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Determination of Chemical Composition of Carob Pod (Ceratonia Siliqua L) and its Morphological Study

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Abstract
In the present investigation, the characterisation of carob pod from (Chefchaouen region in north of Morocco) was studied and evaluate the effect of cold and warm aqueous extraction on chemicals composition of carob kibbles in order to make easy its grinding and also to quantify all compounds of carob seed and give the chemicals properties of its various constituents. The identification and quantification of all components of kibbles and seed were carried out on using many technical instruments like high performance liquid chromatography with various detector and atomic spectrophotometry absorption. The mean values of chemical composition of carob kibbles is calculated on six essays were as follows : (g/100g dry mater) : Protein (2.74±0.03), Ash (3.0±0.03), total and reducing sugar (83.7, 512.5), sucrose(44.64±0.49), fructose (7.2± 0.2), glucose (2.2±0.2), crude fibre(6.90 ± 0.06), tannins (6.7±0.2), total polyphenols (17.0±0.5) and minerals (mg/100g) : P : 76.22, K : 1003.5, Ca : 268.57, Mg : 96.43, Fe : 2.1, Zn : 0.45. The objective of this investigation is to use the carob pod powder as cocao substitute in chocolate industry and the advantage of using carob as chocolate substitute, resides in that carob is an ingredient free from caffeine and theobromine.

Keys word : Ceratonia siliqua, Total polyphenols, HPLC, Composition, Kibbles, Seeds.

1. Introduction
Ceratonia Siliqua.L (carob tree: cesalpinaceae) is a mediterranean and southern Asian evergreen sclophyllous species. but it was successfully introduced in different period in some warm semi arid areas of Australia, California and Arizona (USA), Mexico, South Africa, etc [1].
In Morocco, with the exception of arid zones, carob is naturally present up to 1150 metres in altitude [2].
The carob tree, also called algarroba, it grows throughout mainly in Spain, Morocco, Italy and Portugal. The fruit pod (containing sweet pulp) gives, after removal of the seeds, carob powder [3], often used as a chocolate or cocao substitute [4,5].
The advantage of using carob as a chocolate substitute, resides in that carob is an ingredient free from caffeine and theobromine.
Carob pod provide two important products :
a- Carob kernels or seeds from which carob or locust bean gum is extracted.
b- Carob kibbles or the remaining pulp obtained after the removal of the seeds. This can be used directly in animal and human nutrition or as a raw material for industrial processing.
In Morocco, the carob tree grows in many areas: occidental and oriental Rif, the Gharb, Saiss, Anti Atlas and central plateau [6].
Carob pod is mostly used in food industry for carob bean gum ad locust bean gum, which are polysaccharides (galactomannans) contained in the endosperm of the seeds [1,7]. However, carob pod mainly consists of pulp (90%), which is rich in sugars (48-56%), but it also contains a large amount of condensed tannins (16 - 20%) [1, 8, 9], although lower tannin values have been reported [3] Carob leaves have been reported to contain considerably lower values of 0.7% o dry matter basis [10].
Free sugars, organics acids and amino acids are natural constituents of many fruits and vegetables play an important role in maintaining quality and determine nutritive value [11].
The nature and the concentration of these constituents are also of interest because of their important properties.

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Free sugars are one of the most important constituents of fruits and vegetables, monosaccharide and disaccharides, such as fructose and glucose are considered to be the major sugars in most fruits contributing to the maturity of fruits [12]. Amino acids and their derivatives are important for human nutrition and affect the quality of foods including taste, aroma and colour [13].
The purpose of this study is to evaluate the important characteristics of Moroccan carob taken from the north (Chefchaouen) and to optimize the best condition for elaborating carob pod chocolate and locust bean gum.

2. Materials and methods

Samples of carob were collected from Chefchaouen region of North of Morocco, where they grow naturally
Carob pods (Ceratonia Siliqua.L) were randomly collected from various parts of several trees grown naturally in different locations in North of Morocco especially in Chefchaouen (about 690m above sea level).
The samples were harvested during September 2010 from different regions of Chefchaouen, and carob pods were from the same physiological maturity (dark brown) and of uniform shope and size.
The sampling was realised from each natural areas, the elementary samples (100g per sample) were combined in order to provide the composition samples of 1kg. Samples were stored at room temperature in the suitable conditions, the seeds were removed and the kibbles were ground in a mill (250µm)

- Moisture :
The moisture amount of the carob was determined gravimetrically after heating the material (About 5g shopped carob) in an oven fixed at 103°C during four hours according to method *AOAC International* [14]. The moisture was determined by the International Standard Method.

- **Total Ash** : The ash content was carried out after removed the seed and the analysis was determined gravimetrically after dry mineralisation in furnace fixed at 550°C during six hours using the international standard method [15].

-**Total fat :**
Lipids from the carob kibbles (sample weigh about 10g) were determined by using 150 ml of hexane during 6 hours, lipids were extracted in a soxhlet apparatus, the extraction beakers (containing the extracted lipids)were dried in an oven at 103°C before being weighed. The determination was carried out according to the AFNOR method [16]**.**

- Protein :
The crude protein (N.6.25) content was determined according to Kjeldahl method from 0.5g of carob kibbles, the sample was mineralised in sulphuric acid with Buchi digestion unit K-424, distilled such organic nitrogen in boric acid with Buchi distillation unit K-314, the obtained solution was neutralised by NaOH 0.1N. Protein was calculated using nitrogen to protid conversion factor 6.25 per gram of sample. The protein amount was determined by the International Standard method [17].

- Carbohydrates:
(Nitrogen Free Extract) were estimated by difference.

- Crude Fibber :
The sample weight is treated with 0.26N H_2SO_4 solution during 30min at boiling temperature, then the extract is filtered through the special linen in order to keep the carobs kibbles. The second extraction is carried out with 0.2N KOH solution during 30min at boiling temperature. Let cooling the flask, then filter the content through the crucible N°1 under vacuum and dry it during four hours.
The crude fibber resulting was evaluated such ash resulting after a double treatment.
This determination was carried out using the International method [18].

- HPLC analysis for sugars :
The study was carried using grinded and deseeded carob pod sieved (0.25mm).
The sugar extraction was realised with cold water, weight sample was immersed in the adequate amount of water and mechanically shook in open flask at ambient temperature (20-25°C) until attain extraction equilibrium.

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Then the mixture was filtered and the extract was analysed for its content of sugars by the high performance liquid chromatography. The apparatus is equipped with a differential refractometer, isocratic separation of the compounds was carried out at flow rate of0.7ml/min. The column was an amino-bonded column 4.6DI-250mm-5µm and the mobile phase was a mixture of acetonitrile (75:25) for isocratic elution.
The standards were obtained from JANSSEN CHIMICA and LABOSI.
The sugars are determined by the method as described in AOAC [19].

- Total and reducing sugar :
Sample weight (0.5g) is added with two agents of defecation, complete with distilled water until the gauge (100ml flask), after filter the solution through whatman n°2.
In the first the reducing sugars amount is determined according to Luff-Schoorl as described [20]. The following step consists on hydrolyse of extract in order to determine the total sugar content in milled carob kibbles sieved 0.25mm. The remaining operation was to follow the same step as recommended by Luff-Schoorl method.

-Tannins :
The tannins content was determined with the gravimetric method using the cupper acetate such an agent of association with phenol compounds which are extracted from carob pod in three times meaning the boiled distilled water.
The determination was carried out according to the method decribed in the manuel d'analyses alimentaires et d'expertises usuelles [21].

- Theobromine :
Theobromine extraction from carob pods was carried out with water, 2 grams of grinding carob was immersed in the adequate amount of water. Shake vigorously during 30min in order to exhaust theobromine content from matrix. The slurry was analysed by HPLC according using an appropriate method [22].

- Caffeine :
The sample weight (2g) is placed in filter cartouche; the caffeine was extracted in a soxhlet apparatus with chloroform during 90min, after the solvent was evaporated with rotavapor to dryness. The residue is taken with the warm distilled water, then filtered through Millipore filter paper 0.45µm before injecting in liquid chromatograph equipped with a UV-Visible detector connected to a C18 column 4.6DI.200mm.5µm, the elution was carried out in isocratic mode with a mixture of methanol and water (30 :70).
The caffeine is determined by the method as described in the literature [23].

-Phosphor :
The determination was carried out using the spectrophotometric analysis with the vanadium phosphomolybdate such reagent. The sample preparation follows the same steps like mineral matters determination. A calibration curve was obtained using an interval of phosphor concentrations (25-250ppm)**.** The phosphor content was determined using a method AFNOR [24].

-Total polyphenols :
Total polyphenols were determined using the method of Folin-Ciocalteu (Singleton and al.1965); a calibration curve was obtained using Gallic acid such a range of concentrations solution (25-250ppm). Data were expressed as mg Gallic acid equivalents (GAE/g) dry weight. The total polyphenols were determined according to method described in the literature [25].

- Mineral matters :
The ash residue obtained after having burned the sample weight at 550°C was dissolved in HCl conc. and H_2O_2 on heating the solution during ten minutes.
Filter the solution through Whatman filter paper and complete to the bottom flask with distilled water and then we evaluate the concentrations of the eventual elements presents in carob with Atomic Absorption Spectrophotometer.
It was recommended to pass the calibration standard for each compounds before measurements. We determined the levels of four major elements such (K, Ca, P and Mg…) and five trace minerals in Chefchaouen carob pod

sample such (Cu, Fe, Zn, Na and Mn). The mineral element concentrations are determined according to a method described in the Journal Official de l'Union Européenne [26]. The values are expressed on a dry matter basis.

3. Results and discussion

This investigation was designed not only to evaluate the characteristics of carob pod but also to realize the global morphological study on different part of pod and seed. This work was carried out on taking physical measurements like weight, length and thickness...in order to give information about the portion of each part of carob pod. Sixty randomly selected pods were used for morphological study, length was measured using measuring tape ; width and thickness using vernier calliper, whereas weight was taken using a top-loading balance.

Results of carob pod measurements are shown in table 1, the mean of weight, length, width, thickness and internal thickness were 12,4g ;114,1 ; 22,5 ; 6,6 and 4,4 mm respectively. It is well established that physical measurements of the whole carob pod indirectly indicate the quality of those pods, furthermore it was observed in this study that the higher the thickness, the higher the pulp to kernel ratio are the quality indicators of pods.

3.1 morphology of carob pod

Table1. Measurements of carob pod

Characteristics	***Variation Interval***	***Mean ± SE***
Weight (g/pod)	7.25 - 17.53	12.4 ± 0.4
Length (mm)	82 - 155	114.1 ± 2.8
Width (mm)	15.5 - 27	22.5 ± 0.3
Thickness (mm)	3.5 - 9.0	6.6 ± 0.2
Internal thickness (mm)	2.5 - 5.5	4.4 ± 0.1

3.2. Seed - pulp morphological analysis

Forty randomly selected pods were deseeded for the second morphological study, manually we remove the seed ; whereas weight was taken using a top-loading balance.

Table 2 shows the weight results of carob pod, seed and give the pulp to kernel ratio and the percentage of seed. The pulp to kernel ratio was about 4:5, whereas seeds made about 15% of the total pod weight.

The mean values for all principal fruit and seed morphological characteristics observed were statistically different with the results published in the others studies.

Obviously, the difference between the present study and those published **[**3,28,29,30**]** depends on the cultivar, geographic location, treatment and farming practices.

Table2. Measurements of kibbles /seed

Weight (g/pod)	***Variation Interval***	***Mean ± SE***
Carob pod	7.25 - 17.53	12.4 ± 0.4
Carob kibbles	5.98 - 15.81	10.59 ± 0.38
Carob kernels	1.18 - 2.51	1.77 ± 0.06
Number kernels /pod	6.0 - 12	8.60 ± 0.3
Seed (g/100g)	8.64 - 21.81	14.8 ± 0.5
Pulp (g/100g)	78.2 - 91.4	85.2 ± 0.5

Table 3 shows the chemical characteristics of carob pod sample from several locations representing Chefchaouen (North of Morocco), the study was conducted with many types of International analysis method. The results were expressed as weight percent for each parameter.

As can be showed in this table, the sugar fraction represents a high percentage of the total weight of the carob pod.

Moisture, total ash, crude fibber, protein, fat and carbohydrates contents of the carob are shown in table **3.**

In the carob kibbles the mean values of sucrose, glucose, and fructose were respectively (44± 0.74, 2.16 ± 0.09, 7.04 ± 0.17) ; it was presumed that carob pod contain a low value of fat , crude fibber and protein. Furthermore the carob pod is free from theobromine and caffeine, those properties qualify carob pod to be a deity food.

On the contrary, carob kibbles are especially rich in total and non reducing sugars (sucrose) and in gross energy, making carob kibbles a high energy food for animal nutrition.

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Table3 represents the chemicals result obtained in this study that are in agreement with literature, however a significant difference can be observed at sucrose amount which is important in Chefchaouen carob pod the difference between the present study and those described in the literature **[**3,27,28**]** depends probably on the cultivar, geographic location, treatment and farming practices.

Table 3. Chemical composition of carob kibbles

Parameter	***Variation interval***	***Mean ± ES***
Moisture	9.67 - 10.73	10.2 ± 0.13
Total ash	2.70 - 3.18	3.0 ± 0.05
Fat	0.99 - 1.30	1.15 ± 0.07
Protein	2.68 - 2.85	2.74 ± 0.03
Crude fibber	6.79 - 7.12	6.90 ± 0.06
Aqueous extract	60.7 - 64.3	61.2 ± 0.32
Tannins	6.28 - 6.97	6.70 ± 0.12
Fructose	6.90 - 7.44	7.18 ± 0.17
Glucose	2.0 - 2.26	2.20 ± 0.11
Sucrose	32.6 - 45.4	44.64 ± 0.49
Reducing sugar	78.7 - 87.6	83.7 ± 2.0
Total sugar	492.4- 532.2	516.7 ± 7.2
Total polyphenols (mg/	15.8 - 18.08	16.98 ± 0.42
Theobromine	not detected	---------
Caffeine	not detected	---------

Values mean of five independent extractions and determination (n=5)

Table **4** shows the mineral characterisation of carob pod from Chefchaouen location determined following the Official Journal of European Union.
The results were expressed as weight percent for each parameter, we determined the levels of four major elements as (K , P, Ca and Mg) and five trace (Na, Fe, Cu, Mn and Zn) .
(K ,Ca, P, , Fe and Zn) concentrations (1003, 269, 76 ,2.1 and 0.45 mg/g dry weight respectively were very similar to those previously reported from carob pod **[**29**]** (970 ; 300 ; 71 ; 1,9 and 0,75 mg/g dry weight respectively), but the element Cu and Mn presents a significant differences with those published by Biner and al. **[**29**]** .

Table4. Concentrations (mg/100g) in carob pods.

Minerals	***Interval variation***	***mean± ES***
K	993.6 - 1042	1010.9 ± 8.25
Ca	266.6 - 327	285.4 ± 11.85
Mg	82.75 - 103	94.39 ± 3.38
P	68.2 - 79.7	75.58 ± 1.94
Na	8.47 - 12.78	10.44 ± 0.79
Fe	1.78 - 2.26	2.01 ± 0.1
Zn	0.41 - 0.52	0.46 ± 0.02
Mn	0.23 - 0.30	0.29 ± 0.03
Cu	0,29 - 0,03	0.29 ± 0.03

Values mean of five independent extractions and determination (n =5)

Conclusion

This is a first study that focuses on the chemical characterisation including organic constituents and minerals elements of carob pod.
The results of study showed that carob pod contains high level of carbohydrates, appreciable amount of proteins and low levels of fat. Carob pod contains an important amount of potassium, calcium and polyphenols that play a significant role in human health as it was reported in literature.
From these results, a significant correlation can be realised which is important for the evaluation of agro industrial rentability.

This present investigation reports the first data on the morphological and chemical composition of carob pod in northern Morocco (Chefchaouen region).
In conclusion, our results could have a value of contribution to demonstrate the effects of geographic variety, culture conditions or degree of maturation on the chemical and morphological composition.
As the perspectives, I predict to study some special treatments on the kibbles and seed, furthermore, I will to optimize bio ethanol production and chemical composition of the alcoholic fraction.

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