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BY-PRODUCTS OF PROCESSING CAROB MOLASSES AS SOURCES OF DIETARY FIBRE AND POLYPHENOLS

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Introduction. Formulation of the problem

The carob tree (*Ceratonia siliqua* L.) is a tree typical of the Mediterranean area, mainly of Spain, Morocco, Italy, and Portugal. It produces fruit in the form of an edible bean, or pod [1]. Worldwide, carob pod production amounts to nearly 400,000 tonnes a year from about 200,000 ha. Turkey produces the least of it (5%): 13,500 tonnes a year from 354,000 trees, with differences in the production depending on the cultivar, region, and cultivation practices [2]. Carob pods are high in dietary fibre (DF) and polyphenols (PP) [3]. They are also known as the source of locust bean gum [1]. These compounds have been claimed to have the diverse health benefits as antioxidants, to prevent chronic inflammation, cardiovascular disease, cancer, and diabetes [4, 5]. In recent years, the interest

Abstract. Dietary fibre and polyphenols have been widely used to increase the functionality of some foods because of their potential effects on human health. In this study, extraction of dietary fibre and polyphenols from pomace obtained as a by-product of processing carob molasses has been studied. The dietary fibre and polyphenol extracts were prepared separately. The amount of dietary fibre in the carob molasses pomace was evaluated with two assays: the Association of Official Analytical Chemists' enzymatic-gravimetric method and the enzymatic-chemical method. The methods were compared, each having been preceded by conventional extraction and ultrasound-assisted extraction of carob molasses pomace. It has been found that when the enzymatic-gravimetric method and the ultrasound-assisted extraction method were used, the total dietary fibre contents were significantly higher than after using the enzymatic-chemical method and the conventional extraction method. Conventional extraction of polyphenols from carob molasses pomace has shown a relatively higher polyphenol content and antioxidant activity than ultrasound-assisted extraction. Ultrasound-assisted extraction took less time than conventional extraction did. The polyphenol profile was characterised by means of a high-performance liquid-chromatography diode array detector using 10 phenolic standards. Six compounds, i.e. caffeic, syringic, -epicatechin, trans-cinnamic acid, myricetin, and naringin were determined with the high-performance liquid chromatography-diode array detector using both conventional extraction and the ultrasound-assisted extraction. Our results suggest that carob molasses pomace can be used to prepare low-calorie, high-fibre, and antioxidant-rich foods, nutraceuticals, and pharmaceuticals.

Keywords: antioxidant activity, enzymatic-gravimetric method, enzymatic-chemical method, dietary fibre, polyphenols, carob by-product.

in various carob products has been increasing due to their cheapness [2, 3, 6].

Carob molasses (Pekmez), a kind of fruit juice concentrate, is a traditional Turkish food made from carob fruit [6]. Carob molasses pomace (CMP) is obtained by crushing and squeezing carob fruit. This pomace is still underutilised. It is mostly used as feed for livestock. Accumulation of substantial amounts of it in the production area can cause environmental pollution [7].

Ultrasound-assisted extraction (UAE) is used to hydrolyse the compounds which are found in cell walls as bound [8]. UAE is quite simple, and it is cheaper than other extraction techniques such as microwave-assisted extraction, supercritical fluid extraction, etc. UAE takes significantly less time than conventional extraction methods [9].

The purpose of this study was to characterise the DF and PP contents of CMP. Firstly, the composition of Turkish CMP was analysed. Then, the study was performed in two stages. At the first stage, the conventional extraction (CE) and UAE were compared by determining the dietary fibre contents, because UAE is characterised by an increased extraction yield and decreased extraction time. The two different methods of detecting dietary fibre were used due to the difference of dietary fibre definitions. At the second stage, the effectiveness of UAE was investigated by determining the PP. The phenolic profile, total phenolic content, and antioxidant activity of CMP were compared in extracts obtained by the conventional and the ultrasound-assisted method. The information obtained from this study has shown that CMP will be helpful in developing of value added products.

Research materials and methods

Samples

CMP was provided by a local factory in Turkey in dried state and was stored in perforated and zip lock bags in a refrigerator (+4°C) until analyses were carried out. Before the analyses, the CMP was ground in a mortar in order to make it uniform and increasing the extraction efficiency.

Standards and reagents

The following commercial available phenolic standards were used to determine the chemical compounds: gallic acid (Sigma, G7384), catechin hydrate, (Fluka, 22110), (-) epicatechin (Sigma, E1753), trans-cinnamic acid (Aldrich, 133767), ferulic acid (Fluka, 42280), rutin (Sigma, R5143), myricetin (Sigma, M6760), syringic acid (Sigma, S6881), caffeic acid (Sigma, C0625), quercetin hydrate (Sigma, 337951), p-coumaric acid (Sigma, C9008), naringenin (Sigma, N1376); tri-distilled water; Folin-Ciocalteu phenol reagent (Sigma-Aldrich, E9252), DPPH reagent (2,2 diphenil, 1, picrylhydrazyl) (Sigma, D9132), sodium carbonate (J.T. Baker, 2024), Tween 40 (Sigma, P1504), β -carotene (Roche, 303420), linoleic acid (Sigma L1376), arabinose (Merck, 101492), xylose (Merck, 108689), mannose (Merck, 4440), galactose (Merck, 3455), glucose (Merck, 4074), 3-phenyl phenol (Sigma Aldrich 262250), sulphamic acid (Sigma Aldrich 383120), galacturonic acid (Sigma Aldrich-Fluka 48280), α -amilase (Sigma A3306), pancreatin (Sigma P7545), and amiloglucosidase (Sigma A9913). Other reagents used were: Ba(OH)₂ (Merck 101737), methyl red (Sigma, 250198), H₂BO₃ (Sigma, 703087), bromocresol green (Sigma, 114359), sodium dodecyl sulphate (Merck 817034), acetone (Merck 100014), ethanol (Merck 100983), hexane (Merck 104368), H₂SO₄ (Merck 100713), Na₂HPO₄ (Merck, 106566), NaOH (Merck, 106469) HCl (Merck 100317), methanol (Labscan, A17C11), acetonitrile (Sigma, 34851), acetic acid (Sigma 320099). All other chemicals and solvents for proximate composition were purchased either from Merck or Sigma.

Analysis of the chemical composition of CMP

The moisture content was determined by vacuum oven drying according to AOAC 925.09 [10]. The total protein was determined by the micro-Kjeldahl method according to the AOAC 950.48 standard [11] using a conversion factor of 5.30 [12]. The total fat was extracted with n-hexane for 6 h according to the Folch method [13]. The ash content was determined by incineration in a muffle at 550°C according to TS ISO 5984; the sample was ashed to the white colour. The lignin was evaluated by the Klason lignin method [14]. Determination was performed in triplicate. The carbohydrates were calculated by the difference as follows: carbohydrates % = 100 - (lignin % + moisture% + protein % + fat % + ash %).

Enzymatic-gravimetric method (total dietary fibre)

The total dietary fibre (TDF) was measured by the AOAC 985.29 method [15] determined as gravimetric. This method was also modified using an ultrasound bath. Each enzyme in this protocol is applied at the temperature created by the water bath including ultrasonic prob. In the conventional application, CMP was homogenised in phosphate buffer (0.08M, pH 6.0) and incubated at 100°C with heat-stable α -amylase for 15 min under constant agitation. Then NaOH solution (0.275N, pH 7.5) was added to the water bath at 60°C (Clifton, Weston-super-Mare, North Somerset) for 30 min with protease in order to solubilise proteins. Finally, HCl solution (0.325N, pH 4.5) was added, then amyloglucosidase solution was added, and the samples were incubated for 30min on the water bath at 60°C with continuous agitation. To determine the TDF, the samples were treated with 95% (v/v) ethanol at room temperature for 1 h. In order to precipitate soluble fibre and remove depolymerised protein and glucose, the residues were filtered and washed sequentially with 78% (v/v) ethanol, 95% (v/v) ethanol, and absolute acetone. The TDF was corrected for residual protein and ash. In an ultrasound assistance application, CMP was weighed into a glass bottle, and homogenised with phosphate buffer. Ultrasounding was performed at the amplitude 100% for 5, 15, and 30min (Hielscher UP400S, Teltow, Germany) while adding each enzyme. The following process went on as in the conventional techniques. The conventional dietary fibre extraction was compared with that modified by ultrasound assistance. All samples were prepared and analysed in triplicate.

Enzymatic-chemical method

Neutral sugars were analysed after this residue was hydrolysed with 77% H₂SO₄, followed by 25% H₂SO₄. The hydrolysates were neutralised using Ba(OH)₂ [16]. The standards (arabinose, xylose, mannose, galactose, and glucose) were taken. The neutral sugar composition of the hydrolysates was determined using chromatographic equipment with HPLC (Agilent Technologies Liquid Chromatograph 1200 Series System, US), Agilent ChemStation data software, carbohydrate analysis column Aminex HPX-87P

(250-4.6mm), and a refraction index detector (Waters, mod. 410). The mobile phase was 75% acetonitrile with the flow rate 1.0ml/min. The column temperature was 20°C, 5µl of extract was injected, and the time was 20 min. The experiments were carried out in duplicate. Uronic acids were determined according to the spectrophotometer method. 5mg of CMP was weighed and added 2ml H₂SO₄ with 20min shaking. The mixture was diluted to 10ml with bi-distilled water and centrifuged at 2000g for 10min. 400µl of supernatant was mixed with sulphamic acid/potassium sulphamate solution (4M, pH 1.6). sodium tetraborate (75mM) was added to this solution, which was then boiled in a water bath at 100°C. 3-phenyl-phenol was added. Galacturonic acid was the standard for calibration. The absorbance was measured at 525nm. The experiments were carried out in duplicate. Klason lignin was quantified gravimetrically in the CMP by the modified Theander method [14]. The weighed CMP samples of approximately 0.1g were dispersed into 1.5ml of 72% H₂SO₄ and incubated at room temperature for 24 h with frequent shaking. The samples were further incubated in a temperature-controlled oven set at 100°C for 2.5 h after having been diluted with 18.5ml of water. After centrifugation, lignin was recovered as residue. The residue was dried until a constant weight was achieved, and the Klason lignin content was calculated gravimetrically. The experiments were carried out in triplicate.

Extraction of polyphenols (PP)

For both CE and UAE, 80% aqueous methanol was chosen as the extraction liquid. In CE, 10 g of CMP was mixed with 50 ml of the extraction liquid at 30°C for 20 min in an Erlenmeyer flask in a shaking water bath. The samples were centrifuged (6000g, 15 min) and washed two more times with fresh solvent. The supernatants were combined and used for the analyses [17]. In UAE, the same was performed during 5, 10, 15, 20, and 30min. An ultrasound device (Hielscher UP400S with 14 mm diameter H14 Sonotrod, Germany) was used instead of a water bath in CE at the amplitude 100%.

Total phenolic content

To estimate the total phenolic content, the Folin–Ciocalteu method modified by Singleton and Rossi [18] was used. A calibration curve was prepared by using gallic acid and catechin standard solutions with different concentrations (10-50 mg kg⁻¹). The sample extract (50µl) was mixed with the Folin–Ciocalteu reagent (250µl). This mixture was kept in a dark room at room temperature for 5min. Then a 7% Na₂CO₃ (750µl) was added to the mixture. It was diluted to 5ml with bi-distilled water. The reaction was kept for 120 min at ambient temperature in the dark. The same procedures were applied to the standard solutions. The absorbance was measured at 765nm using a diode array spectrophotometer (Varian Cary 50 Bio UV-Vis Spectrophotometer, the path length of the cuvette 10 mm, Australia). The total phenolic content was

expressed as mg of gallic acid/equivalents (mg GAEg⁻¹ dry basis) and catechin/equivalents (mg CEg⁻¹ dry basis). Each determination was performed at least in triplicate.

Determination of the antioxidant activity

DPPH radical scavenging method: the DPPH (1.1-diphenyl-2-picrylhydrazyl hydrate) radical scavenging activity of CMP was measured according to the method of Brand-Williams et al. [19] with some modifications. 50µl of the extract was mixed with 2ml of 6x10⁻⁵M DPPH. The mixture was kept in the dark at room temperature for 20min. The absorbance was measured at 515nm with a UV spectrophotometer. Gallic acid was used as the standard reference antioxidant. The antioxidant capacities of the sample extract were measured using a calibration curve prepared by using different concentrations (10–100ppm) of gallic acid solution. The experiment was carried out in triplicate. The scavenging activity of the extracts was expressed as the percentage of inhibition of the DPPH radical. The absorbance was measured both initial and after 20 min. The antioxidant activity was calculated by the following equation 1:

$$\text{Antioxidant activity}(\%) = \frac{Abs_{initialDPPH} - Abs_{finalDPPH}}{Abs_{initialcontrol} - Abs_{finalcontrol}} \times 100 \quad (1)$$

where A_{control} is the absorbance of the control (containing all reagents except the sample), A_{DPPH} is the absorbance of the sample, both measured at 515nm.

β-carotene-linoleat model system: the antioxidant activity of CMP was evaluated according to the procedure described [20,21]. β-carotene solution was prepared by dissolving 25mg of β-carotene in 5ml of chloroform. Chloroform was removed from three millilitres of this solution under nitrogen. 40mg of linoleic acid, 400mg of Tween 80 emulsifier, and 100 ml of distilled water were added to the concentrate. The samples were mixed in an orbital shaker for 5min. Three millilitres of this emulsion was transferred into 2ml of CMP extracts. The samples were shaken and incubated at 50°C for 2 h in a water bath. 80% methanol was the control, and emulsion without β-carotene was used to zero the spectrophotometer. Both the initial and the final absorbance was recorded to be 470nm. The assays were carried out in triplicate. The antioxidant activity was calculated using the following equation 2:

$$\text{Antioxidant activity}(\%) = \frac{Abs_{initial\beta\text{-carotene}} - Abs_{final\beta\text{-carotene}}}{Abs_{initialcontrol} - Abs_{finalcontrol}} \times 100 \quad (2)$$

where A_{control} is the absorbance of the control (containing all reagents except the sample), A_{β-carotene} is the absorbance of the sample, both measured at 470nm.

Identification and quantification of the phenolic compound

The qualitative and quantitative phenolic characteristics of the samples were determined by using an HPLC-DAD, with both conventional and ultrasound-assistance extraction. The method described by Monagas et al. [22] was used with some modifications. An HPLC (Agilent Technologies Liquid

Chromatograph 1200 Series System, US), equipped with a diode array detector (DAD), Agilent ChemStation data software, and a μ -Bondapak C18 column (3.9x300mm) were used for the analysis of phenolic compounds. Separations were performed at 40°C. The extracts were filtered through a 0.45 μ m filter. Acetic acid in high-purity water (2:98; mobile phase A) and acetonitrile and acetic acid in high-purity water (25:2:75; mobile phase B) were used as solvents with the flow rate 1ml/min⁻¹. The gradient programme started with 100% A to reach 20% A and 80% B in 55min, 10% A and 90% B in 57min, 10% A and 90% B in 70min, 5% A and 95% B in 80min, 100% B in 90min. The column was washed with 100% A for 5min. Five microlitres of each sample was injected for analysis, and the chromatogram monitored at the wavelength 254–370nm. The samples were analysed in duplicate. The phenolic compound identified were quantified on the basis of their peak areas and comparison with the calibration curve obtained with the corresponding standards such as (-)-epicatechin, trans-cinnamic acid, ferulic acid, rutin, myricetin, syringic acid, caffeic acid, quercetin hydrate, p-coumaric acid, and naringenin.

Statistical analysis. The values were averaged and given with the standard deviation. They were analysed by Statistical Package SPSS, version 16.0 for Windows. The data from the different methods were statistically analysed by one-way analysis of variance to choose the optimum value of ultrasound assistance. Duncan's multiple-range test was applied to establish differences between samples and methods ($\alpha > 0.05$).

Results of the research and their discussion

The CMP chemical composition has been presented as follows. The moisture content has been found to be 105.9g/kg sample. Protein (65.1g/kg sample), ash (50g/kg sample), and complex carbohydrates (471.1g/kg sample) are the major components, while fat (34.9g/kg sample) has been found to be the minor component, but these values depend on the cultivar. Lignin has been determined as 273g/kg sample. Since the composition of different cultivars depends on genetic, climatic, and ecological factors, their variability [23] and the use of fertilisers and fungicides can affect it. The chemical composition can be high [24].

Dietary fibre content. In order to investigate the total dietary fibre content of the CMP, two different methods and two different extraction techniques have been performed and compared: the enzymatic-gravimetric and the enzymatic-chemical methods as well as the conventional and the ultrasound-assistance extraction techniques. The two methods are based on different dietary fibre definitions. The aim of the enzymatic-chemical method is to measure the dietary fibre content as non-starch polysaccharides and lignins, whereas the enzymatic-gravimetric method measures

cellulose, some hemicellulose, pectin, and the amount of other non-starch dietary fibre polysaccharides.

According to the first method of detecting dietary fibre, the results of the effect of extractions by the enzymatic-gravimetric method are shown in Table 1. The content of total dietary fibre in CMP has been found as 511.7 \pm 2.4g kg⁻¹ in CE and ranged from 217.4 \pm 1.68 to 536.1 \pm 3.17g kg⁻¹ in UAE. UAE was performed at three different point: 5, 15, and 30min. The amount of total dietary fibre obtained with each enzyme applied in CE during 30min has been found as similar to that of UAE done for 15min for each enzyme according to SPSS 16.0 for Windows using Duncan ($p > 0.05$). The results presented show that UAE is more rapid and efficient than CE (Table 1). However, when the UAE duration extends, enzyme deterioration can occurred, and the yield of extraction can diminish.

Table 1 – Comparison of the durations of conventional treatment and ultrasound treatment

Treatment	Duration (min)	Total dietary fibre (g kg ⁻¹ dm)
CE	30	511.7 \pm 2.4 ^b
UAE	5	217.4 \pm 1.68 ^c
UAE	15	536.1 \pm 3.17 ^b
UAE	30	466.5 \pm 0.28 ^a

The data are mean values of duplicate determination \pm standard deviation. Means are not significantly different ($p > 0.05$).

Another method of detecting dietary fibre is the enzymatic-chemical method. It was performed to determine the amount of dietary fibre using both CE and UAE during 15min. The neutral sugar compositions of CE and UAE are presented in Fig. 1.

The details of the sugar compositions are given in Table 2. The amount of dietary fibre was measured as 489.6 \pm 2.42g kg⁻¹ according to CE, whereas it was found as 510.4 \pm 1.43g kg⁻¹ in UAE. No significant differences between the two applications in the dietary fibre content have been observed (Table 2).

To sum up, the total dietary fibre (TDF) accounted for 511.7g kg⁻¹ according to the enzymatic-gravimetric method and 489.6g kg⁻¹ (w/w) according to the enzymatic-chemical method with CE. The total dietary fibre was calculated as 536.1g kg⁻¹ and 510.4g kg⁻¹, respectively when UAE was performed. No good agreement was found between the enzymatic-gravimetric method and the enzymatic-chemical method with both CE and UAE.

According to Alba et al. [25], this difference can be explained either by overestimation of the amount of fibre in the AOAC method (co-precipitation of oligosaccharides and Maillard reaction products) or by the underestimation of the amount of fibre in the enzymatic-chemical method (loss of polysaccharides during hydrolysis) or both. Similar results were shown in literature in the case of dietary fibre determination using both the enzymatic-chemical and the AOAC methods.

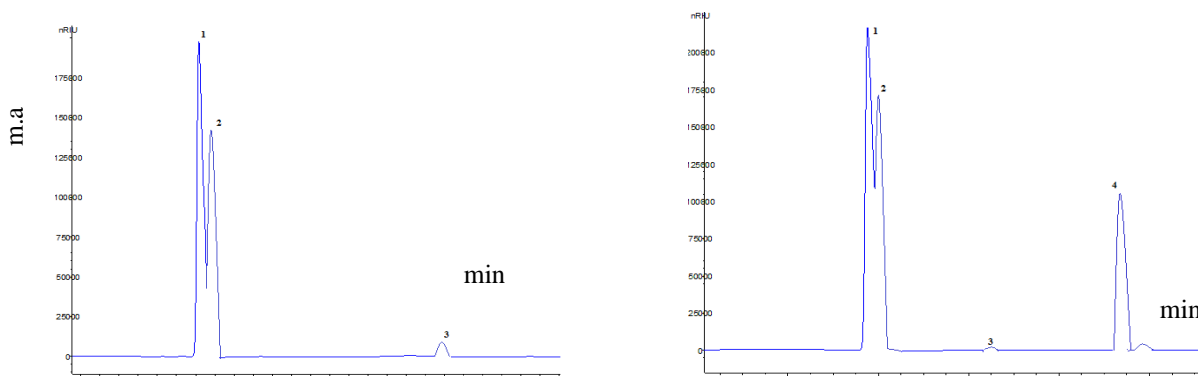


Fig. 1. HPLC chromatogram of (a) CE, 1. arabinose, 2. xylose, 3. galactose (b) UAE in 20 min, 1. arabinose, 2. xylose, 3. mannose, 4. glucose, 5. Galactose

Table 2 – Dietary fibre content of CMP obtained by the enzymatic-chemical method

	Neutral sugar					Uronic acid (g kg ⁻¹)	Klason lignin (g kg ⁻¹)	DF (g kg ⁻¹)
	Ar	Xy	Man	Gal	Glu			
Conventional	132.5±0.78	88.5±0.78	-	trace	-	0.092±0.0001	268.5±1.88	489.6±2.42
Ultrasound	129 ±1.41 ^a	101±0.99 ^b	trace	trace	7.3±0.12	0.117±0.0005	273±0.51	510.4±1.43

The data are mean values of duplicate determination ± standard deviation. Means are not significantly different (p>0.05).

Total phenolic content

In this study, the total phenolic contents varied between 112.0134±0.46 mg GAEg⁻¹ and 218.71±6.08mg GAEg⁻¹. The contents of total phenol compounds obtained by CE and UAE of CMP have been determined and presented in Table 3. The extraction time was selected to be 10min, because the total phenolic content with 10 min of UAE was similar to that of CE according to SPSS 16.0 for Windows using Duncan. These extracts were used for other analyses. Comparison of the two extraction techniques has shown that UAE ensures more rapid and efficient extraction than CE. However, as long as the duration of UAE extended, solvent losses occurred, and the extraction yield diminished. The content of total phenolic compounds of CMP with CE was 148.93mg GAEg⁻¹.

Table 3 – Content of total phenols in CMP after extraction

Sample	Duration	mg GAE/g dm	mg CE/g dm
CE	20	148.93±0.6 ^d	290.46±1.26 ^d
UAE	5	112.0134±0.46 ^e	223.323±1.04 ^e
UAE	10	144.70±4.72 ^d	297.4±10.7 ^d
UAE	15	167.22±10.13 ^c	348.396±22.96 ^c
UAE	20	190.6628±7.12 ^b	401.52±16.138 ^b
UAE	30	218.71±6.08 ^a	465.08±138 ^a

The findings presented make it obvious that phenolic compounds are best extracted from CMP by 10min UAE. Therefore, this extract was selected to determine the antioxidant activity and further identify individual phenolic compounds, and it has been compared with that obtained by CE.

Determination of the antioxidant activity

DPPH radical scavenging method: DPPH assay is one of the widely used methods to test the antioxidant

activity of a sample, because this method is reliable, simple, and takes but a short time for analysis [19]. The antioxidant activity of the samples is measured by their ability to reduce the DPPH• by donating the hydrogen atom. The results of the DPPH *free radical scavenging activity* of these extracts are expressed as mg GAE kg⁻¹ sample. The antioxidant activity of CE (205.6±0.27mg GAEkg⁻¹ sample) was higher than UAE in 10min (199.27±2.47mg GAEkg⁻¹ sample). There was no significant difference between the antioxidant activity of the CE and UAE in 10 min of CMP. The results were also reported as antioxidant activity (AA %). AA % of CE was found to be 84.96±2.29 %, and UAE in 10min was 87.41±5.41%. The antioxidant activity of CE appeared to be lower than that of UAE. The high phenolic content was usually correlated with high radical scavenging activity [26]. Hence, the AA of both CE and UAE was most probably due to the presence of polyphenols, such as flavonoids and phenolic acids in the extract, which have the hydrogen-donor ability to scavenge the free radicals.

B-carotene-linoleat model system: formation of conjugated diene is an indication of lipid oxidation during the initiation phase. As oxidation progressed, the absorbance of β-carotene at 470nm decreased and its yellow colour faded. In this study, the results have also been reported as AA % (antioxidant activity %). The antioxidant activity of CE (61.92±3.74 %) was established to be lower than that of UAE in 10min (70.12±3.69%). With this method used, the trends in the antioxidant activity of the extracts were not similar to that of the DPPH• assay. Furthermore, the concentrations used were much smaller as compared to the DPPH• method, indicating the effectiveness of the extracts in inhibiting the oxidation process in a lipid model system.

Identification and quantification of phenolic compounds

Fig. 2 shows the HPLC profiles of standard phenolic compounds. Six phenolic compounds were identified in both CE and UAE in 10 min (Fig. 3). Table 4 shows the amount of each phenolic compound detected. It should be mentioned that the phenolic profiles in HPLC analysis are not related to the extraction method used, but the amount of the phenolic compounds depends on the extraction techniques.

Table 4 – Phenolic compounds identified in CMP after extraction

	CE	UAE in 10 min
Syringic acid	375.98±4.915 ^a	3315.36±36.56 ^b
Trans-cinnamic acid	7.18±0.0005 ^a	13.745±0.485 ^b
Myricetin	7.975±0.61 ^a	14.675±0.095 ^b
Caffeic acid	33.94±12.162 ^a	31.6±2.3 ^a
-epicatechin	0.861±0.1525 ^a	1.021±0.151 ^a
Naringin	1.191±0.0005 ^a	2.256±0.032 ^b

The data are mean values of duplicate determination ± standard deviation.

Means are not significantly different (p>0.05).

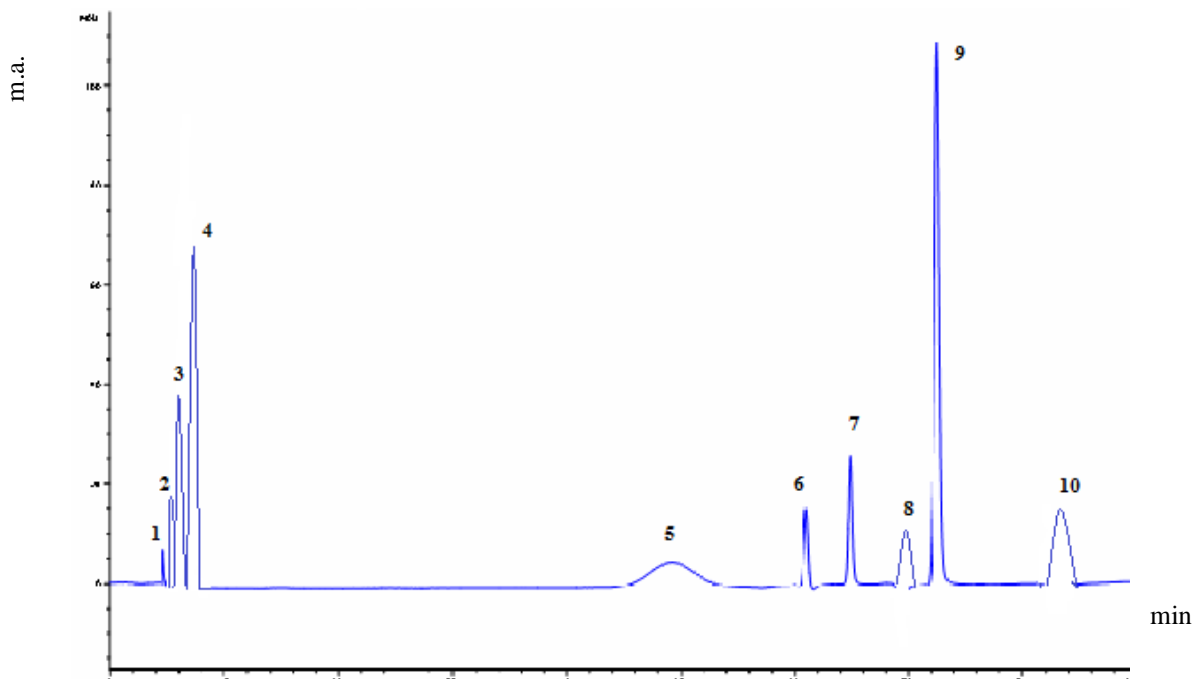


Fig. 2. HPLC chromatogram of antioxidant standards and retention time of these standards.

1. caffeic acid, rt: 2.814; 2. syringic acid, rt: 3.518; 3. -epicatechin, rt: 4.045; 4. ferulic acid, 4.476;
5. trans-cinnamic acid, rt: 42.432; 6. myricetin, rt: 46.647; 7. naringenin, rt: 61.157; 8. quercetin, rt: 70.322; 9. p-coumaric acid, rt: 72.709; 10. rutin, rt: 83.024

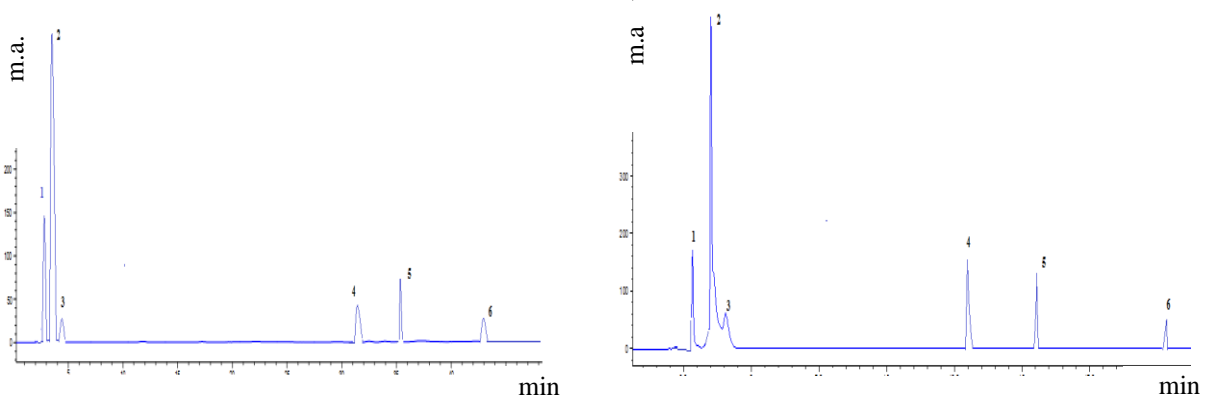


Fig. 3. HPLC chromatogram of (a) CE, (b) UAE in 10 min

- (1 – caffeic, 2 – syringic, 3 – epicatechin, 4 – trans-cinnamic acid, 5 – myricetin, 6 – naringin)

Conclusion

CMP can actually be regarded as by-products of processing carob molasses, because this product is intended for animal livestock. Carob pods and seeds are, therefore, a cheap source of natural polyphenolic phytochemicals and fibre, but their nature and importance are, so far, poorly investigated. The study presented here indicates that efficient polyphenol extraction from CMP might be achieved by employing conventional extraction. The extracts obtained with this procedure exhibit appreciable antioxidant capacity. Moreover, dietary fibre extraction can be achieved by conventional assistance. CMP proves the high potential of carobs as a cost-effective

source of value-added dietary fibre. However, ultrasound applied to CMP could become a more efficient strategy for obtaining phenolic compounds and dietary fibre. The influence of the time parameters on the extraction yields has been evaluated. The ultrasound assistance technique was a way to produce antioxidant and dietary fibre-rich extracts in a shorter period of time and in an energy-effective way. This study has revealed that CMP is rich in phenolic compounds and fibre. CMP could potentially be used as a dietary supplement to improve different physical, chemical, and nutritional properties of the foods to which it is added. CMP can be used to prepare low-calorie, high-fibre, and antioxidant-rich foods.

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