

# Evaluation of Proximate Composition, Minerals Content and Antioxidant Properties of Carob (*Ceratonia Siliqua L.*) Pod

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## Abstract:

Carob (*Ceratonia siliqua L.*) has been used by humans since very ancient times; this study aimed to evaluate the proximate composition, the minerals content and antioxidant properties of the *C. siliqua L.* (carob) pods. The carob pods samples were collected from local market prepared and subjected to proximate composition analysis using standard methods, also minerals content determination via spectrophotometer and flame photometer; beside assessment of antioxidant activity of carob pods extract using DPPH free radical scavenging methods. The obtained results of proximate composition indicated that carob pods had a high content of carbohydrate ( $59.46 \pm 1.22\%$ ), protein ( $13.27 \pm 0.1\%$ ), moisture ( $8.79 \pm 0.24\%$ ), fibers ( $8.62 \pm 0.40\%$ ), fat ( $6.32 \pm 0.41\%$ ), and ash ( $3.55 \pm 0.27\%$ ). As well, the results of elemental analysis showed that calcium content is higher (1989.61 mg/100 g), followed by magnesium (1482.19 mg/100 g), potassium (470.00 mg/100 g), iron (66.38 mg/100 g), manganese (8.67 mg/100 g), zinc (4.30 mg/100 g) and finally sodium (3 mg/100 g). The ethanolic carob pod extract showed good antioxidant activity with the percentage inhibition of  $50 \pm 0.04\%$ . Based on the obtained results carob pods have a good potential as nutritional and antioxidant sources and, it can be used in human consumption and food industry.

**Keywords:** Carob, Minerals, Proximate composition, Antioxidant, Nutrition.

## 1. Introduction

*Ceratonia siliqua L.*, commonly known as carob tree, is a Mediterranean evergreen tree that belongs to the family Fabaceae (subfamily of *Caesalpinioideae*) [1, 2]. The scientific name derives from the Greek keras meaning horn and the Latin Siliqua alluding to the hardness of the pod [1]. The pod is dark brown, with a straight, curved or twisted shape and it might be elongated or compressed in structure [2, 3]. The pod mass ranges between 5 and 30 g, up to 25 cm long and up to 1.3 cm thick [2–4]. The two main carob pod constituents are pulp (90%) and seed (10%) [3, 5, 6]. Chemical composition of the pulp depends on the cultivar, origin and harvesting time. Carob pulp has a high content in total sugar, consisting of mainly sucrose, glucose, fructose and maltose [5]. In addition, it contains about 18% of cellulose and

hemicellulose. Constituents of the carob seed are coat (30-33%), endosperm (42-46%) and embryo or germ (23-25%) [5]. Carob has been used as an ingredient in different foods (bread, sweets or drinks) due to its flavor (cacao-like) and sweetness, as well as its high content of dietary fiber (soluble and insoluble fractions), minerals (mainly, Ca, P, Mg, Na and K), vitamins (vitamin A, B1, B2 and B3), and antioxidants (proanthocyanidins, gallo- and ellagitannins, quercetin glycosides) [7]. Several studies suggest that carob may be helpful in treating diarrhea in infants [8]. Carob powder is a natural sweetener with flour and an appearance similar to chocolate; therefore, it is often used as cocoa substitute [9, 10]. The advantage of using carob as a chocolate resides in that carob is an ingredient free from caffeine and theobromine [10]. Carob trees are found in great abundance in Sudan and in the past mostly used as fodder for animals, but recently people started using it widely as food. Therefore, this study was aimed to investigate the proximate composition, mineral content and antioxidant properties of carob pods grown in Sudan.

## 2. Materials and methods

### 2.1. Plant material

The sample of the carob pods used in this research was purchased from the local market in Bahri city, Khartoum – Sudan, authenticated in the National Centre for Research by Dr. Yahiya Suleiman. The collection of samples was done during December 2019.

### 2.2. Sample preparation

The seeds were manually separated from the carob pods, pounded using a pestle and pod powder pouched in a sealed plastic container and stored in desiccator at room temperature for further work.

### 2.3. Proximate analysis

The proximate composition analysis of the carob pods which includes the moisture content, ash content, crude fat, crude protein and crude fiber were determined according to the standard methods described by AOAC [11]. Each analysis was performed three times and the results obtained were reported as mean values.

#### 2.3.1. Moisture content

About 2 g of the carob pod powder was weighed accurately into pre-weighed porcelain crucible and dried inside a hot air oven set at 105 °C temperature until a constant weight was achieved. The percentage of water loss (moisture) is calculated using equation 1:

$$\% \text{ Moisture content} = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

Where:  $W_i$  = initial weight in (g) of the carob pod sample,  $W_f$  = final weight in (g) of the sample after drying.

#### 2.3.2 Ash content

The total ash content was determined using standard AOAC [11] method. about 1 g of the carob pulp sample was transferred into a clean, dry, and pre-weighed porcelain crucible. Then the sample was incinerated in a muffle furnace at the temperature of 550 °C – 600 °C for 3 h. The percentage of the weight loss to the initial weight gives the ash content. The crucible was then removed from the furnace, left to cool in a desiccator and weighed again. % ash content was estimated by equation 2:

$$\% \text{ Ash content} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100 \quad (2)$$

### 2.3.3. Crude protein content

The crude protein was estimated using modified micro-Kjeldahl method for determining total nitrogen, whose value is multiplied by a factor of 6.25 to give the crude protein content according to AOAC [11] as follows:

About 0.2 g of the sample was accurately weighed and transferred into 50 mL micro-Kjeldahl digestion flask. About 0.4 g of catalyst (a mixture of 96% anhydrous sodium sulfate and 3.5% copper sulfate) and then 3.5 mL of 98% H<sub>2</sub>SO<sub>4</sub> were added to the flask. The contents of the flask were heated in an electric heater for 2 h until the color of the solution changed to blue. The content of the digestion flask was then allowed to cool to room temperature. Then 20 mL of a 40% NaOH solution was added to the digested sample in a distillation unit and the mixture was heated. The liberated ammonia was received in a 10 mL of 2% boric acid with 3-4 drops of (methyl red + bromo-cresol green) indicator in 100 mL Erlenmeyer flask, the distillation was maintained until the volume reached 50 mL. Then the content of the flask was titrated against 0.02 N HCl till the color changed to pink. A blank titration was also run. The crude protein was calculated using equation 3:

$$\% \text{ Crude Protein} = \frac{(V_S - V_B) \times N \times 14 \times 100 \times 6.25}{WS \times 1000} \quad (3)$$

Where:  $V_S$  = volume in mL of standard acid used for sample titration,  $V_B$  = volume in mL of standard acid used for blank titration, N = normality of HCl, WS = Weight (g) of sample, 1000 = to convert to mg.

### 2.3.4. Fat content

The fat content was determined by extracting the carob pod with petroleum ether for 8 h with using a Soxhlet extractor. The solvent was evaporated and then dried at 105 °C in an oven and then cooled in a desiccator and weighed. The remaining crude fat was determined using equation 4:

$$\% \text{ Fat content} = \frac{\text{weight of the extract}}{\text{weight of sample}} \quad (4)$$

### 2.3.5. Crude fiber

Approximately 2 g of an air dried fat-free sample was weighed and transferred into a dry 500 mL digestion flask. The sample was digested with 200 mL of boiling H<sub>2</sub>SO<sub>4</sub> (0.128 M) for 30 min. Then the solution was filtered using Buchner set under light vacuum. The residue was washed with hot water and then collected into separate conical flask. Then 200 ml of sodium hydroxide solution (0.23 M) was added to the residue and allowed to boil on a hot plate for 30 min, and the digest filtered through Buchner funnel. The remaining residue was washed with hot water until it was base free. The residue was collected into a pre-weighed crucible and dried in an oven at 110 °C overnight, then cooled in desiccator and reweighed. The fibre was then ignited in a muffle furnace at 550 °C for 2 h and allowed to cool in a desiccator and the final weight of the ashed fibre was measured and recorded. The crude fibre was calculated using equation 5.

$$\% \text{ Crude Fiber} = \frac{\text{loss in weight on ignition}}{\text{weight of sample}} \times 100 \quad (5)$$

### 2.3.6. Carbohydrate content

The total carbohydrate content of carob pod was determined by difference [12], as in equation 6.

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Fat} + \% \text{ Fibre} + \% \text{ Protein}) \quad (6)$$

## 2.4. Minerals analysis

Elements; calcium, magnesium, zinc, iron, and manganese were analyzed with atomic absorption spectrophotometer equipped with air-acetylene flame and elements [13]; sodium, potassium were analy-

zed with flame photometer.

### 2.5. Sample extract preparation

The crude extract was obtained from prepared carob pods using a slightly modified method reported by Oussaid et al. [14]. The extractions were carried out by maceration of a mass of fine powder of the prepared carob pod in ethanol as solvent, in a ratio of 1: 5 (w:v), under mechanical stirring at room temperature for 72 hours. The extract solution was filtered with filter paper, then pooled and concentrated using a rotary evaporator in 35-55 °C. The extract obtained was then stored at a temperature of -4 °C in the absence of light until was used.

### 2.6. Antioxidant activity

The DPPH radical scavenging assay was determined by the method reported by Maliki et al. [15], with some modifications. In 96-wells plate, the methanolic solution of the samples were allowed to react with 300 µM DPPH (2,2-Di(4-tert-octylphenyl)-1-picryl-hydrazyl) stable free radical dissolved in ethanol. A control standard solution of DMSO, was added to a DPPH methanolic solution, and the mixtures were vigorously shaken and incubate in the dark for half an hour at 37 °C. After incubation, discoloration was monitored in triplicate at 517 nm using spectrophotometer, and radical scavenging capacity was computed as a percentage effect (E%) using equation 7:

$$E\% = \frac{\text{Abs. Control} - \text{Abs. Sample}}{\text{Abs. Control}} \times 100 \quad (7)$$

### 2.7. Statistical analysis

Statistical analysis of the results was done using MS Excel version (2010). The 95% confidence level ( $\mu$ ) for the obtained data of the different parameter values was determined using equation 8 [16].

$$\mu = \bar{x} \pm \frac{ts}{\sqrt{n}} \quad (8)$$

Where:  $\bar{x}$  is the mean;  $t$  is Student's  $t$ ;  $s$  is the sample standard deviation and  $n$  is a number of data.

The data were exhibited as mean  $\pm$  standard deviation (SD).

## 3. Results

### 3.1. Proximate compositions

In the present study samples of carob pod from Bahri area, were analyzed to evaluate its nutritional value for domestic consumption and industrial utilization. The percentage of proximate compositions (mg/100 g) presented on Table 1.

**Table 1. Proximate composition of carob pod.**

Constituents	Percentage composition (%)
Moisture	8.79 $\pm$ 0.24
Ash	3.55 $\pm$ 0.27
Crude Fiber	8.62 $\pm$ 0.40
Crude Fat	6.32 $\pm$ 0.41
Crude Protein	13.27 $\pm$ 0.10
Carbohydrate	59.46 $\pm$ 1.22

The obtained result of carob moisture content was 8.79  $\pm$ 0.24%, where it found closed to results 7.56 $\pm$ 0.16% [17], and lower than 10.2  $\pm$  0.13 and 11.07 $\pm$  0.38% [18, 19]. The ash content was 3.55  $\pm$

0.27%, where it's closed to results 3.16 [9, 20], and higher than  $2.25 \pm 0.02$  and  $2.79 \pm 0.22\%$  [17, 18]. Besides the fiber content was  $8.62 \pm 0.40\%$ , where its higher than  $6.90 \pm 0.06$  and  $7.30\%$  [9, 19], and lower than  $10.99 \pm 0.51\%$  [18]. The carob crude fat content was determined to be  $6.32 \pm 0.41$ , where it found higher than  $0.30 \pm 0.04 - 1.15 \pm 0.07\%$  [18–20]. In addition, the crude protein was  $13.27 \pm 0.10\%$ , where its higher than  $5.54 \pm 0.38 - 6.34\%$  [9, 17, 18]. The carbohydrate content was  $59.46 \pm 1.22\%$ , where it found higher than  $45.0 \pm 0.30\%$  [18]; and lower than  $75.92 - 85.50 \pm 0.02\%$  [9, 17].

### 3.2. Mineral contents

The prepared samples of carob pods were also analyzed to evaluate their minerals content and the obtained results presented as mg/100 g of dry sample in Table 2.

**Table 2. Minerals contents of carob pods.**

Minerals	Concentration (mg/100 g)
Na	3.00
Mg	1482.19
K	470.00
Ca	1989.61
Mn	8.67
Fe	66.38
Zn	4.30

The results of the mineralogical analysis showed that the carob pods contained a significant amount of potassium, calcium, magnesium, sodium, iron, manganese and zinc. However, calcium (1989.61 mg/100 g) was the most common mineral, where its result was in the range of previous studies  $212.30 - 285.4 \pm 11.85$  mg/100 g [9, 19]. Follow by magnesium (1482.19 mg/100 g), where its higher than the results  $94.39 \pm 3.38$  and  $26.50$  mg/100 g [3, 19]; and potassium (470 mg/100 g), where its lower than the results  $8637.64$  and  $1010.9 \pm 8.25$  mg/100 g [9, 19]. The result of iron (66.38 mg/100 g), it's higher than the results  $1.68, 2.01 \pm 0.1$  and  $38.18$  mg/100 g [3, 9, 19]. The manganese result (8.67 mg/100 g), it's higher than  $0.29 \pm 0.03$  and  $1.024$  mg/100 g; beside the result of Zinc (4.30 mg/100 g), it's higher than  $0.46 \pm 0.02$  and  $2.471$  mg/100 g; as well the result of sodium (3.00 mg/100 g), its lower than  $10.44 \pm 0.79$  and  $50.597$  mg/100 g [9, 19].

### 3.3. Antioxidant activity

The prepared sample of carob pods were also subjected to an antioxidant activity test and the obtained result is given in Table 3.

**Table 3. Antioxidant activity of Carob pod extract.**

No.	Assayed Type	DPPH scavenging %RSA $\pm$ SD
1	Carob pods sample	$50 \pm 0.04$
2	PG Standard	$90 \pm 0.01$

The obtained result in Table 3, indicated that carob has a good antioxidant activity, as shown in previous studies [5, 9, 21, 22], its antioxidant activity may attributed to its content of polyphenol compounds. In addition, some previous studies demonstrated that carob leaves had higher antioxidant capacity than the fruit [23, 24], thus the high polyphenolic content found in carob leaves; the variation of our obtained results is attributed to environmental and climate issues.

#### 4. Conclusion

The obtained results of proximate composition indicated that carob pods had a high content of carbohydrate, protein, moisture and fibers. In addition, the results of elemental analysis showed that calcium content is higher, followed by magnesium, then potassium, iron, manganese, zinc and finally sodium. The obtain result also showed that carob had remarkable antioxidant activity and this result was found agreed to previous studies.

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#### Conflict of interests

Authors declare no conflict of interest exists.

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