using the Association of Official Analytical Chemists AOAC procedure (AOAC, 2007). Samples were dried at 105 ± 1 ºC until reaching constant weight. The moisture content was expressed as a percentage, from which dry weight was calculated for each sample (Nielsen, 2010).

Sample Preparation

Finely ground carob pods or carob syrup was weighed (1 g) in 50 ml centrifuge tubes with screw caps. Distilled water (25 ml) was added to the sample and sonicated in an ultrasonic bath brand VWR (Malaysia) with ultrasonic frequency 45 kHz, power 30 W (Petkova, Ivanov, Denev, & Pavlov, 2014b) at 30 °C for 20 minutes. The obtained extracts were filtered through 0.45 μm filter paper and the collected samples were kept at −18 °C for further analysis.

Total Soluble Carbohydrate Content

The total soluble carbohydrate content in the carob pods and syrups was estimated according to the spectrophotometric method of Dubois, Gilles, Hamilton, Rebers and Smith (1956). In brief, 0.1 ml of each extract was mixed with 1 ml of 5% phenol and 5 ml of sulphuric acid. The samples were then placed in a water bath at 30 °C for 20 minutes. Next, the absorbance was measured at 490 nm against a blank, prepared using the same process as distilled H_2O was used.The amount of presented carbohydrates was determined as previously described by Dimirova, Petkova, Denev and Aleksieva (2015) from the calibration curve for glucose and a standard where $y = 0.0098x – 0.0465$ ($R^2 = 0.998$), with results calculated in dry weight (g/100 g dw).

Reducing Sugars Content

The reducing sugars in the carob samples were estimated by the p-Hydroxybenzoic Acid Hydrazide PAHBAH method, described by Lever (1972). The analysis was carried out using 0.750 ml of PAHBAH reagent, added to 0.250 ml properly diluted carob extract. The samples were then heated at boiling point for 5 min in a water bath and next, cooled in an ice bath for 5 min before absorbance was measured at 410 nm against the blank, prepared with distilled H_2O. The assay was designed by preparing a glucose standard in the concentration range of 5–100 μg/ml.

Identification of Carbohydrate Composition by Thin Layer Chromatography (TLC)

For elucidation of carbohydrate composition in the carob pods and syrups, a TLC analysis was performed. Standard solutions of glucose, fructose, sucrose, fructooligosaccharides, and inulin, each in concentrations of 3 mg/ml, were used. Each sample (5 μl) was analyzed on silica gel 60 F_{254} plates (Merck, Germany) with mobile phase n-BuOH: i-Pro:H_2O:CH_3COOH (7:5:4:2) and spots were then detected by dipping the plates in diphenylamine-aniline-H_3PO_4–acetone reagent, heated at 120 °C and scanned as previously described (Petkova & Denev, 2013).

High Performance Liquid Chromatography (HPLC) Analysis of Carbohydrates

Chromatographic separations and determination of glucose, fructose, sucrose, and 1-kestose in carob pods and prepared syrups was carried out according to Petkova, Vrancheva, Denev, Ivanov, and Pavlov (2014a) on a HPLC Shimadzu, coupled with LC-20AD pump, refractive index detector, and the software LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan). The analysis of carob and product extracts were performed on a Shodex® Sugar SP0810 with Pb^{2+} guard column (50 × 9.2 mm inside dimension; i.d.), an analytical column (300 mm × 8.0 mm i.d.) at 85 °C, mobile phase distilled H_2O with flow rate 1.0 ml/min, and the injection volume 20 μl. The time for the HPLC analysis was 15 min.

Statistical Analysis

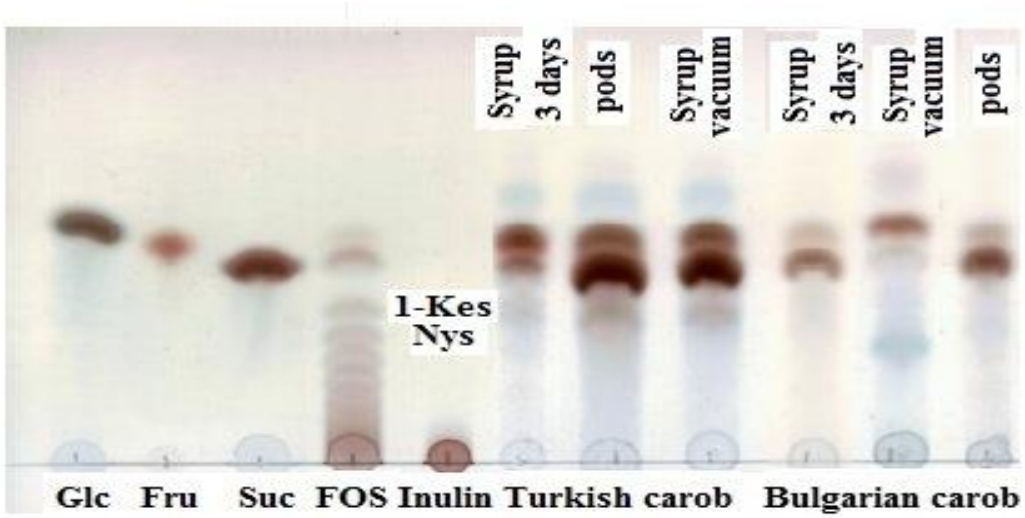
The moisture content was carried out in triplicate, while the spectrophotometric and HPLC analysis of carbohydrate content was performed in duplicate. Data were expressed as an averages and standard deviations. An analysis of results was prepared using Microsoft Excel 2010.

Results and Discussion

Detailed information about carbohydrate profiles of Turkish and Bulgarian carobs and their syrup products were obtained after TLC analysis of obtained extracts (Figure 1).

The TLC analysis showed that all investigated carob samples were characterized by a presence of monosaccharide fructose ($R_f = 0.50$) and disaccharide sucrose ($R_f = 0.44$). Furthermore, in Turkish carob and syrup products, from all tests for fructooligosaccharides (FOS), only the form, 1-kestose ($R_f = 0.37$), was detected. No presence of inulin or other fructooligosaccharides were found in all tested carob samples (Figure 1). For more detailed analysis of sugar composition, carob samples were analyzed by the HPLC-RID method (Figures 2 & 3). The presence of glucose, fructose, sucrose, and 1-kestose was established in Turkish carob and syrup products (Table 1).

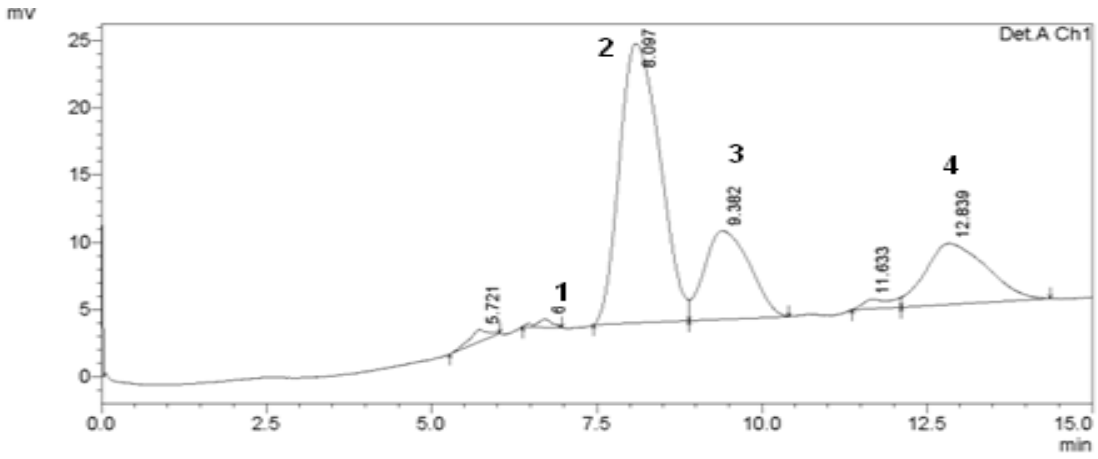
Figure 1: Thin-layer chromatogram of extracts from carob pods and syrups



Glc: glucose
Fru: fructose
Suc: sucrose
FOS: fructooligosaccharides (DP =7-9)

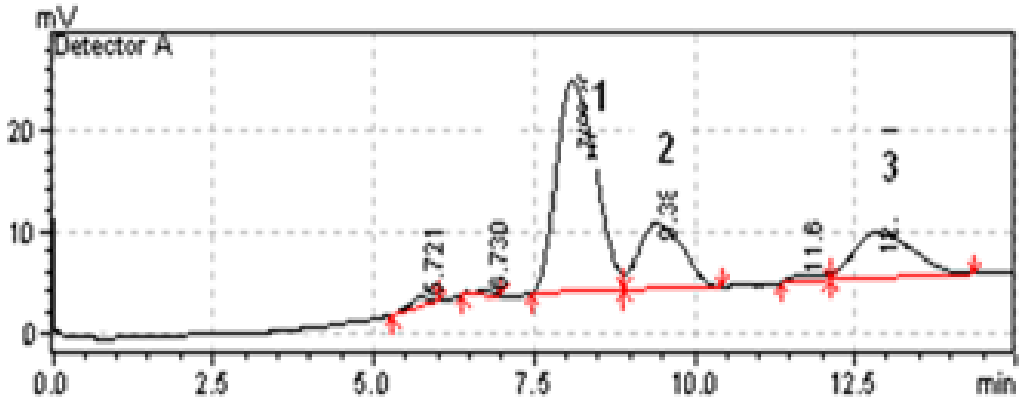
Source: Authors

Figure 2: HPL-RID chromatograms of carbohydrates composition in Turkish carob pods showing peaks for 1-kestose (1), sucrose (2), glucose (3), and fructose (4).



Source: Authors

Figure 3: HPLC-RID chromatograms of syrup obtained from Bulgarian carob pods showing peaks for sucrose (1), glucose (2), and fructose (3).



Source: Authors

In carob samples from Bulgaria, only glucose, fructose, and sucrose were detected (Table 1; Figure 3). The results of the total carbohydrate and reducing sugar content in carob pods and their syrup products were summarized in Table 1.

Table 1: Means ± standard deviations of carbohydrate content in carob (*Ceratonia siliqua* L.) pods and their syrups (g/100g dry weight)

Sample	Total carbohydrates	Reducing sugars	Fructose	Glucose	Sucrose	1-Kestose
Turkish carob	40.8 ±1.5	20.6 ±2.7	6.5 ±0.3	11.1 ±1.7	34.2 ±0.7	0.5 ±0.1
Bulgarian carob	21.0 ±1.2	5.2 ±1.1	2.1 ±0.6	3.4 ±0.2	16.5 ±0.4	n.d.
Syrup from Turkish carob (vacuum)	66.7 ±0.2	37.1 ±1.0	15.1 ±0.1	16.5 ±0.3	30.2 ±0.5	0.3 ±0.1
Syrup from Bulgarian carob (vacuum)	47.8 ±2.0	21.2 ±2.0	7.8 ±0.8	10.5 ±0.3	26.2 ±0.5	n.d.
Syrup from Turkish carob after three days	53.6 ±0.5	41.2 ±1.4	18.5 ±0.2	19.7 ±0.6	45.8 ±1.1	0.2 ±0.1
Syrup from Bulgarian carob after three days	71.5 ±1.2	6.4 ±2.4	2.5 ±0.2	3.6 ±0.1	16.2 ±1.2	n.d.

n.d.: not detected

Source: Author

The total carbohydrate and reducing sugar contents showed slight variations among the studied carobs collected from the different countries of Bulgaria and Turkey. The total carbohydrates content in products varied in the range from 21 to 71g/100 g dw. The reducing sugars content varied between 5.2 and 41.8 g/100 g dw. The Turkish carob pods were evaluated as the richest source of carbohydrates at 37.1 g/100 g dw**.** Our results were in accordance with earlier reported data for carob species from Marconian locations of 31.5 and 50.1 g/100 g dw (El Batal et al., 2011) and of 49–53 g/100 g dw (Khlifa, Bahloul, & Kitane, 2013), for wild varieties from the territory of Turkey (Gubbuk et al 2010), for Libyan carob of 500 g/kg (Haddarah, 2013), respectively. The quantity of reducing sugars was

determined to be 20.6 g/100 g that coincided with reported by Kahkah Zouhair, Diouri, Ait Chitt, and Errakhi (2015) results (19–25 g/100 g dw

More detailed characteristics about sugar profiles were obtained after HPLC-RID analysis. The presence of sucrose, glucose, and fructose were detected in all investigated samples (Table 1 & Figure 2). Sucrose dominated in carob pods collected from the territory of Bulgaria and Turkey with 34.2 ±0.7 and 16.5 ±0.4 g/100 g dw, respectively. Similar to our results were those reported for Sicilian and Turkish carobs (Avallone et al. 1997; Ayaz et al., 2009). Carobs collected from Bulgaria contained fructose 2.1 ±0.6 g/100 g; glucose 3.4 ±0.2 g/100 g, and sucrose 16.5±0.4 g/100 g dw and they showed lower sugar content in comparison to those collected from Turkey.The sucrose and fructose content in carob pods obtained from Turkey was close to results reported by Avallone et al (1997) of 34 ±3.6% dw for sucrose and 6 ± 2% dw for fructose. Similarly, the carob pods from Anatolian origin (Ayaz et al., 2009) were in the agreement with our samples with fructose content also low. However, the presence of the fructooligosacchride, 1-kestose, was detected only in Turkish carob pods. The average sugar content in analyzed carob syrup samples prepared from Turkish carob was close to that reported by Tetik, Turhan, Oziyci, and Karhan (2011) of quantities for sucrose of 38.5, glucose of 15.2.4, and fructose of 16.2g/100 g dw, whereas the carob syrup prepared from Bulgarian carob pods was evaluated as having lower sugar composition.

To the best of our knowledge, up until now, no information about the presence of 1-kestose has been published. Moreover, with results from our study, the information about sugar composition of carob pods was enriched. The Turkish carob pods and syrups evaluated in this study were a source of prebiotic, because of the presence of 1-kestose. The levels of this prebiotic compound were 0.5 g/100 g dw in carob pods and this remained significantly constant throughout their processing into syrups (Table 1). The carob syrup prepared by vacuum process contained lower reducing sugars than the three-day-processed products. This may be due to the differences in heat treatment and storage condition of the syrups.

Conclusion

The carob pods from Turkish and Bulgarian plants were identified as rich sources of carbohydrates. For the first time, the presence of 1-kestose was detected in *C. siliqua* grown in Turkey. The carbohydrates measured in the syrup products derived from carob pods suggest these products are highly nutritional and a source of energy and health benefits especially due to the presence of the prebiotic compound. During the processing of carob, increases in reducing sugars were observed and this increase could be from carbohydrate hydrolysis during storage or heat treatment during syrup production.

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