Food and Public Health 2013, 3(6): 304-308
DOI: 10.5923/j.fph.20130306.06

Assessment of Proximate Chemical Composition, Nutritional Status, Fatty Acid Composition and Phenolic Compounds of Carob (*Ceratonia Siliqua L.*)

M. Kamal E. Youssef[1], Moshera M. El-Manfaloty[2], Hend M. Ali[2,*]

[1]Food Science & Technology Department, Faculty of Agric., Assiut University, Assiut, A. R. Egypt
[2]Home Economic Department, Nutrition and Food Science, Faculty of Specific Education, Assiut University, Assiut, A. R. Egypt

Abstract The Carob is the fruit of an evergreen (*Ceratonia Siliqua L.*) cultivated in the Mediterranean area. The pulp represents 90% of the fruit. It has a high content of sugars and tannins and low contents of protein and fat. Carob powder or syrup is used as an ingredient in cakes and cookies and chocolate substitute. Carob powder contained high levels of carbohydrates (75.92%), (6.34% protein) and low level of fat (1.99%). The crude fiber content recorded 7.30%. Carob powder was rich source of Fe, Ca, Na, K, P and S as well as E, D, C, Niacin, B6 and folic acid. Carob powder consisted of 11 phenolic compound. Pyrogallol, catechol, chlorogenic and protocatechuic recorded the highest values, while coumarin, cinnamic, ferulic, gallic acid and vanillic recorded the least values of the phenolic compounds. Carob powder oil consisted of 17 fatty acids, but consisted mainly of four fatty acids namely: oleic, linolic, palmitic and stearic acids recording 40.45%, 23.19%, 11.01% and 3.08%, respectively. Carob powder is acclaimed ingredient with a marked nutritional value due to its high dietary fiber and phenol compounds. The soluble fibers exert a preventative role against heart disease and lowering serum cholesterol.

Keywords Carob Powder, Chemical Composition, Minerals, Vitamins, Phenolic Compounds, Fatty Acid Composition

1. Introduction

The Carob is the fruit of an evergreen (*Ceratonia Siliqua*) cultivated in the Mediterranean area. The seeds of the carob are utilized in food industry for their gum content. However, the use of the whole fruit in human consumption is rather limited, due to a high level of tannins causing astringency[1]. The fruit pod (containing sweet pulps) which is giving, after removal of the seeds and the carob powder[2] often used as a chocolate or cacoa substitute[3]. Carob fruits are used in food industry as a source of many products such as gum, sugar and alcohol[4],[5].

Recently investigators isolated and identified the major polyphenols in carob fibers[6, 7, 8] studied the variation and the composition of phenolic compounds of carob pods grown in different regions of Morocco.

The chemical composition of carob was studied by[9]. The two principal components of the carob fruit are the pulp and seed. The seeds represent 10% of the weight of the fruit and the pulp represent the other 90% of the fruit, and its composition depends on the variety, climate and growing techniques[10, 11, 12 and 13].

Carob is typically dried or roasted, and is mildy sweet. In powdered, chip, or syrup from it is used as an ingredient in cakes and cookies, and is used as a substitute for chocolate. Crushed pods may be used to make a beverage; compote, liqueur, and syrup are made from carob in Turkey, Malta, Portugal, Spain and Sicily[14]. Several studies suggest that carob may add in treating diarrhea in infants[15]. Carob powder is a natural sweetener with flour and appearance similar to chocolate; therefore it is often used as cocoa substitute. The advantage of using carob as a chocolate resides in that carob is an ingredient free from caffeine and theobromine[16]. Carob germ flour is used as dietetic human food[3], or as a potential ingredient in cereal – derived foods for celiac people[17].

Furthermore, the protein content of carob germ flour seeds is higher than those observed for other beans such as faba bean, pea and soy beans[18]. Carob pulp is high (48 – 56%) in total sugar content that include many sucrose, glucose, fructose and maltose. In addition it contains about 18% cellulose and hemi cellulose. Ripe carob pods contain a large amount of condensed tannins (16 – 20%) on dry weight basis[19].

Tradionally important main products of carob are pods, seed gums and derived products like carob bean flour, pekmez (concentrated carob syrup / molasses), health foods

* Corresponding author:
hendma20@yahoo.com (Hend M. Ali)
Published online at http://journal.sapub.org/fph

(as a chocolate substitute), carob syrup and medicines such as laxatives and diuretics[20, 21, 22, 23, 24, 25 and 26].

The present investigation was carried out in an attempt to clarify the proximate chemical composition, the nutritional status, as well as, the fatty acid composition and the phenolic compounds of carob powder.

2. Materials and Methods

2.1. Materials

5 Kg. of carob powder were procured from Aswan Governorate in November 2012 where carob is cultivated. The seeds were removed and the carob was ground to particles of 1 ≤ mm. samples were stored at 18^{o}C and analyzed within 2 months.

2.2. Methods

2.2.1. Determination of Gross Chemical Composition

Moisture, crude protein, crude oil, crude fiber and ash contents were determined according to the procedures described in the AOAC[27]. The total carbohydrates were calculated by difference according to[28]. Caloric value was calculated according to[29].

2.2.2. Determination of Minerals Contents

The samples were wet acid-digested using a nitric acid and perchloric acid mixture (HNO3; HCSO4; 2 : 1 v / v). The amounts of iron, zinc, copper and manganese in the digested sample were determined using a GBC Atomic Absorption 906 A, as described in[30]. Sodium and potassium were determined by a flame photometer 410, calcium was determined by titration with version 0.0156 N according to[31]. Phosphorus, sulphur and selenium were determined according to the methods described by[30].

2.2.3. Determination of Vitamins Contents

Vitamin C was determined according to[32]. On the other vitamins E, D and A were determined according to the methods described by[33].

2.2.4. Determination of Vitamin B-complex

A new reversed-phase chromatographic method was described for the separation and quantification of thiamine, folic acid, B_{12}, pyridoxine, nicotinic acid and riboflavin applying HPLC technique as outlined by[34].

2.2.5. HPLC-separation and Identification of Phenolic Compounds

Phenolic compounds were determined by HPLC according to the method of[35]. As follow: 5 g of sample were mixed with methanol and centrifuged at 10,000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Hewllet Packared (series 1050) equipped with autosampliing injection, solvent degasser, ultraviolet (UV) detector set at 280 nm and quarter HP pump (series 1050). The column temperature was maintained at 35℃. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of Hewllet Packared software.

2.2.5.1. Preparation of Methyl Ester of Fatty Acids

The methyl esters of fatty acids were prepared from aliquots of total lipids using 5 ml 3% H_2SO_4 in absolute methanol and 2 ml benzene as mentioned by[36].

2.2.5.2. GLC-of Methyl Ester of Fatty Acids

The methyl esters of fatty acids were separated using a PYE Unicam Pro-GC gas liquid chromatography with a dual flame ionization carried out on (1.5m×4mm) SP-2310 column, packed with 55% cyanopropyl phenyl silicone dimensions. Column temperature: At first increasing the temperature from 70 190℃ at the rate of 8^{o}C/minute and then isothermal for 10 minute at 190℃. The injector and detector temperature were 250℃ and 300℃; respectively.

Carrier gas: Nitrogen at the rate 30 ml/minute, hydrogen and air flow rate were 33 and 330 ml/minute; respectively. The chart speed was 0.4 cm/minute. Peak identifications were established by comparing the retention times obtained with standard methyl esters. The areas under chromatographic peak were measured with electronic integrator as mentioned by[36].

3. Results and Discussion

3.1. Gross Chemical Composition and Caloric Value

Carob powder had been considered as a food supplement in various cultures and it is eaten for their edibility and delicacy. Carob powder fall between the best vegetables and animal protein source[1, 2 and 3].

Table (1). Mean values of gross chemical composition and caloric value of carob powder*

Chemical composition	%
Moisture	5.29
Protein a**	6.34
Crude fat	1.99
Ash**	3.16
Crude fiber**	7.30
Carbohydrates b**	75.92
Caloric value^{C} Kcal. / 100 g.	346.95

* Mean of three replicates.
** Calculated on dry weight basis.
(a) Protein = % Nitrogen × 6.25
(b) Carbohydrate : calculated by difference.
(c) Calories : Calculated as K cal/100 g dry matter.

The data of gross chemical composition of carob powder and caloric value are presented in table (1).

The carob powder samples contained low level of fat (mean value 1.99%) and an appreciable amount of protein (mean value 6.34%). The crude fiber content (mean value 7.30%) was not high compared to the carbohydrate content (mean value 75.92%) which was extremely high. The data are in good agreement with[1, 3 and 9].

The caloric value of the carob powder was 346.95 K caloric / 100 g dry matter.

3.2. Minerals Content

The data of the average values of minerals content in carob powder are outlined in Table (2).

Table (2). Mean values of minerals content of carob powder (mg / kg)*

Minerals	mg / Kg.
Mn	10.24
Zn	24.71
Fe	381.80
Cu	4.84
Se	9.79
Ca	2123.00
Na	505.97
K	8637.64
P	2255.21
S	17577.80

* Calculated on dry weight basis

The data revealed that the carob powder is considered as a rich source of Fe, Ca, Na, K, P and S. The data are in good agreement with[37] and[38].

The trace element Cu, Zn and Se act as cofactors of antioxidant enzymes to protect the body from oxygen free radicals that are produced during oxidative stress[39].

3.3. Vitamins Content

The data outlined in Table (3) represented the mean values of vitamins content in carob powder.

The data revealed that carob powder is a good source of vitamins E, D, C, Niacin, B_6 and folic acid. Meanwhile, carob powder contained lower levels of vitamins A, B_2 and B_{12}. Such data are in agreement with[38].

Table (3). Mean values of vitamins content of carob powder

Vitamins	Test units
Fat soluble vitamins	**µg / 100 g.**
A	1.407
E	5.377
D	4.9
Water soluble vitamins	**mg / 100 g**
C	830.08
B_2	0.38
Niacin	185.68
B_6	23.80
Folic acid	41.97
B_{12}	1.30

3.4. Phenolic Compounds Content

The phenolic compounds content of the carob powder is presented in Table (4). The data revealed that the phenolic compounds of the carob powder consisted of 11 compounds. Phrogallol, catechol, chlorogenic, and protocatechuic recorded the highest values, while coumarin, cinnamic, ferulic, gallic acid, and vanillic recorded the least values of the phenolic compounds. The data are in agreement with[40, 41 and 42].

Both chlorogenic acid and caffeic acid are antioxidants and inhibit the formation of mutagenic and carcinogenic N-nitroso compounds in vitro as reported by[42, 43].

Moreover, some phenolic acids (Caffeic acid, ferulic acid, gallic acid, and protocatechinic acid contribute against various types of cancer such as breast, lung and gastric cancer as reported by[44, 45, 46, 1, and 7].

Table (4). Phenolic compounds content of carob powder (ppm)

Phenolic compounds	ppm
Gallic acid	10.21
Pyrogallol	4970.18
Protocatechuic	79.47
Chlorogenic	101.09
Catechin	27.97
Catechol	164.67
Cinnamic	7.78
Caffeine	48.23
Vanillic	13.92
Ferulic	10.17
Coumarin	4.49

3.5. Fatty Acid Composition

The fatty acid composition data of carob powder are presented in Table (5). The obtained data revealed that the carob powder oil considered of 17 fatty acids, but consisted mainly of four fatty acids namely: oleic, linoleic, palmitic and stearic recording 40.45%, 23.19%, 11.01% and 3.08%. The data are in good accordance with[26, 3 and 18].

The total unsaturated fatty acids recorded 66.98%, while the total saturated fatty acids recorded 29.46% . Besides, Table (5) revealed that the saturated fatty acids / unsaturated fatty acids ratio accounted to 0.44.

Generally, there are two most important parameters related to fatty acid composition of any oil, one is the ratio of total saturated fatty acid / total unsaturated fatty acid, which is related to the oxidation stability of the oil, while the second is oleic / linoleic ratio, which has a positive effect on the taste of the oil[47].

In conclusion the carob powder is being acclaimed as an ingredient with a marked nutritional value due to its high levels of dietary fibre and phenol compounds. The soluble fibres in particular are thought to exert a preventative role against heart disease, as they appear to have the ability to lower serum cholesterol. Besides the polyphenols have antioxidant activity, which mainly enhances the prevention or delay the oxidative damage. Consequently, polyphenols are involved in protection against several diseases (cardiovascular and neuronal, among others). As a result, carob powder should be increasing interest as an ingredient in the food industry such as functional and healthy foods formulations as Biscuits, bread, and cakes.

Table (5). Fatty acid composition of carob powder (% of total fatty acids)

Fatty acids	Carbon chain	% of total fatty acids.
Lauric acid	C 12 : 0	0.75
Myristic acid	C 14 : 0	1.11
Palmitic acid	C 16 : 0	11.01
Palmitolic acid	C 16 : 1	0.65
Heptadecanoic acid	C 17 : 0	0.30
	unknown	1.07
Heptadecenoic acid	C 17 : 1	0.15
Stearic acid	C 18 : 0	3.08
Oleic acid	C 18 : 1	40.45
Linoleic acid	C 18 : 2	23.19
Linolenic acid	C 18 : 3	2.47
Arachidic acid	C 20 : 0	1.51
Gadoleic acid	C 20 : 1	2.68
	Unknown	2.12
Behenic acid	C 22 : 0	0.55
	C 24 : 0	0.43
	Unknown	4.36
Total unknown		7.55
Total saturated fatty acids (SFA)		29.46
Total unsaturated fatty acids (USFA)		66.98
SFA / USFA		0.44

REFERENCES

[1] Avallone, R., Plessi, M., Baraldi, M. and Monzan A. (1997). Determination of chemical composition of carob (Ceratonia Siliqua): protein, fat carbohydrates and tannins. J. of Food Composition and Analysis. 10, 166 – 172.

[2] Yousif, A. K. and Alghzawi, H. M. (2000). Processing and characterization of carob powder. Food Chemistry.

[3] Dakia, P. A., Wathelet, B., and Paquot, M. (2007). Isolation and chemical evaluation of carob (Ceratonia Siliqua L.). Food Chemistry, 102, 1368 – 1374.

[4] Carlson, W. A. (1986). The carob: Evaluation of trees, pods and Kernels. Int. Tree Crops J. 3, 281 – 290.

[5] Tous, J. (1992). Isozyme polymorphism in carob cultivars. Hort. Sci. 27. 257 – 258.

[6] Owen, R. W., Haubne, R. Hull, W. E., Erben, G., Spiegelhalder, B., Bartsch, H. and Haber, B. (2003). Isolation and structure elucidation of the major individual polyphenols in carob fiber. Food Chem. Toxicol. 41, 1727.

[7] El Hajaji, H., Lachkar, N., Alaoui, K., Cherrab, Y., Farah, A. Ennabili, A., El Bali, B., and Lachkar, M. (2011). Antioxidant activity, phytochemical screening, and total phenolic content of extracts from three genders of carob growing in Morocco. Arab. J. of Chem. 4. 321 – 324.

[8] Papagiannopoulos, M. Woll seifen, H. R., Mellenthin, A., Haber, B. and Galensa, R. (2004). Identification and quantification of polyphenols in carob fruits (Ceratonia Siliqua L.) and derived products by HPLC – UV – ESI / MS. J. Agric. Food chem.. 52, 3784 – 3791.

[9] Bouzouita, N., Khaldi, A., Zgoulli, S., Cheli, L., Chekki, R., Chaabouni, M. M. and Thonart, P. (2007). The analysis of crude and purified locust bean gum: A comparison of samples from different carob tree populations in Tunisia. Food Chem. 101. 1508 – 1515.

[10] Tous, J. (1990). El garrobo, Ed. Muniperna. Madrid, pp. 27-43.

[11] Petit, M. D. and Pinilla, J. M. (1995). Production and purification of a sugar syrup from carob pods. Lebensm. -Wiss. U – Technol. 28. 145-152.

[12] El-Shatnawi, M. J. and Mohawesh, Y. (2000). Seasonal chemical composition of saltbuck in semiarid grasslands of Jordan. J. Range Manage. 53: 211 – 214.

[13] Morton, J. F. (1987). Carob. In Fruits of warm climates. C F Dowling, Jr. (ed.). Miami, Fl. PP 121 – 124.

[14] Burg, B. (2007). Good treats for dogs cookbook for dogs: 50 Home-cooked treats for special occasions. Quarry Books. P. 28.

[15] Fortier, D. Lebel, G., Frechette, A. (1953). Carob flour in the treatment of diarrhoeal conditions in infants Candian Medical Association J., 68 (6): 557 – 561.

[16] Bengoechea, C., Romero, A., Villanueva, A., Moreno, G., Alaiz, M., Millan, F., Guerrero, A., and Puppo, M. C. (2008). Composition and structure of carob (Ceratonia Siliqua L.) germ proteins. Food chem.. 107, 675 – 683.

[17] Fillet, P. and Roulland, I. M., (1998). Caroubin: A gluten-like protein isolate from carob bean germ. Cereal Chem., 75. 488 – 492.

[18] Maza, M. P., Zamora, R., Alaiz, M., Hidalgo, F. J., Millan, I., and Vioque, I. (1989). Carob bean germ seed (Ceratonia Siliqua L.). Study on the oil and proteins, J. of the Sci. of Food and Agric. 46. 495 – 502.

[19] Karababa, E. and Coskunder, Y. (2013). Physical properties of carob bean (Ceratonia Siliqua L.) : An industrial germ yielding crop. Industrial crops and Products. 42. 440 – 446.

[20] Eski, A., and Artik, N. (1986). Harnup (Kecibonuzu) meyvesi ve pekmezinin Kimyasal hilesimi. A. U. Ziraat Fakultest Yilligi. 36 (1) : 77 – 82.

[21] Artik, N. and Erbas, S. (1988). The amino acid profile of carob beans. Ind. Obst. Gemus. 3 (3). 83 – 86.

[22] Tous, J. Romero, A., Hermoso, J. F., Ninot, A., Plana, J., and Batlle, I. (2009). Agronomic and commercial performance of four Spanish carob cultivars. Hort. Techn. 19. 463 – 470.

[23] Tunahoglu, R. and Ozkaya, M. T. (2003). Keciboynuzi. T. E. A. E. Bakis. 3 (5). 1 – 4.

[24] Turhan, I., Tetik, N., Aksu, M., Kahan, M., Certel, M. (2006). Liquid solid extraction of soluble solids and total phenolic compounds of carob bean (Ceratonia Siliqua L.). J. Food Processing Eng. 29 (5). 495 – 502.

[25] Biner, B., Gubbuk, H., Karhan, M., Aksu, M., and Pekmezci, M. (2007). Sugar profiles of the pods of cultivated and wild types of carob bean (Ceratonia Siliqua L.) in Turkey. Food

Chem. 100 (4). 1453 – 1455.

[26] Matthaus, B. and Ozcan, M. M. (2011). Lipid evaluation of cultivated and wild carob (Ceratonia Siliqua L.) seed oil growing in Turkey. Scientia Horticulturae, 130. 181 – 184.

[27] AOAC (1995). Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists. Washington. D. C.

[28] Howard, O., and Leonard, W. A. (1963). Food Composition and Analysis. Van Nostrond Reinhold Company (VNR). New York.

[29] Osborne, D. R. and Voogt, P. (1978). The analysis of nutrients in foods. Academic Press. INC. London. GB.

[30] AOAC (1990). Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists. Washington. D. C.

[31] Jakson, M. L. (1967). Soil Chemical Analysis. Prentice-Hall of India. Private Limited. New Delhi.

[32] Bajaj, K. L. and Kaur, G. (1981). Spectrophotometeric determination of L-ascorbic acid in vegetables and fruits. Analyst. 106, 117 – 120.

[33] Principal Central Lab. of Cairo University. (2008). Vitamin Assay. Faculty of Agriculture. Cairo Univ.

[34] Batifoulier, F. M. A., Besson, C., Demigne, C. and Remesy, C. (2005). Determination of thiamine and its phosphate esters in rat tissues analyzed as thiochromes on a RP-amide C16 column. J. of Chrom. Vol. 816. P. 67 – 72.

[35] Goupy, P. Hugues, M. Biovin, P. and Amiot, M. J. (1999). Antioxidant composition and activity of barley (Hordum vulgare) and malt extracts of isolated phenolic compounds. J. Food Sci. Agric. 79. 1623 – 1634.

[36] Rossell, J. B., King, B. and Downes, M. J. (1983). Detection of adulteration. J. Am. Oil Chem. Soc. 60 : 333.

[37] El-Shatnawi, M. J. and Erifi, K. I. (2001). Chemical composition and livestock injestion of carob (Ceratonia Siliqua L.)seeds. J. of range management. 54 (8): 669 – 673.

[38] Anon. (1986). The useful plants of India. Publications and Information Directorate. CSIR. New Delhi. Inida.

[39] Barakat, H. A. (2009). Efficiency of licorice and mustard extracts as anticancer, antimicrobial and antioxidant agents. Ph. D. Thesis. Faculty of Agriculture - Cairo university.

[40] Smith, H. A. E. (2007). Biochemical studies on some flavonoides, essential oils and saponins extracted from medical plants. Ph. D. Thesis. Faculty of Agriculture - Cairo University.

[41] Hiramo, R., Sasamots, W., Itakura, A., Igarachi, O., and Kondo, K. (2001). Antioxidant ability of various flawonoids against DPPH radicals and LDL oxidation. J. Nutr. Sci. and Vitaminol. 47: 357 – 362.

[42] Han, X. Z., Shen, T. and Lou, H. X. (2007). Dietary polyphenols and their biological significance. Int. J. Mol. Sci., 8 : 950 – 988.

[43] Ortega, N., Macia, A., Romero, M., Reguant, J. and Motilva, M. (2011). Matrix composition effect on the digestibility of carob flour phenols by an in-vitro digestion model. Food Chemistry. 124 : 65 – 71.

[44] Stagos, D., Kazantzoglou, G., Theofanidue, D., Kakalopoulou, G., Magiatis, P., Mitaku, S., and Kouretas, D. (2006). Activity of grape extracts from Greek varieties of vitis vinifera against mutagenicity induced by bleomycin and hydrogen peroxide in Salmonella typhimurium strain TA 102. Mutat Res-Gen Tox. En., 609 : 165 – 175.

[45] Bravo, L., Grados, N. and Saura - Calixo, F. (1998). Characterization of syrups and dietary fiber obtained from Mesquite pods (Prosopis pallida L). J. Agric. Food Chem. 46 (5). PP 1727 – 1733.

[46] Kumazawa, S., Taniguchi, M., Suzuki, Y., Shimura, M., Kwon, M. and Nakayama, T. (2002). Antioxidant activity of polyphenols in carob pods. J. Agric. Food Chem. 50 (2). PP 373 – 377.

[47] Ranalli, A., Contento, S., Schiavone, C. and Simone, N. (2001). Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. European of lipid Sci. and Techn. 130 : 228 – 238.