Effect of Rosting Process on Polyphenols Content of Carob Powder

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Abstract: The objectives of the present investigation were: (1) to identify and quantify the polyphenols compound of carob powder, (2) to study the effect of roasting at 180ºC for 30 minutes on the polyphenols compound of carob powder by using high-performance liquid chromatography (HPLC). Results showed that, roasting treatment of carob powder at 180˚C for 30 min. was decreased total phenols,carotenoids, tannins and antioxidant activity. The HPLC-chromatograms of the two ethanolic extracts of carob powder before roasting and after roasting at 180ºC for 30 min. were showed that, the phenolic compounds were decreased by roasting treatment, however, some flavonoid compounds increased by roasting treatment such as: narengin from 13.90 to 138.34 ppm/g, rutin from 31.90 to 244.31 ppm/g, hisperidin from 23.73 to 246.09 ppm/g, and 31.90 to 244.31 ppm/g, hisperidin from 23.73 to 246.09 ppm/g and hispertin from 11.61 to 68.14 ppm/g.Other flavonoid compounds were decreased by roasting treatment such as quercetrin from 50.38 to 21.86 ppm/g.
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1. Introduction:

Carob is the beanlike of *Ceratonia Siliqua*, which grows widely in the Mediterranean region and belongs to the genus *Leguminosae.* The world production of carob pods is 374,800 – 441,000 tons/year, the leading producer being Spain followed by Italy, Portugal, Morocco, Greece, Cyprus, Turkey and Algeria. The production of Carobpods varied depending on thecultivar, region, and farming practices (Karababa and Coşkuner, 2013).

Due to its chemical composition, Carob is used in the food industry and in medicine. In terms of the medicinal uses, Carob has revealed interesting Lipid-lowering (Zunft, *et al,* 2001), nephroprotective (Ahmed, 2010), anti-cardiovascular, anti-proliferative (Corsi, *et al*., 2002 and Roseiro, *et al*, 2013a), in vitro and in vivo antioxidant properties, apparently related to its phenolic compounds (Vekiari, *et al,* 2012 and Sebai, *et al*, 2013). Carob pods are a rich source of natural antioxidants, which may, by different mechanisms; act as an effective defense against reactive oxygen species including free radicals such as superoxide anion and hydroxyl radicals and non-free radical species such as hydrogen peroxide.

Oxidative stress is the common causative factor of many health problems such kidney diseases, cardiovascular diseases and neurodegenerative diseases by underlying mechanisms of inducing oxidation reaction of lipids, proteins and nucleic acids (Palipoch, *et al*., 2013). Bioactive compounds such as phenolics present in Carob are able of acting as chemical antioxidants and therefore possess the ability to reduce the oxidative damage (Roserio, *et al*., 2013a,b).

Carob Kibbles contain 448mg/kg extractable polyphenols comprising gallic acid (174 mg/kg), hydrolyzable tannins (26 mg/kg), condensed tannins (15 mg/kg) and derivatives of myricetin (171 mg/kg), quercetin (53 mg/kg) and kaempferol (9mg/kg) (Hilal Şahin *et al*., 2009).

To the best of our knowledge there are no studies undertaken to maximize the phenolic compound extraction and antioxidant activity of Carobpulp. Hence, the objectives of the present investigation were: (1) to identify and quantify the polyphenols compound of carob powder, (2) to study the effect of roasting at 180ºC for 30 minutes on the polyphenols compound of carob powder by using high-performance liquid chromatography (HPLC).

2. Materials and Methods:

Carob pods (*Ceratonia Siliqua L.*) were obtainedfrom localmarket in Jeddah, Saudi Arabia. All chemicals reagents were purchased from El-Gomhoria Co. in Cairo, Egypt.

Methods:

Five kg carob were obtained and seeds were removed, pods then were kibbled, milled and roasted in a drying oven at 180ºC for 30 minutes. The roasted sample was then ground in amill (Molineux) and passed through the screen (60 meshes)to provide uniform particle size. Carob powder sample was then placed into polyethylene bags and kept at 4ºC until analysis.

Determination of Total Phenolic Compounds:

Total soluble phenolicsin carob powder before and after roasting at 180ºC for 30 minutes, were determined with Folin-Ciocalteu reagent according to

the method of (Slinkard and Singleton, 1977) using gallic acid as standard phenolic compound. Briefly, 1.0 ml of extract solution containing 1.0g extracts in a volumetric flask was dilutedwith dis. water (46ml). One mil liter of Folin-Ciocalteau reagent was added and the content of the flask was mixed thoroughly. Three minutes later, 3 mol of Na_2CO_3 (2%) was added and the mixture which was allowed to stand for 2 hrs with intermittent shaking. The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the samples extracts were determined as microgram of gallic acid equivalent using an equation obtained from the standard gallic acid graph:

Absorbance = 0.0028 x gallic acid (mg)

Antioxidant activity:

The reaction mixture contained 2mL of ethanol, 125 µM DPPH and test samples. After 2 minutes incubation at room temperature, the absorbance wasrecorded at 517nm. All experiments were carried out in triplicate and repeated at least three times. Results were expressed as percentage decrease with respect to control values (Chen and Ho, 1995).

Determination of Tannins (as tannic acid):

Total tannins were determined calorimetrically as described by A.O.A.C (2000). Sample (5gm.) was mixed with 50ml methanol in a closed conical flask and left for 20hrs at room temperature (25± 2ºC). The mixture was then centrifuged for 30 minutes at 3000 rpm. The tannins in the supernatant were measured at 760 nm using Jen way 6505 UV/VIS spectrophotometer. Tannic acid was used to prepare the standard curve.

Determination of Total Carotenoids:

The total carotenoids were determined according to Heinonen and Marina (1989).

Separation of Polyphenols and Flavonoids by HPLC:

High performance liquid chromatography (HPLC) technique using HPLC Agilent 1100 series equipped with Quaternary pump, set at flow 1 ml/min. Autosampler, degaser, column compartment set at 35ºC and variable wavelength detector set at 330 for flavonoid compounds and 280 for phenolic compounds, column: Hypersil ODS 5 µm, 250x4 mm was used. Pure phenolic compounds: gallic, pyrogallol, 3-Hydroxy-Tyrosol, Protocatchouic, 4-Amino Benzoic acid, Chrorogemic, Catechol, Catechin, Epi-catechin, Caffeine, P-OH-Benzoic, Caffeic, Vanillic, Chicoric, Ferulic, Iso-ferulic, E-Vanillic, Resveratrol, Oleuropein, Ellagic, Alpha-coumaric, Benzoic, 3,4,5 Methoxycinnamin, Salicylic, Coumerin, P-Coumaric and Cinammic and pure flavonoid compounds: Naringin, Rutin, Hespirdin, Rosmarinic, Quecetrin, Quecitin, Kaempferol, Hespirtin, Apegenin, and 7-Hydroxy Flavone were used as standard obtained from El-Gomhoria-chemical company, Egypt.

3. Results and Discussion

Antioxidant Contents:

Phenolic compounds are well-known secondary metabolic compounds are of high interest as alternative for synthetic antioxidant to prevent lipid peroxidation in food systems. The quantification of total phenolic compound contents is the first step used when assessing the antioxidant activity of plant and food extracts.

Total phenols, carotenoids and tannins were determined and results are presented in Table (1). The table shows that total phenolic compounds content of carob powder before and after roasted were 186.07 and 202.89 mg/100g, respectively. While, carotenoids contents were 18.66 and 11.93mg/100g, respectively. Results in the same table revealed that, tannins content in the same samples represented 106.67 and 643.43 mg/100g, respectively. Roseiro *et al*.(2013) reported that, carob kibbles contained total phenolics ranged from 1.55 to 3.96% and tannins ranged from 0.81 to 2.62%.

There is growing interest in the antioxidant properties of phytochemical compounds of plant extracts due to their significant role in disease prevention and health benefits. No standardized method is available to evaluate the antioxidant capacity of plant extracts. Numerous methods have been employed to estimate the antioxidant potential. In the present study, DPPH radical scavenging effect was used to assess the antioxidant activity of Carob powder before and after roasted at 180ºC for 30 minutes.

The DPPH assay is commonly used to the assessment of free radical-scavenging ability of plant and health food extracts due to its simplicity, stability and reproducibility. Regarding the DPPH tests, the results shown in table (1) were 95.82% and 27.09%, respectively.

Fractionation of natural phenolic and flavonoid compounds in carob powder before and after roasted at 180ºC for 30 minutes.

Data illustrated in fig. (1) and (2) and presented in tables (2) and (3) showing the HPLC-chromatograms of the two ethanolic extracts of carob powder before roasting and after roasting at 180ºC for 30 min. and representing the identified components monitored at 280nm for phenolic compounds and at 330nm for flavonoid compounds. Tables (2) and (3) show that Rt and the concentrations by ppm/g of the identified phenolic and flavonoid compounds, respectively. As seen in Table (2) the phenolic compounds were decreased by roasting treatment, however, some flavonoid compounds increased by

roasting treatment such as: narengin from 13.90 to 138.34 ppm/g, rutin from 31.90 to 244.31 ppm/g, hisperidin from 23.73 to 246.09 ppm/g, and 31.90 to 244.31 ppm/g, hisperidin from 23.73 to 246.09 ppm/g and hispertin from 11.61 to 68.14 ppm/g.Other flavonoid compounds were decreased by roasting treatment such as quercetrin from 50.38 to 21.86 ppm/g.

Table (1): Total phenols, carotenoids, tannins (mg/100g) and antioxidant activity (%) content of carob powder before and after roasted at 18 ° C for 30 min.

Constituents (mg/100 g)	Before roasting	After roasting
Total phenols (as mg gallic acid)	186.07	102.89
Total carotenoids	18.66	11.93
Total tannins	706.67	643.43
Antioxidant activity (%)	95.82	27.09

Table (2): Phenolic compounds contents of carob powder before and after roasted at 18 ° C for 30 min.

Phenolic compounds	Before roasted		After roasted	
	Rt	ppm/g	Rt	ppm/g
Gallic	7.477	326.01	7.487	75.46
Pyrogallol	7.609	603.64	7.553	3674.30
3-OH Tyrosol	8.620	184.38	8.580	179.87
4-Aminio Benzoic	8.704	9.06	8.680	28.39
Protocatchnic	8.840	71.53	8.873	560.42
Chlorogenic	9.531	151.30	9.594	180.60
Catechol	9.861	12.88	9.873	170.89
Catechein	10.013	81.63	9.938	109.89
Caffeine	10.163	16.59	10.140	62.08
P-OH-Benzoic	10.243	44.10	10.298	66.64
Caffic	10.580	6.38	10.540	29.77
Vanillic	10.635	4.97	10.620	29.08
Ferulic	12.102	19.13	12.107	21.87
Iso-Ferulic	12.490	19.61	12.467	26.26
e-Vanillic	12.746	304.93	12.739	248.63
Reversetrol	12.854	24.31	12.953	16.73
Effagic	13.236	45.57	13.248	76.38
Alpha-Coumaric	13.607	16.17	13.551	66.34
Benzoic	13.687	161.59	13.687	158.90
3,4,5-Methoxy Cinnamic	14.128	5.70	14.115	13.54
Salycilic	14.247	36.70	14.233	105.65
Coumarin	14.328	16.49	14.320	18.95
P-Coumaric	14.767	1.31	14.877	9.55
Cinnamon	15.293	15.83	15.278	9.94

Table (3): Flavonoid compounds content of carob powder before and after roasted at 18 ° C for 30 min.

Flavonoid compounds	Before roasted		After roasted	
	Rt	ppm/g	Rt	ppm/g
Narengin	12.363	13.90	12.341	138.34
Rutin	12.493	31.38	12.504	244.31
Hisperidin	12.573	23.73	12.569	246.09
Rosmarinic	12.875	6.49	12.869	51.55
Quercetrin	13.390	50.38	13.292	21.86
Quercotin	14.707	3.11	14.707	33.04
Hispertin	15.220	11.61	15.127	68.14
kampferol	15.398	7.83	15.198	7.01
Apegenin	16.263	16.20	16.237	29.01
7-OH-Flavone	16.731	0.08	16.748	2.18

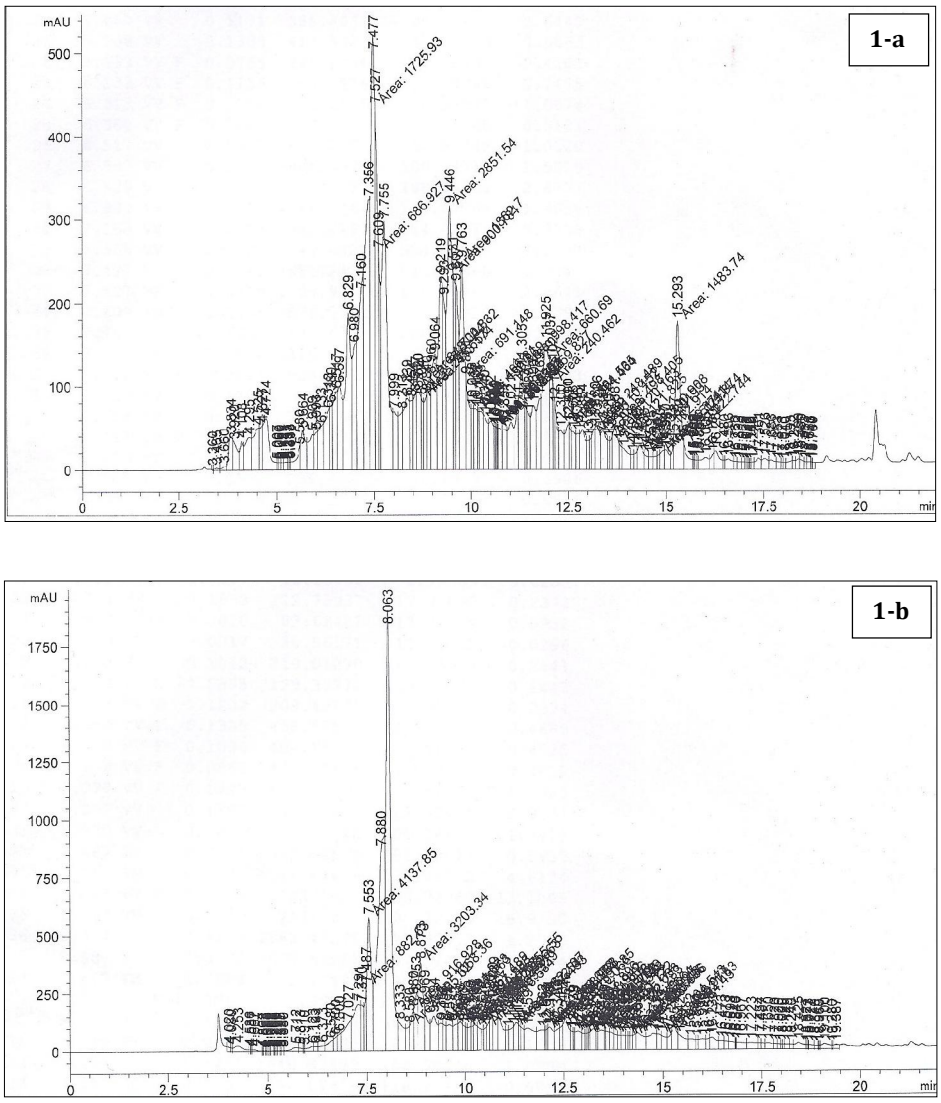
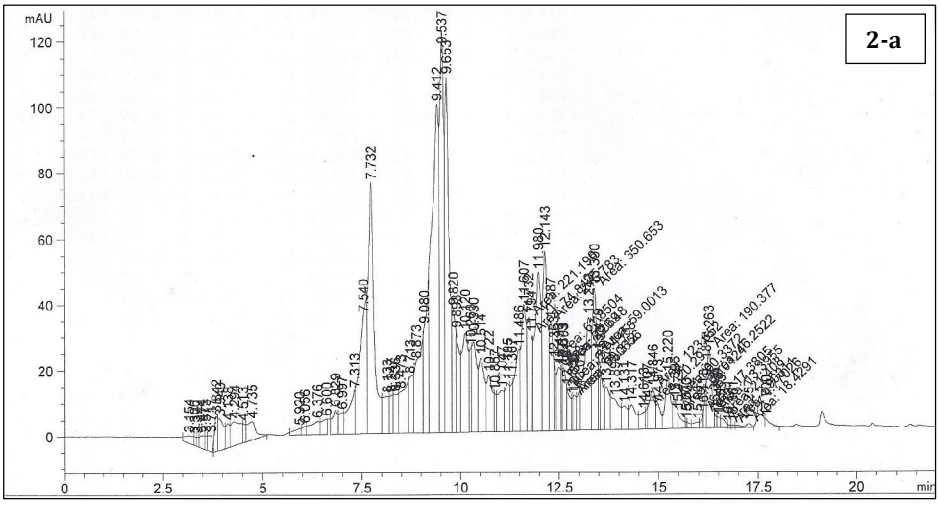


Figure (1) - Identification of phenolic compound contents of carob powderbefore (a) and after (b) roasted at 180˚ C for 30 min.



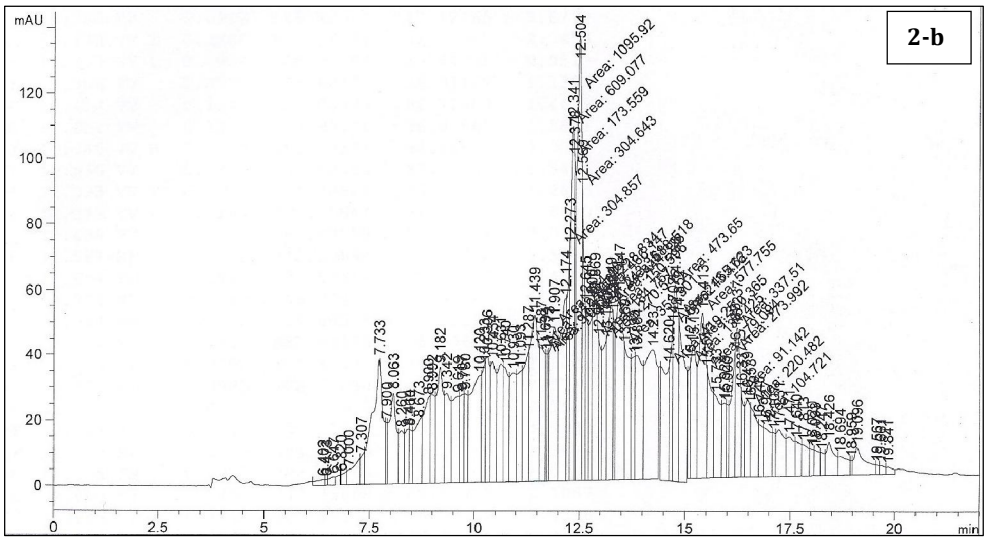


Figure (2) – Identification of Flavonoid compounds contents of carob powder before (a) and after (b) roasting at 180^0 C for 30 min.

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