Research Article

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Determination of chemical composition, antibacterial and antioxidant properties of products obtained from carob and honey locust

Keçiboynuzu ve glediçyadan elde edilen ürünlerin kimyasal bileşimi, antibakteriyel ve antioksidan özelliklerinin incelenmesi

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Abstract

Objective: The objective of this study was to characterize flours and syrups, obtained from pods of carob (*Ceratoniasiliqua* L.) and honey locust (*Gleditsia triacanthos*).

Method: Flours and syrups, produced by carob and honey locust were analyzed for moisture, ash, protein content, dietary fibers, minerals composition, total phenolic content, as well as antibacterial and antioxidant activity.

Results and discussion: Carob flour contained high amounts of protein (22.56%) and dietary fibres (28.17%), respectively. Dietary fibers in honey locust flour (33.12%) were higher than that of carob flour (28.17%). The total phenolic content of carob flour (4.53 ± 0.08) was lower than this of honey locust (25.31 ± 0.06) (mg gallic acid equivalent [GAE]/g dry weight). *Gleditsia triacanthos* flour

showed higher antioxidant potential – from 127.52 ± 2.43 to 540.28 ± 2.47 $\mu\text{M TE/g dw}$. Carob syrup in an amount of 0.15 cm^3 demonstrated pronounced antibacterial activity against *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus*, respectively.

Conclusion: The current study demonstrated that flour and syrup, obtained from carob (*C. siliqua* L.) and honey locust (*G. triacanthos*) pods presented products rich of protein and dietary fiber (both above 20%), good sources of antioxidants, especially polyphenolic compounds and minerals (Mg, Fe and Zn).

Keywords: Antibacterial and antioxidant activity; Carob; Honey locust; Flour; Syrup; Chemical composition.

Öz

Amaç: Bu çalışmanın amacı, keçiboynuzu (*Ceratoniasiliqua* L.) ve glediçya (*Gleditsia triacanthos*) meyvelerinden elde edilen un ve şurupları analiz etmektir.

Yöntem: Keçiboynuzu ve glediçya tarafından elde edilen unlar ve şuruplar nem, kül, protein içeriği, diyet lifleri, mineraller, toplam fenolik içeriği ve ayrıca antibakteriyel ve antioksidan aktivite açısından analiz edilmiştir.

Sonuçlar ve tartışma: Keçiboynuzu unu, sırasıyla yüksek miktarda protein (% 22.56) ve diyet lif (% 28.17) içerir. Glediçya unu içerisindeki diyet lifleri (% 33.12), keçiboynuzu unundan (% 28.17) daha yüksektir. Keçiboynuzu ununun toplam fenolik içeriği (4.53 ± 0.08) glediçyadan (25.31 ± 0.06) daha düşük olduğu belirlenmiştir (mg GAE/g kuru madde). *Gleditsia triacanthos* unu daha yüksek antioksidan potansiyeli göstermekle birlikte (127.52 ± 2.43 – 540.28 ± 2.47 $\mu\text{M TE/g}$), 0.15 cm^3 ’lük miktardaki ekstrakt,

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sırasıyla *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica* ve *Staphylococcus aureus*'a karşı belirgin antibakteriyel aktivite göstermektedir.

Sonuç: Bu çalışma, keçiyoynuzu (*C. siliqua* L.) ve glediçya (*G. triacanthos*) meyvelerinden elde edilen un ve şurubun, protein ve diyet lifi (her ikisi de % 20'den fazla) bakımından zengin ürünler olduğu belirlenmiş, özellikle de polifenol miktarı bakımından iyi bir antioksidan ve mineral (Mg, Fe ve Zn) kaynağı oldukları görülmüştür.

Anahtar Kelimeler: Antibakteriyel ve antioksidan aktivite; Keçiyoynuzu; Glediçya; Un; Şurup; Kimyasal bileşim.

Introduction

Carob (*Ceratonia siliqua* L.) or locust bean is considered as phylogenetically primitive species with tropical origin. It is a slow-growing tree distributed in Arabia and then introduced into California, Mexico and South Australia [1].

The carob pod consists pulp (90%) and seed (10%). Depending on the genotype, there are wild and cultivated carob species, which are distinguished by their seeds and pulp [1]. The pulp is rich of sugars (48–56%, mainly sucrose) and contains about 16–20% tannin [2]. The cultivated carob had higher amount of total soluble sugars than the wild species [3].

In most of the cases, pulp is assumed as a by-product. The seeds are considered to be the most valuable part of the fruit because of the polysaccharide is widely used in the food industry [4, 5]. Carob (the pulp and its seeds) is used for the production of flour, extract, especially concentrated syrup form pulp [6] and locust bean gum [7, 8]. Pulp carob flour is a major ingredient in the production of biscuits and pastries, cereal products, chocolate confectionery, ice cream and dietary products. Due to its low price, it is widely used as a substituent of cocoa. Carob water extract showed inhibitory activity against certain strains of *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella enteritidis* [9].

Honey locust (*Gleditsia triacanthos* L.), is a leguminous, moderately fast growing tree, known also as sweetlocust or thornylocust. The pods are of significant interest to many industries, as well as livestock feeding, erosion control, windbreaks, shelterbelts [10]. The pods are edible and can be used for consumption or for production of alcoholic or non-alcoholic beverages. In Bulgaria it is pods were consumed by processing as syrup. Fruits of *G. triacanthos* L. contain very low values of condensed tannins, hydrolysable tannins and total phenols. Similarly to *C. siliqua*, the total sugar fraction of GT pods is the preferential energetic

source [11]. The leaves and pods of *G. triacanthos* have good nutritional properties as; crude protein (24.16%), crude fibre (16.01%), total ash (7.35%), phosphorus (0.30%), potassium (2.04%), calcium (1.08%), sulphur (0.15%), magnesium (523.13 ppm), zinc (19.82 ppm) and iron (231.33 ppm) [12]. There are several reports of variations in the chemical composition of carob and honey locust from different parts of the world. The aim of this study was to determine the content of protein, dietary fibre, minerals, antioxidant and antibacterial activity of carob and honey locust derived products – flour and syrup.

Materials and methods

Samples

Carob pods were harvested from randomly chosen wild growing plants during the summer of 2016 from Turkey (Mersin province), while honey locust pods were collected from Bulgaria (Plovdiv region) during October the same year. Flours were obtained by removing the seeds and pods, milled to a fine powder with a particle size of up to 1 mm. Both flours were extracted with boiling water (1:20, v/v) under reflux for 30 min prior to the total polyphenolic and antioxidant activity analyses.

Carob and honey locust syrup preparation – carob and honey locust pods were used for syrup preparation, as follows: the samples were separated from the seeds and dried at 40°C for 1 day. The dried mass was suspended in water (1:2 w/v) for 55 h at 22°C. The solids were removed. The obtained juice was concentrated under vacuum to the soluble solids (66.00–75.00°Brix).

Determination of total polyphenolic content (TPC)

The total polyphenol content was measured at 765 nm according to Folin-Ciocalteu method [13]. The TPC was expressed as mg GAE per g dry weight.

Determination of antioxidant activity

DPPH radical scavenging activity

DPPH radical scavenging activity was determined by method of [14]. The absorbance was measured at 517 nm. Trolox equivalent antioxidant capacity (TEAC) was

defined as the concentration of Trolox having equivalent AOA expressed as the μM Trolox per g dw.

ABTS radical cation decolorization assay

The scavenging activity against radical cation 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) was estimated according to [15]. The results were expressed as TEAC value (μM TE/g dw).

Ferric reducing antioxidant power assay (FRAP)

The FRAP method was performed [16] and the absorbance was recorded at 593 nm. The results were expressed as μM TE/g dw

Cupper reduction assay (CUPRAC)

CUPRAC assay was performed [17] Absorbance was measured at 450 nm. Trolox was used as standard and total antioxidant capacity was measured as μM TE/g dw.

Protein determination

Protein content was determined as nitrogen by elemental analysis using a LECO CHNS-932 analyzer (St. Joseph, MI, USA) and it was calculated using 6.25 nitrogen to protein correction factor.

Determination of total, soluble and insoluble dietary fibres

The total, soluble and insoluble dietary fibres was determined [18], using the total dietary fibre enzymatic-gravimetric assay kit Bioquant 1.12979.0001 (Merck, Germany) and the instructions provided by the manufacturer.

Mineral analysis

Macro (magnesium-Mg) and micro (iron- Fe, copper-Cu, manganese-Mn, zinc-Zn, selenium-Se) minerals were determined according to validated laboratory method (in Food Research and Development Institute Plovdiv) using microwave mineralization of 1 g carob sample with 3 mL of 0.2% HNO_3 and 2–3 mL H_2O_2 . Mineralized sample was filtered and analysed by ICP-OES SPECTROFLAME (Spectro Analytical

Instruments) with monochromator, linear range from 165 to 440 nm, Nebulizer type Minhard, coolant gas- 42 bar, auxiliary gas – 26 bar. The measured intensities were compared with the intensity of series of standard solutions.

Antimicrobial activity

Antibacterial activity was tested against Gram-positive bacteria – *L. monocytogenes* NCTC 11994 and *S. aureus* ATCC 25093, and Gram-negative bacteria – *Escherichia coli* ATCC 8739 and *Salmonella enterica* subsp. *enterica* serovar Abony NCTC 6017. The selective growth media were: Listeria Oxford Agar Base/Merck/; Baird Parker Agar Base with Egg Yolk Tellurite emulsion supplement/Merck/, Rapid' *E. coli* 2 Agar/BioRad/ and Mac CONKEY Agar/Merck/, respectively.

The media were inoculated with 24-h suspension of the bacterial species.

Melted and cooled to 45°C selective media were inoculated with the tested microorganisms and next equally dispensed into Petry dishes. After setting of the media, sterile rings ($\varnothing$ 6 mm) were placed on, and different amounts of each sample (0.05; 0.10 and 0.15 mL) were put into the rings. Petry dishes were incubated at 37°C for 24 or 48 h according to the bacterial species, and then the distinct zone of growth inhibition around the rings was measured. The used inoculums have resulted as an actual concentration cells of – *L. monocytogenes*, *S. aureus*, *E. coli*, *S. enterica* into the responding selective medium about 1×10^4 CFU/mL. The total plate count was estimated by the conventional plate-counting technique using appropriate dilution.

Statistical analysis

All samples were performed in triplicate. The results were expressed as mean $\pm$ SD and statistically analysed using MS-Excel software.

Results and discussion

Total phenolic content and antioxidant activity of flour and syrup obtained by carob and honey locust

The results for total polyphenolic content and antioxidant activity of flour and syrup samples were presented in Table 1.

The total phenolic content in carob and honey locust flours ranged from 4.53 ± 0.08 to 25.31 ± 0.06 mg GAE/g dw.

Table 1: Total phenolic content (mg GAE/g dw) and antioxidant activity ($\mu\text{M TE/g dw}$) of flours and syrups (mean $\pm$ SD), obtained by carob and honey locust.

Assay/Sample	TPC	FRAP	CUPRAC	ABTS	DPPH
Carob flour	4.53 ± 0.08	109.14 ± 1.11	34.78 ± 3.72	64.70 ± 4.00	75.49 ± 2.52
Honey locust flour	25.31 ± 0.06	540.28 ± 2.47	436.85 ± 11.36	127.52 ± 2.43	257.50 ± 1.85
Carob syrup	2.14 ± 0.07	41.86 ± 1.24	149.10 ± 4.88	441.11 ± 4.57	223.35 ± 0.88
Honey locust syrup	9.74 ± 0.06	225.71 ± 1.41	279.59 ± 4.37	234.56 ± 2.82	147.98 ± 0.63

In the syrup, obtained by carob and honey locust, the values were 2.14 ± 0.07 and 9.74 ± 0.06 mg GAE/g dw, respectively. Our data for TFC of honey locust flour were near to a previous report [11]. The honey locust samples showed higher phenolic content compared to the carob samples. The honey locust flour had the highest polyphenolic content – 25.31 ± 0.06 mg GAE/g dw. However, our results for the carob flour were comparable to a report [19] in Morocco and higher than reported values (17.0 mg/g) for carob kibbles in North Morocco [20]. The differences could be due to extraction methods and environmental conditions.

In accordance with the recommendations for at least two methods for antioxidant activity [21], four reliable assays were used in the present study.

The highest antioxidant potential showed the honey locust flour – from 127.52 ± 2.43 to 540.28 ± 2.47 $\mu\text{M TE/g dw}$. However, the antioxidant potential of carob flour evaluated by DPPH and FRAP method was lower than previous report [22] – (70.45 ± 3.29 and 84.23 ± 5.08 mM TE/g dw). The syrup derived from honey locust consisted of more total phenolic compounds and showed higher antioxidant capacity evaluated by FRAP and CUPRAC assays (Table 1). The prevalence of the antioxidant potential according to ABTS and DPPH assays was established for the carob syrup. The solvent and extraction conditions may influence the quantity, antioxidant composition and biological activity.

However, the results revealed the potential of the honey locust as a source of biologically active substances. This slightly explored in honey locust plant is worth to be investigated for detailed profile and application. The syrups, obtained by carob and honey locust could be considered as natural sources of antioxidant compounds.

Chemical characteristics of flour and syrup, obtained by carob and honey locust

Mineral analysis

One major element (Mg) and four trace (Fe, Cu, Mn, Se and Zn) minerals were found in honey locust and carob samples (Table 2). The both flours of carob and honey locust could be

Table 2: Mineral content in flour and syrup obtained by carob and honey locust.

Mineral content (mg/kg dw)	Carob flour	Carob syrup	Honey locust flour	Honey locust syrup
Se	1.16	1.24	1.44	1.26
Mg	249.81	234.95	238.50	237.16
Fe	55.30	16.06	15.12	14.77
Cu	4.91	1.45	5.04	1.42
Mn	6.43	4.17	3.98	3.87
Zn	8.59	6.02	9.72	6.24

considered as a source of Mg, whereas these derived from carob were with the highest mineral content. The amounts of the other minerals were considerably lower in the both samples. Fe, Cu and Zn content in honey locust flour (15.12, 5.04 and 9.72 mg/kg dry weight, respectively) were similar with previously report for carob pod [23]. These three elements were reported as dominante in honey locust [12]. It was reported that among the micro elements, Fe had the highest concentration (32.28 ± 6.04 mg/kg) in the grafted carob fruit seeds from Antalya province (Turkey) [24]. The syrups, obtained from the raw materials of carob and honey locust had similar mineral composition and were close to the mineral composition of the pods of the respective raw material. The exception is the presence of iron in carob flour, as its concentration was higher in the flour than the syrup. The environmental factors could highly influence these values.

The chemical composition of carob and honey locust flour were presented (Table 3).

The moisture contents in carob and honey locust samples were below 10%. Fresh stage pods moisture content ranged between 10 and 20% [25]. In our case ash content was 2.18 and 3.02%. Our values were lower than reported ash content in Turkish honey locust [26]. In comparison ash content was 1.38–5.21% in pods of wild and grafted carob fruits from Antalya Province, while protein content was 0.76–0.61% [27]. The reported total nitrogen content (2.13–2.69%) in the carob pods from South Africa [28] was higher than our results. The values obtained in current study were similar to above

Table 3: Chemical composition of flour and syrup obtained by carob and honey locust.

Parameter	Carob	Honey locust
Total dietary fibers (%)	28.17	33.12
Soluble dietary fibers (%)	4.79	5.91
Insoluble dietary fibers (%)	23.36	27.21
Protein ^a (%)	22.56	16.70
Dry matter (%)	91.00	89.84
Ash (%)	3.02	2.18

^aProteins = % nitrogen $\times$ 6.25.

mentioned values. Protein content in honey locust was near to values reported [26] and slightly lower by some other authors [11].

The content of dietary fiber in honey locust flour (33.12%) was higher than that of carob flour (28.17%) (Table 3). These values were comparable to dietary fibre contents in various cultivars [25] – 29.88 and 36.07 g 100 g⁻¹. Previously reported results for the total dietary fibre content of carob flour were observed to be 35.2% which is consistent with our values [29]. However, some authors reported lower content of dietary fibre in raw carob – 23.15 $\pm$ 0.39 g/100 g dw [30]. In our study the content of dietary fiber was higher than some previous reports [11, 12, 26]. The water soluble carbohydrate of honey locust pods obtained from different areas of Turkey ranged from 122.3 to 152.2 g kg⁻¹ DM [27].

The high content of dietary fiber in locust bean determines the health benefits of its consumption as lowering total and LDL cholesterol and reducing the risk of gastrointestinal diseases [31, 32].

The carob flour contained the highest protein content (22.56%) (Table 3). A research reported higher protein content for carob samples – between 54.7% and 67.1% [33]. Furthermore, our results for protein content was higher than these in carob from South Africa (3.07–4.42 g/100 g) [5, 25].

According to the subdivision of foods into categories honey locust powder could be referred to the group of “foods source of protein” or for carob “high-protein foods” – foods, in which the protein provides more than 12% or not less than 20% of their energy value. The basis for this finding is the calculation of the energy value based on the substantial nutrients, as follows: protein content about 16%, total sugars – 21% and dietary fiber – 33% for honey locust and protein content about 22.5%, total sugars – 40.8% and dietary fibers – 28% for carob.

Antibacterial activity of flour and syrup obtained by carob and honey locust

Based on the previous studies [22, 34, 35] where carob pods and their products were analysed for mineral composition and carbohydrate content, this work aimed to determine the antibacterial, antioxidant and chemical properties as well as antibacterial and antioxidant activities of the carob and honey locust derived products.

Carob and honey locust have been considered as food sources and they are eaten for its nutritive values. Therefore, the antimicrobial properties of carob and honey locust extracts toward *L. monocytogenes*, *E. coli*, *S. enterica* and *S. aureus* were assessed (Table 4).

The extract of carob and applied in an amount of 0.15 mL, possessed the most pronounced antibacterial activity with inhibition zones: 22 mm against *L. monocytogenes*; 12 mm against *E. coli*, 10 mm against *S. enterica* and 13 mm against *S. aureus*. These results were in agreement with previously reported [32], where the antimicrobial activity of *C. siliqua* pods essential oil against the tested Gram-positive and negative bacteria was qualitatively assessed by the presence or absence of inhibition zone diameters and the inhibition zones were in the range of 9–25 mm. The carob extract was also found to inhibit the growth of clinically important Gram-negative bacteria,

Table 4: Antibacterial activity of syrup, obtained by carob and honey locust.

Sample	Extract amount	Diameter of zone of growth inhibition (mm)			
		<i>L. monocytogenes</i> NCTC 11994	<i>E. coli</i> ATCC 8739	<i>S. enterica</i> NCTC 6017	<i>St. aureus</i> ATCC 25093
Carob	0.15 mL	22	12	10	13
	0.10 mL	16	9	8	11
	0.05 mL	15	a	a	9
Honey locust	0.15 mL	9	8	a	10
	0.10 mL	a	a	a	8
	0.05 mL	a	a	a	a

^anot detected.

such as *E. coli* and food contaminants -*S. enterica* and *L. monocytogenes*.

Honey locust extract showed lower antibacterial activity against three of the tested pathogenic bacteria, compared with carob. We found that the activity of the carob extract depended on its concentration and the tested bacteria. Gram-positive bacteria were more sensitive to plant extracts than Gram-negative ones. These could be referred to the structural differences and the great complexity of the double membrane of Gram-negative bacteria [36].

Conclusion

In the present study for the first time were compared flours and syrups obtained by carob (*C. siliqua* L.) and honey locust (*G. triacanthos*). It is established that carob and honey locust products are good source of dietary fibres, which are thought to exert a preventative role against heart disease. Additionally, the antioxidant activity, measured in the flours and syrups, obtained by carob and honey locust suggest these products are with high nutritional value and would raise increasing interest as ingredients in the food industry for functional and healthy food formulations.

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