International Journal of Food Science and Nutrition Engineering 2017, 7(4): 91-103
DOI: 10.5923/j.food.20170704.04

Mineral Composition of Mature Carob (*Ceratonia siliqua* L.) Pod: A Study

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Abstract In the present study, the mineral macroelements and microelements content of the different parts of mature carob (*Ceratonia siliqua* L.) pod (pulp and seed) were analyzed for the first time using a multi-element technique called neutron activation analysis (NAA). It is one of the most reliable non-destructive nuclear methods to determine the overall contents of mineral elements in a material. Five macroelements and thirty microelements were identified in the whole pod carob (pulp and seed and its various constituents). Mineral content of pod is very variable; potassium predominates, with 90.1 mg/pulp and 0.569 mg/germ. Tegument is very rich in calcium (0.156 mg), as well as germ (0.162 mg) and pulp (35.1 mg). Chlorine content is high in pulp (20.9 mg) and endosperm (0.113 mg). Magnesium is concentrated in the germ (0.132 mg) and absent in the endosperm. Two microelements are remarkable in the fruit: aluminum (457.5 µg/pulp, 5.5 µg/ endosperm and 2.1 µg/tegument) and iron, mainly in the pulp (702 µg/pulp, 4.2 µg/germ and 2.6 µg/tegument).

Keywords *Ceratonia siliqua*, Mature carob pod, Macroelements, Microelements, Neutron activation analysis (NAA)

1. Introduction

Carob (*Ceratonia siliqua* L.) is an evergreen tree belonging to Leguminosae family (subfamily Caesalpinioideae). The scientific name comes from the Greek name "keras" meaning horn and the Latin name siliqua, alluding to the hardness and shape of the pod [1]. The tree is native to the Mediterranean region and has been cultivated since ancient times throughout the Mediterranean basin, usually in soft and dry areas [2]. It can be grown on low productive land with low rainfall (250-500 mm/year). Some cultivars have been introduced in the United States (California), Mexico, South Africa and Australia [3, 4].

The maturation of carob fruit occurs generally in summer, at the end of June. Mature pods are brown with specific flavors. They consist mainly of pulp (about 90% of the weight of the fruit) and seeds (10%). The number of seeds is between 3 and 20 [5]. The chemical composition of pods, as well as their content of different elements, depend on several factors, including the origin of the sample, its variety, the period of its harvest and culture practices, but also on edaphic and climatic conditions [6-10].

Carob pulp has a high sugar content of between 30 and 60%, the main sugars are sucrose (65 to 75% of total sugars), fructose and glucose (15 and 25% of total sugars, respectively) [11-13]. It also contains appreciable amounts of fiber (up to 40%), proteins (2 to 7%) and low fat content (0.9 to 1.3%) [6, 14-21]. In addition to its nutritive qualities, the pulp contains more than 1.5% of condensed tannins, which are widely used as extracts in nutrition and medicine [22-24].

Carob powder is one of the main products obtained from pulp. It is intended for human consumption and is produced by roasting, grinding and sifting of ground carob. It is mainly used in the food industry as a substitute for cocoa, devoid of caffeine and theobromine [9, 25-27]. It is also used for the production of pharmaceuticals, sugars and ethanol [1, 9, 26].

Carob seed consists of seed tegument (30-33%), rich in antioxidants and phenolic compounds (tannins), endosperm (42-46%) and embryo or germ (23-25%) [28, 29].

The seeds are a source of gum [30], called carob gum or E410 [31], obtained from seed endosperm and containing galactomannans. This gum is added to a variety of products as a thickener, stabilizer or flavoring agent [32, 33], in addition to several other applications in non-food industries

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Published online at http://journal.sapub.org/food

[34, 35]. 100 kg of seeds give an average of 20 kg of pure and dry gum [1].

The germ (=embryo) contains 34.4% of oleic acid, 44.5% of linoleic acid, 16.2% of palmitic acid and 3.4% of stearic acid [36]. The germ flour is used in dietary supplements [36] or as an ingredient in foods derived from cereals for celiacs [37, 38].

Ash refers to the inorganic residue remaining after the complete combustion or oxidation of organic matter in a foodstuff and gives an indication of the total mineral content in the food [39]. Some minerals (calcium, phosphorus) are part of the human skeleton and if the body is not supplied in sufficient quantities of these minerals, some physical malfunctions or deformities can be observed [40]. Other minerals regulate metabolic and circulatory systems, among other biological functions in the body. More than 100 μg of macro-minerals (Ca, P, Na, K, Mg, Cl and S) are required per day, whereas less than 10 μg of trace elements (Fe, I, Zn, Cu, Cr, Mn, Fr, Se and Si) are required in daily human diet [41].

Researches on the mineral composition of carob pulp allowed the detection of the following mineral elements: potassium (K), phosphorus (P), calcium (Ca), sodium (Na), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), selenium (Se), boron (B), barium (Ba), cobalt (Co), chromium (Cr), nickel (Ni), lead (Pb), strontium (Sr), titanium (Ti), aluminum (Al), lithium (Li), silver (Ag) and vanadium (V). The contents of these elements are variable [11, 42-50]. For the seed and its constituents (tegument, endosperm and germ), they have variable levels of potassium, sodium, phosphorus, boron, selenium, manganese, iron, copper, zinc, sulfur, magnesium and calcium [45, 48-52].

The objective of the present study was to determine mineral composition of different components of mature carob (*Ceratonia siliqua* L.) pod (pulp, tegument, endosperm and germ seed).

2. Material and Methods

2.1. Plant Material

The experimental material used is represented by mature fruits (pods) of carob (*Ceratonia siliqua* L.). They come from a selected domesticated tree located 30 km on the road Tetouan-Chefchaoun (Amtel region, West Rif, Morocco). This tree benefits from a subhumid bioclimatic microclimate and is found on a drained marl-limestone soil. The pods were collected in August 2016 and stored in canvas bags in the dark and at room temperature.

In this study, 90 pods taken randomly from the chosen carob tree were used to measure the weight of fresh material (pulp + seeds) using an analytical balance with an accuracy of 0.01 g (Precisa, XB 620C), the length (mm) of the pod using a fabric tape without including the peduncle and stigma, width (mm) and thickness (mm) with a digital read caliper (OTMT, Orascom Telecom Media and Technology Holding SAE). After these measurements, each pod was manually shelled and analyzed individually, which gave the number of seeds, their weight (g), the number of aborted seeds and the fresh weight of the pulp (g). The seed-free pods were dried for about 15 days in an oven (WiseVen® model WOF-155) at 60°C until a constant dry weight (g) was obtained.

From the seeds of pods, 90 seeds randomly selected were measured. The seeds were first weighed individually using an analytical balance with an accuracy of 0.001 g (Precisa, 125 A) and the length, width and thickness were measured (mm) using a caliper with digital reading.

The seeds were previously soaked for 48 hours after scarification according to the technique of Correia and Martins-Loução [53] with 36N sulfuric acid for one hour, followed by three successive rinses with sterile distilled water, 10, 10 and 15 min. After, the different components of the seed were easily separated (teguments, endosperm and germ), weighed to determine their fresh weights (g) and then placed in an oven at 60°C, until weight stabilization (about 15 days).

2.2. Mineral Neutron Activation Analysis (NAA)

2.2.1. Sample Preparation

The pulp and the various parts of the seed dried beforehand for about 15 days in an oven at 60°C until the dry weight was stabilized, were ground separately in an agate mortar. The homogeneous powder obtained was analyzed by a multi-element technique, by neutron activation (NAA). It is one of the most reliable non-destructive nuclear methods to determine the overall contents of mineral elements in a material [54].

Table 1. Conditions of neutron activation analysis

	Short irradiation	**Long irradiation**
Sample weight	100 mg	100 mg
Envelope	Polyethylene pots	Pure aluminum foil
Flow monitor	Au (gold)	Fe (iron)
Reactor	Orpheus	Orpheus + Osiris
Irradiation time	30 to 180 seconds	7 to 8 hours
Counts	Directly after irradiation 1st count: 2 min 2nd count: 5 min 3rd count: 15 min 4th count: 1 h	1st count: 1 h after 7 days 2nd count: 4 h after 15 days 3rd count: 8 h after 30 days

Neutron activation analysis (NAA) was performed on pellets (0.1 mm thickness and 10 mm diameter) formed of 50 mg of powder mixed with the same weight of ultra-pure cellulose. The pellets were wrapped in aluminum foil for long irradiation or placed in plastic pots for short irradiation. They were then irradiated separately in short irradiation for the analysis of short period elements (Al, Mg, Cl, Mn and Cu) and long irradiation for long period elements (Na, K, Ca, Sc, Cr, Fe , Cu, Zn, As, Se, Br, Rb, Sr, Zr, Mo, Ag, Sb, Cs, Ba, La, Ce, Nd, Sm, Eu, Tm, Hf, Ta, Hg, Au, Th...) under the conditions mentioned in **Table 1**.

For this work, two reactors of the CEA of Saclay were used, which Pierre Süe (CEA-CNRS) laboratory has direct access: Orpheus (available flux: about 2.1013 n.cm^{-2}.s^{-1}) and Osiris (7 to 9.10^{13} n.cm^{-2}.s^{-1}).

2.2.2. Measurement of Various Elements of the Sample

After 2 minutes, in the case of the short-irradiation analysis and 7 days in the case of the long-irradiation analysis, the elements were measured using a gamma spectrometer, using Ge(Li). The relative efficiencies of the detectors were about 10% with resolutions of 1.8 to 1.9 keV (1 keV=10^{3}eV) at the gamma line of 1332 keV of the ^{60}Co. The spectra obtained were processed by the InterWinner4 software. The concentrations of the elements were determined by the Ko-Labsüe software method at the Pierre Süe laboratory of the CEA Saclay in France.

3. Results

3.1. Morphological Characteristics of Pod and Seed

The measured morphological characteristics of the pods presented in **Table 2** show that the fresh and dry pod weight are11.89 and 10.31 g, respectively, while the length, width and thickness are 137.4, 20.7 and 7.41 mm. Seeds number is 11.04 per pod (0.75 aborted seeds) with a dry and fresh weight of 2.00 and 1.53 g of seeds/pod, and a dry and fresh weight of pulp/pod of 9.89 and 8.77 g, respectively.

Table 3 shows the different morphological characteristics of the seeds. The values corresponding to length, width, thickness, fresh and dry weight of seed are 9.39, 7.22, 4.08 mm, 0.2052 and 0.1442 g, respectively. The separation of the structural elements of carob seed shows average fresh and dry weights of seed tegument of 0.0278 and 0.0224 g, endosperm of 0.1332 and 0.0890 g and germ of 0.0442 and 0.0329 g, respectively.

Table 2. Agro-morphological characteristics of domesticated carob pods*

Pod properties	Mean ± SD	Minimum	Maximum	CV %
Pod length (mm)	137.40 ± 1.1	82	188	11.93
Pod width (mm)	20.70 ± 0.15	15.87	28.49	6.41
Pod thickness (mm)	7.41 ± 0.07	4.02	10.03	10.34
Number of seeds per pod (aborted)	11.04 ± 0.13 (0.75)	4	16	16.53
Fresh weight of the pod (g)	11.89 ± 0.21	2.99	23.34	18.43
Dry weight of the pod (g)	10.31 ± 0.20	1.52	21.35	20.70
Fresh matter weight of a pulp (g)	9.89 ± 0.19	2.64	20.25	19.55
Fresh matter weight of seeds per pod (g)	2.00 ± 0.03	0.35	3.13	19.50
Dry matter weight of a pulp (g)	8.77 ± 0.18	1.22	18.89	22.23
Dry matter weight of seeds per pod (g)	1.53 ± 0.03	0.30	2.46	19.93

*Results are mean values of 30 determinations of three individual procedures (n=30; 3) ± standard deviation (SD); CV (%): coefficient of variation

Table 3. Agro-morphological characteristics of domesticated carob pod seeds*

Seed properties	Mean ± SD	Minimum	Maximum	CV %
Seed length (mm)	9.39 ± 0.02	9.19	10.00	4.18
Seed width (mm)	7.22 ± 0.02	6.85	7.83	4.41
Seed thickness (mm)	4.08 ± 0.01	3.90	4.63	5.60
Fresh weight of a seed (g)	0.2052 ± 0.003	0.1834	0.2404	6.16
Dry weight of a seed (g)	0.1442 ± 0.003	0.1145	0.1743	7.15
Fresh weight of tegument (g)	0.0278 ± 0.0008	0.0248	0.0379	10.23
Dry weight of tegument (g)	0.0224 ± 0.0007	0.0165	0.0305	8.90
Fresh weight of endosperm (g)	0.1332 ± 0.002	0.1141	0.1657	8.52
Dry weight of endosperm (g)	0.0890 ± 0.002	0.0694	0.1110	8.80
Fresh weight of germ (g)	0.0442 ± 0.001	0.0405	0.0526	8.97
Dry weight of germ (g)	0.0329 ± 0.0007	0.0286	0.0389	10.29

*Results are mean values of 30 determinations of three individual procedures (n=30; 3) ± standard deviation (SD); CV (%): coefficient of variation

3.2. Mineral Neutron Activation Analysis

The neutron activation analysis (NAA) allowed identifying the majority of macroelements and microelements present in the whole pod, pulp, carob seed and its various constituents. The results for each mineral macro- and microelement (**Tables 4, 5, 6, 7, 8 and 9**) are expressed in g/kg of dry and fresh matter (**Tables 4, 5, 7 and**

8), in mg (**Table 6**) and in µg (**Table 9**) by different parts of pod and seed.

The content of different macro- and microelements of pod, seed and of the four analyzed parts (pod pulp, seed tegument, endosperm and germ), shows a great variation according to the element under consideration.

Long-irradiation analysis has the advantage of determining the majority of the mineral elements present in the pulp and seeds in spite of their very low level: 38 elements have been analyzed, 30 of which have been revealed at least in one of the analyzed parts.

Some elements are present in large quantities, such as potassium, abundant in the four analyzed parts, in particular seed germ (17.3 g/kg of dry matter (DM), 12.9 g/kg of fresh matter (FM) and 0.569 mg/germ) and pulp (10.3 g/kg DM, 9.1 g/kg FM and 90.1 mg/pulp). Calcium is in the second place with a relatively high concentration, particularly in the germ (4.9 g/kg DM, 3.7 g/kg FM and 0.162 mg/germ) and the seed tegument (6.9 g/kg DM, 5.6 g/kg FM and 0.125 mg/tegument). Other elements are also present in a good amount such as sodium, iron, strontium, zinc and rubidium. Others are poorly represented such as scandium, cobalt... Some elements are not detected such as gallium, tin, terbium...

Short-irradiation analysis shows that pulp is particularly rich in chlorine (2.4 g/kg DM, 2.1 g/kg FM and 20.9 mg/pulp) and magnesium (0.71 g/kg DM, 0.63 g/kg FM and 6.2 mg/pulp). Aluminum is placed in the third position (52.2 mg/kg DM, 46.3 mg/kg FM and 457.5 µg/pulp), followed by copper (16.4 mg/kg DM, 14.5 mg/kg FM and 143.5 µg/pulp) and manganese (4.4 mg/kg DM, 3.9 mg/kg FM and 38.5 µg/pulp) with very low levels.

Seed germ has a very high magnesium content (3.99 g/kg DM, 2.98 g/kg FM and 0.132 mg/germ), relatively high content in chlorine (0.93 g/kg DM, 0.70 g/kg FM and 0.031 mg/germ) and low content in copper and aluminum. The endosperm is mainly rich in chlorine (1.3 g/kg DM, 0.85 g/kg FM and 0.113 mg/endosperm) and aluminum (61.3 mg/kg DM, 41.0 mg/kg FM and 5.5 µg/endosperm) like pulp. The tegument is mainly rich in magnesium (0.99 g/kg DM, 0.81 g/kg FM and 0.022 mg/tegument), chlorine (0.43 g/kg DM, 0.34 g/kg FM and 9.5 10-3 mg/tegument), aluminum (91.5 mg/kg DM, 73.7 mg/kg FM and 2.1 µg/tegument) and low in copper and manganese. Some elements such as iodine, thallium and vanadium are not detected.

Generally, carob pod is rich in chlorine (2.2 g/kg DM, 1.9 g/kg FM and 17.9 mg/pod) and poor in manganese (5.6 mg/kg DM, 4.6 mg/kg FM and 33.5 g/pod). On the other hand, the seed showed high and equal magnesium and chlorine contents (1.1 g/kg DM, 0.75 g/kg FM and 0.081 mg/seed) and very low manganese (12.7 mg/kg DM, 8.9 mg/kg FM and 0.885 µg/seed) and copper content (12.5 mg/kg DM, 8.8 mg/kg FM and 0.852 µg/seed).

Potassium is the most abundant of macroelements (**Tables 4, 5 and 6; Figures 1 and 2**), particularly in germ (17.3 g/kg DM, 12.9 g/kg FM and 0.569 mg/germ) and pulp (10.3 g/kg DM, 9.1 g/kg FM and 90.1 mg/pulp). Calcium is also found in large amounts in the seed tegument (6.9 g/kg DM, 5.6 g/kg FM and 0.156 mg/tegument), in the germ (4.9 g/kg DM, 3.7 g/kg FM and 0.162 mg/germ) and in the pulp (4.0 g/kg DM, 3.6 g/kg FM and 35.1 mg/pulp). Magnesium is mainly concentrated in the germ (3.99 g/kg DM, 2.98 g/kg FM and 0.132 mg/germ), whereas it is absent in the endosperm. The chlorine content was relatively high in the pulp (2.4 g/kg DM, 2.2 g/kg FM and 20.9 mg/pulp) and endosperm (1.3 g/kg DM, 0.85 g/kg FM and 0.113 mg/endosperm). Finally, sodium concentrates abundantly in the tegument (0.47 g/kg DM, 0.38 g/kg FM and 0.01 mg/tegument) and the germ (0.17 g/kg DM, 0.13 g/kg FM and 5.4 10^{-3} mg/germ).

For microelements (**Tables 7, 8 and 9; Figures 3 and 4**), aluminum is most abundant in the tegument (91.5 mg/kg DM, 73.7 mg/kg FM and 2.1 µg/tegument), the endosperm (61.3 mg/kg DM, 41.0 mg/kg FM and 5.5 µg/endosperm) and pulp (52.2 mg/kg DM, 46.3 mg/kg FM and 457.5 µg/pulp), while iron is more concentrated in the germ (126 mg/kg DM, 93.8 mg/kg FM and 4.2 µg/germ) and the seed tegument (118 mg/kg DM, 95.1 mg/kg FM and 2.6 µg/tegument). Other elements are also present in a good amount such as strontium in the tegument (38.3 mg/kg DM, 30.9 mg/kg FM and 0.858 µg/tegument), zinc in the germ (71.4 mg/kg DM, 53.2 mg/kg FM, 2.4/µg germ), rubidium in the germ (35.5 mg/kg DM, 26.4 mg/kg FM and 1.2 µg/germ), manganese in the germ (45.4 mg/kg DM, 33.8 mg/kg FM and 1.5 µg/germ) and copper in the pulp (16.4 mg/kg DM, 14.5 mg/kg FM and 143.5 µg/pulp). Others are poorly represented (<1 mg/kg) in different parts of carob pod and seed such as selenium, cobalt…

Table 4. Macroelements content (g/kg of dry matter) in different parts of pod and seed of carob

Elements*	Pod	Different parts of pod		Different parts of seed		
		Pulp	Seed	Tegument	Endosperm	Germ
Potassium, K	9.7 ± 0.06	10.3 ± 0.06	6.2 ± 0.05	2.2 ± 0.04	3.0 ± 0.04	17.3 ± 0.09
Calcium, Ca	3.9 ± 0.59	4.0 ± 0.40	2.9 ± 0.69	6.9 ± 0.91	1.2 ± 0.20	4.9 ± 0.50
Chlorine, Cl	2.2 ± 0.11	2.4 ± 0.14	1.1 ± 0.09	0.43 ± 0.07	1.3 ± 0.11	0.93 ± 0.10
Magnesium, Mg	0.76 ± 0.06	0.71 ± 0.11	1.1 ± 0.30	0.99 ± 0.07	nd	3.99 ± 0.52
Sodium, Na	0.16 ± 0.002	0.16 ± 0.0007	0.17 ± 0.002	0.47 ± 0.002	0.10 ± 0.005	0.17 ± 0.0008

*Results are mean values of triplicate determinations (n=3) ± standard deviation (SD); nd: not detected

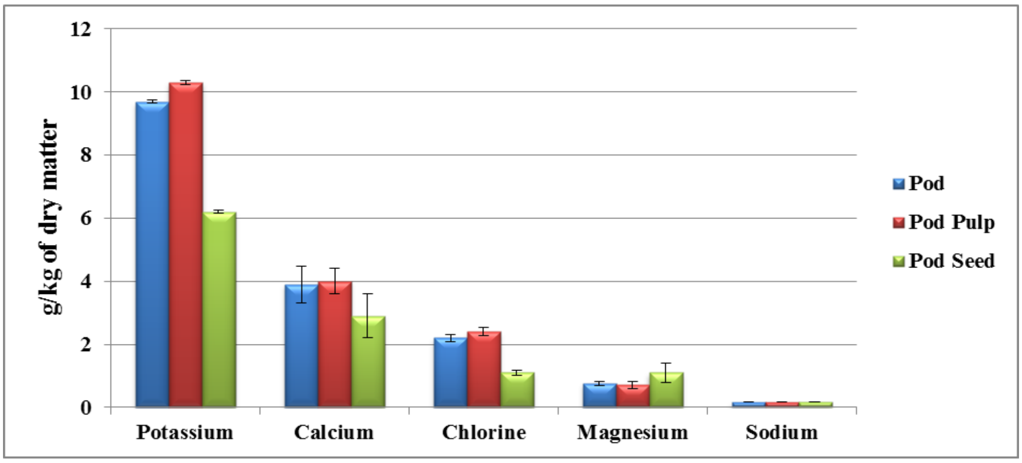

Figure 1. Macroelements content (mg/kg of dry matter ± SD) in different parts of domesticated carob pod

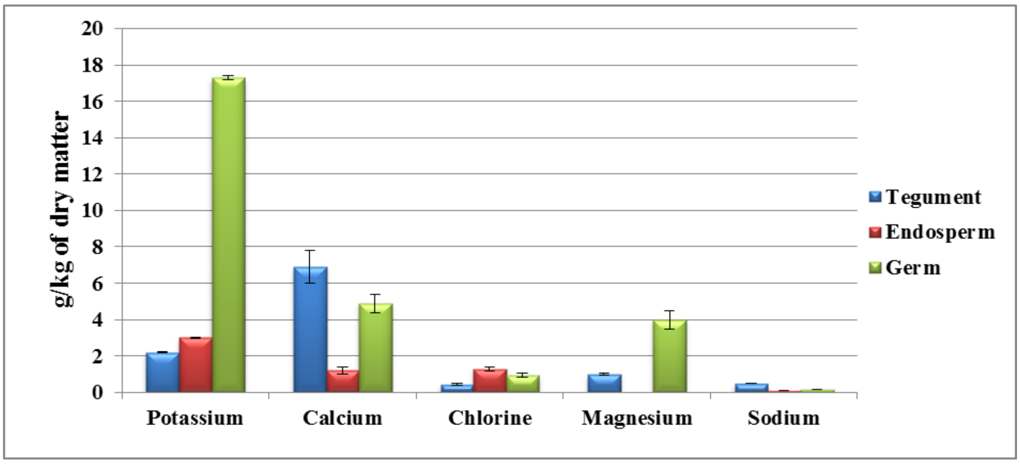

Figure 2. Macroelements content (mg/kg of dry matter ± SD) in different parts of domesticated carob seed

Table 5. Macroelements content (g/kg of fresh matter) in different parts of pod and seed of carob

Elements*	Pod	Different parts of pod		Different parts of seed		
		Pulp	Seed	Tegument	Endosperm	Germ
Potassium, K	8.3 ± 0.05	9.1 ± 0.05	4.3 ± 0.04	1.7 ± 0.03	2.0 ± 0.03	12.9 ± 0.07
Calcium, Ca	3.3 ± 0.48	3.6 ± 0.35	2.1 ± 0.54	5.6 ± 0.73	0.80 ± 0.17	3.7 ± 0.35
Chlorine, Cl	1.9 ± 0.08	2.1 ± 0.13	0.75 ± 0.07	0.34 ± 0.06	0.85 ± 0.07	0.70 ± 0.08
Magnesium, Mg	0.65 ± 0.18	0.63 ± 0.1	0.75 ± 0.22	0.81 ± 0.06	nd	2.98 ± 0.39
Sodium, Na	0.14 ± 0.002	0.14 ± 0.0006	0.13 ± 0.002	0.38 ± 0.001	0.07 ± 0.003	0.13 ± 0.0006

*Results are mean values of triplicate determinations (n=3) ± standard deviation (SD); nd, not detected

Table 6. Macroelements content in different parts of pod and seed of carob

Elements*	mg/pod	Different parts of pod		Different parts of seed		
		mg/pulp	mg/seed	mg/tegument	mg/endosperm	mg/germ
Potassium, K	75.5 ± 0.13	90.1 ± 0.5	0.305 ± 0.002	0.048 ± 9 10^{-3}	0.271 ± 0.003	0.569 ± 0.003
Calcium, Ca	30.1 ± 1.17	35.1 ± 3.5	0.125 ± 0.02	0.156 ± 0.02	0.106 ± 0.01	0.162 ± 0.02
Chlorine, Cl	17.9 ± 0.3	20.9 ± 1.3	0.081 ± 0.005	9.5 10^{-3} ± 0.001	0.113 ± 0.01	0.031 ± 0.003
Magnesium, Mg	5.4 ± 0.3	6.2 ± 0.99	0.081 ± 0.009	0.022 ± 0.002	nd	0.132 ± 0.02
Sodium, Na	1.2 ± 0.002	1.4 ± 0.006	0.008 ± 1 10^{-4}	0.01 ± 3 10^{-3}	9 10^{-3} ± 0.004	5.4 10^{-3} ± 3 10^{-4}

*Results are mean values of triplicate determinations (n=3) ± standard deviation (SD); nd, not detected

Table 7. Microelements content (mg/kg of dry matter) in different parts of pod and seed of carob

Elements*	Pod	Different parts of pod		Different parts of seed		
		Pulp	Seed	Tegument	Endosperm	Germ
Iron, Fe	78.4 ± 8	80.1 ± 8	69.4 ± 8	118 ± 9	36.1 ± 6	126 ± 8
Aluminium, Al	52.4 ± 9	52.2 ± 12	44.1 ± 8	91.5 ± 11	61.3 ± 9	8.9 ± 0.4
Strontium, Sr	21.2 ± 3	22.3 ± 5	15.0 ± 0.5	38.3 ± 4	5.6 ± 0.3	24.7 ± 0.8
Rubidium, Rb	15.9 ± 0.9	16.7 ± 1	12.0 ± 0.8	6.1 ± 0.8	4.9 ± 0.5	35.5 ± 1
Copper, Cu	15.8 ± 1	16.4 ± 0.9	12.5 ± 0.6	9.4 ± 0.9	12.5 ± 0.8	14.6 ± 0.8
Zinc, Zn	13.7 ± 0.8	10.9 ± 0.5	29.5 ± 0.9	25.8 ± 0.8	14.9 ± 0.6	71.4 ± 1
Manganese, Mn	5.6 ± 1.2	4.4 ± 0.6	12.7 ± 1.4	9.0 ± 0.9	1.5 ± 0.1	45.4 ± 3
Zirconium, Zr	2.9 ± 0.2	3.4 ± 0.5	0.59 ± 0.08	3.8 ± 0.08	nd	nd
Barium, Ba	2.3 ± 0.02	2.7 ± 0.03	$4.8\ 10^{-3} \pm 110^{-4}$	0.03 ± 0.001	nd	nd
Thorium, Th	2.2 ± 0.2	2.4 ± 0.4	1.2 ± 0.1	0.32 ± 0.01	0.65 ± 0.08	3.1 ± 0.1
Bromine, Br	1.9 ± 0.05	2.1 ± 0.06	0.72 ± 0.05	0.69 ± 0.06	0.74 ± 0.04	0.67 ± 0.06
Chromium, Cr	0.85 ± 0.1	0.77 ± 0.01	1.3 ± 0.1	3.3 ± 0.1	1.2 ± 0.1	0.38 ± 0.02
Thulium, Tm	0.85 ± 0.2	0.91 ± 0.02	0.54 ± 0.02	nd	0.36 ± 0.02	1.4 ± 0.1
Selenium, Se	0.34 ± 0.02	0.33 ± 0.06	0.38 ± 0.01	nd	0.29 ± 0.02	0.90 ± 0.01
Cerium, Ce	0.25 ± 0.07	0.28 ± 0.09	0.05 ± 0.005	0.08 ± 0.004	0.06 ± 0.001	nd
Molybdenum, Mo	0.06 ± 0.04	nd	0.37 ± 0.04	nd	0.60 ± 0.04	nd
Lanthanum, La	0.05 ± 0.009	0.06 ± 0.001	0.04 ± 0.009	0.05 ± 0.001	0.03 ± 0.007	0.05 ± 0.008
Caesium, Cs	0.05 ± 0.001	0.06 ± 0.001	0.03 ± 0.001	0.04 ± 0.001	0.01 ± 0.008	0.06 ± 0.001
Antimony, Sb	0.04 ± 0.001	0.05 ± 0.002	0.02 ± 0.001	0.04 ± 0.002	0.03 ± 0.001	nd
Mercury, Hg	0.04 ± 0.002	0.04 ± 0.002	0.04 ± 0.002	0.06 ± 0.002	0.06 ± 0.002	nd
Neodymium, Nd	0.04 ± 0.008	0.04 ± 0.002	0,02 ± 0.008	0.04 ± 0.002	0.02 ± 0.005	nd
Arsenic, As	0.03 ± 0.002	0.03 ± 0.002	nd	nd	nd	nd
Cobalt, Co	0.03 ± 0.008	0.03± 0.007	0.06 ± 0.009	0.09 ± 0.009	0.03 ± 0.006	0.12 ± 0.01
Scandium, Sc	0.01 ± 0.003	$9.6\ 10^{-3} \pm 8\ 10^{-4}$	0.02 ± 0.004	$6.4\ 10^{-3} \pm 8\ 10^{-4}$	0.03 ± 0.005	0.01 ± 0,004
Tantalum, Ta	$9.5\ 10^{-3} \pm 510^{-4}$	$4.8\ 10^{-3} \pm 8\ 10^{-4}$	0.04 ± 0.005	$8.2\ 10^{-3} \pm 110^{-4}$	0.05 ± 0.001	$7.4\ 10^{-3} \pm 110^{-4}$
Gold, Au	$5.510^{-3} \pm 110^{-3}$	4.9 10-3 $\pm 9\ 10^{-4}$	$8.5\ 10^{-3} \pm 110^{-4}$	0.03 ± 0.001	$6.3\ 10^{-3} \pm 8\ 10^{-4}$	$3.3\ 10^{-3} \pm 8\ 10^{-4}$
Hafnium, Hf	$3.8\ 10^{-3} \pm 310^{-4}$	$4.4\ 10^{-3} \pm$ 310-4	$6.4\ 10^{-4} \pm 110^{-5}$	$4.2\ 10^{-3} \pm 1\ 10^{-4}$	nd	nd
Samarium, Sm	$3.3\ 10^{-3} \pm 210^{-4}$	$3.8\ 10^{-3} \pm 210^{-4}$	$5.5\ 10^{-4} \pm 210^{-5}$	$3.6\ 10^{-3} \pm 210^{-4}$	nd	nd
Silver, Ag	$1.7\ 10^{-3} \pm 410^{-4}$	nd	0.01 ± 0.004	0.07 ± 0.004	nd	nd
Europium, Eu	$1.2\ 10^{-3} \pm 5\ 10^{-4}$	$1.4\ 10^{-3} \pm 5\ 10^{-4}$	nd	nd	nd	nd

*Results are mean values of triplicate determinations (n=3) ± standard deviation (SD); nd, not detected

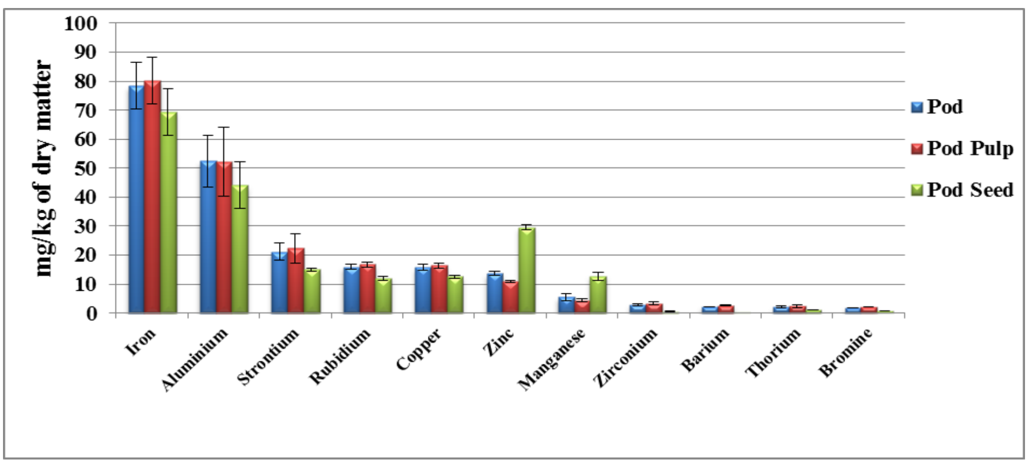

Figure 3. Main microelements content (mg/kg of dry matter ± SD) in different parts of domesticated carob pod

Table 8. Microelements content (mg/kg of fresh matter) in different parts of pod and seed of carob

Elements*	Pod	Different parts of pod		Different parts of seed		
		Pulp	Seed	Tegument	Endosperm	Germ
Iron, Fe	67.7 ± 6.6	71.0 ± 7.6	48.7 ± 6.3	95.1 ± 7.6	24.1 ± 4.6	93.8 ± 6.6
Aluminium, Al	45.0 ± 7.5	46.3 ± 11.3	38.0 ± 6.2	73.7 ± 8.9	41.0 ± 6.6	6.7 ± 3.1
Strontium, Sr	18.4 ± 4.1	19.8 ± 4.4	10.6 ± 4.1	30.9 ± 3.4	3.7 ± 2.2	18.4 ± 6.6
Rubidium, Rb	13.9 ± 0.7	14.8 ± 0.9	8.5 ± 0.6	4.9 ± 0.6	3.2 ± 0.3	26.4 ± 0.9
Copper, Cu	13.7 ± 0.8	14.5 ± 0.7	8.8 ± 0.5	7.6 ± 0.8	8.4 ± 0.6	10.9 ± 0.5
Zinc, Zn	11.4 ± 0.6	9.8 ± 0.5	20.7 ± 0.7	20.8 ± 0.6	9.9 ± 0.4	53.2 ± 0.9
Manganese, Mn	4.6 ± 0.9	3.9 ± 0.6	8.9 ± 1.1	7.3 ± 0.7	1.0 ± 0.1	33.8 ± 2.4
Caesium, Cs	4.2 ± 0.01	4.9 ± 0.01	0.02 ± 0.009	0.04 ± 0.001	9.5 10^{-3}± 5$10^{-4}$	0.04 ± 0.001
Zirconium, Zr	2.6 ± 0.1	3.0 ± 0.4	0.41 ± 0.05	3.0 ± 0.1	nd	nd
Barium, Ba	2.0 ± 0.01	2.4 ± 0.02	3.4 10^{-3} ± 1$10^{-4}$	0.03 ± 0.001	nd	nd
Thorium, Th	2.0 ± 0.2	2.2 ± 0.4	0.82 ± 0.08	0.26 ± 0.09	0.43 ± 0.06	2.3 ± 0.09
Bromine, Br	1.7 ± 0.04	1.9 ± 0.05	0.50 ± 0.04	0.55 ± 0.05	0.49 ± 0.03	0.50 ± 0.04
Thulium, Tm	0.74 ± 0.2	0.80 ± 0.2	0.38 ± 0.01	nd	0.24 ± 0.01	1.0 ± 0.1
Chromium, Cr	0.72 ± 0.01	0.68 ± 0.01	0.93 ± 0.1	2.7 ± 0.1	0.79 ± 0.06	0.28 ± 0.02
Selenium, Se	0.29 ± 0.01	0.29 ± 0.04	0.27 ± 0.08	nd	0.19 ± 0.02	0.67 ± 0.07
Cerium, Ce	0.22 ± 0.06	0.25 ± 0.09	0.03 ± 0.004	0.07 ± 0.004	0.04 ± 0.008	nd
Lanthanum, La	0.05 ± 0.008	0.05 ± 0.001	0.02 ± 0.007	0.04 ± 0.001	0.02 ± 0.005	0.04 ± 0.006
Antimony, Sb	0.04 ± 0.001	0.04 ± 0.002	0.02 ± 0.009	0.03 ± 0.02	0.02 ± 0.001	nd
Molybdenum, Mo	0.04 ± 0.003	nd	0.26 ± 0.02	nd	0.40 ± 0.02	nd
Cobalt, Co	0.03 ± 0.007	0.03 ± 0.006	0.04 ± 0.007	0.07 ± 0.007	0.02 ± 0.004	0.09 ± 0.009
Mercury, Hg	0.03 ± 0.002	0.03 ± 0.002	0.03 ± 0.002	0.04 ± 0.02	0.04 ± 0.002	nd
Neodymium, Nd	0.03 ± 0.005	0.04 ± 0.001	0.01 ± 0.006	0.03 ± 0.001	0.01 ± 0.003	nd
Arsenic, As	0.02 ± 0.002	0.03 ± 0.002	nd	nd	nd	nd
Scandium, Sc	9.5 10^{-3} ± 2$10^{-4}$	8.5 10^{-3} ± 7 10^{-4}	0.02 ± 0.003	5.2 10^{-3} ± 6 10^{-4}	0.02 ± 0.004	8.3 10^{-3} ± 3$10^{-4}$
Tantalum, Ta	7.4 10^{-3}± 3$10^{-4}$	4.3 10^{-3} ± 6 10^{-4}	0.03 ± 0.004	6.6 10^{-3} ± 1$10^{-4}$	0.04 ± 0.008	5.5 10^{-3} ± 8 10^{-4}
Gold, Au	4.5 10^{-3}± 8$10^{-4}$	4.4 10^{-3} ± 8 10^{-4}	6 10^{-3} ± 7 10^{-4}	0.02 ± 0.001	4.2 10^{-3} ± 5 10^{-4}	2.5 10^{-3} ± 6 10^{-4}
Hafnium, Hf	3.4 10^{-3}± 3$10^{-4}$	3.9 10^{-3}± 3$10^{-4}$	4.5 10^{-4} ± 7 10^{-5}	3.3 10^{-3} ± 7 10^{-4}	nd	nd
Samarium, Sm	2.9 10^{-3}± 2$10^{-4}$	3.4 10^{-3} ±2$10^{-4}$	3.9 10^{-4} ± 2$10^{-5}$	2.9 10^{-3}± 2$10^{-4}$	nd	nd
Silver, Ag	1.2 10^{-3}± 3$10^{-4}$	nd	7.9 10^{-3} ± 2.5 10^{-4}	0.06 ± 0.003	nd	nd
Europium, Eu	1.1 10^{-3} ± 3 10^{-4}	1.3 10^{-3} ± 3 10^{-4}	nd	nd	nd	nd

*Results are mean values of triplicate determinations (n=3) ± standard deviation (SD); nd, not detected

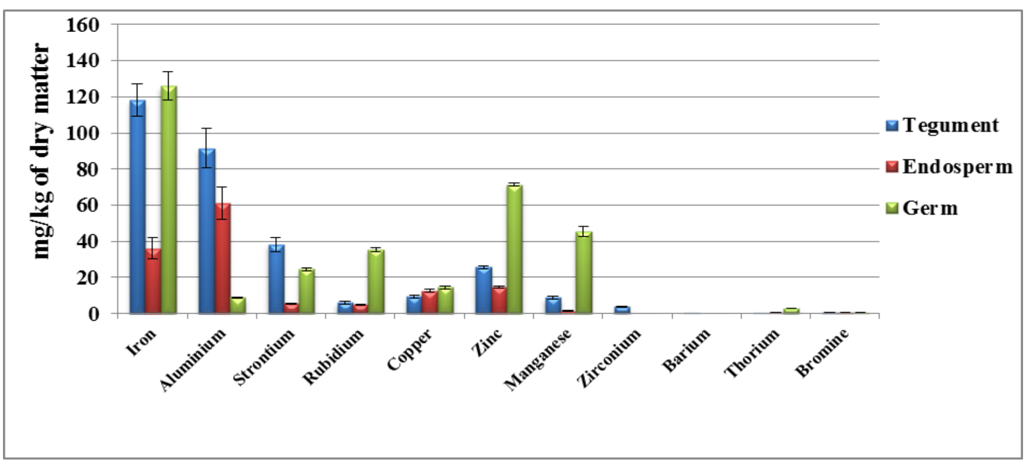

Figure 4. Main microelements content (mg/kg of dry matter ± SD) in different parts of domesticated carob seed

Table 9. Microelements content in different parts of pod and seed of carob

Element*	µg/pod	Different parts of pod		Different parts of seed		
		µg/pulp	µg/seed	µg/tegument	µg/endosperm	µg/germ
Iron, Fe	602.6 ± 20	702 ± 70	3.3 ± 0.4	2.6 ± 0.2	3.2 ± 0.6	4.2 ± 0.2
Aluminium, Al	395.5 ± 30	457.5 ± 50	3.9 ± 0.4	2.1 ± 0.2	5.5 ± 0.9	0.294 ± 0.01
Strontium, Sr	167.4 ± 10	195.6 ± 43	0.613 ± 0.2	0.858 ± 0.09	0.496 ± 0.02	0.813 ± 0.02
Rubidium, Rb	125.7 ± 2	146.7 ± 8.9	0.551 ± 0.03	0.137 ± 0.02	0.432 ± 0.04	1.2 ± 0.04
Copper, Cu	123.5 ± 1.7	143.5 ± 6.3	0.852 ± 0.04	0.210 ± 0.03	1.1 ± 0.04	0.48 ± 0.01
Zinc, Zn	84.4 ± 1	96.4 ± 4.8	1.5 ± 0.038	0.578 ± 0.02	1.3 ± 0.05	2.4 ± 0.04
Manganese, Mn	33.5 ± 1	38.5 ± 5.6	0.885 ± 0.05	0.202 ± 0.02	0.134 ± 0.01	1.5 ± 0.1
Zirconium, Zr	25.3 ± 0.8	29.7 ± 0.8	Trace	Trace	nd	nd
Barium, Ba	19.9 ± 0.9	23.4 ± 0.9	Trace	Trace	nd	nd
Thorium, Th	18.2 ± 1	21.3 ± 3.9	0.022 ± 0.005	Trace	Trace	0.103 ± 0.004
Bromine, Br	15.7 ± 0.1	18.4 ± 0.5	Trace	Trace	Trace	Trace
Thulium, Tm	6.8 ± 0.1	7.9 ± 0.3	Trace	nd	Trace	Trace
Chromium, Cr	6.1 ± 0.3	7 ± 1	0.069 ± 0.006	Trace	0.106 ± 0.008	Trace
Selenium, Se	2.5 ± 0.3	2.9 ± 0.3	Trace	nd	Trace	Trace
Cerium, Ce	2.1 ± 0.4	2.4 ± 0.8	Trace	Trace	Trace	nd
Lanthanum, La	0.426 ± 0.02	0.501 ± 0.09	Trace	Trace	Trace	Trace
Caesium, Cs	0.414 ± 0.02	0.487 ± 0.09	Trace	Trace	Trace	Trace
Antimony, Sb	0.338 ± 0.04	0.397 ± 0.02	Trace	Trace	Trace	nd
Neodymium, Nd	0.300 ± 0.03	0.353 ± 0.03	Trace	Trace	Trace	nd
Mercury, Hg	0.263 ± 0.07	0.309 ± 0.02	Trace	Trace	Trace	nd
Cobalt, Co	0.212 ± 0.01	0.249 ± 0.06	Trace	Trace	Trace	Trace
Arsenic, As	0.212 ± 0.01	0.249 ± 0.02	nd	nd	nd	nd
Scandium, Sc	Trace	Trace	Trace	Trace	Trace	Trace
Molybdenum, Mo	Trace	nd	Trace	nd	Trace	nd
Tantalum, Ta	Trace	Trace	Trace	Trace	Trace	Trace
Gold, Au	Trace	Trace	Trace	Trace	Trace	Trace
Hafnium, Hf	Trace	Trace	Trace	Trace	nd	nd
Samarium, Sm	Trace	Trace	Trace	Trace	nd	nd
Silver, Ag	Trace	nd	Trace	Trace	nd	nd
Europium, Eu	Trace	Trace	nd	nd	nd	nd

*Results are mean values of triplicate determinations (n=3) ± standard deviation (SD); nd, not detected; Trace < 0.1 µg

4. Discussion

For experts, agro-morphological characteristics make it possible to differentiate spontaneous carob from the domesticated one [55-58]. Although the shape, color and size of pods are generally influenced by environmental factors, pods of domesticated types are distinguished from spontaneous types by their larger size and bulked seeds [1, 57].

The morphological data obtained on the pods and seeds by Gharnit *et al.* [28], Konate [59], Sidina *et al.* [60] and El Kahkahi *et al.* [61] for domesticated trees in Morocco, compared with our results, show similarities for pod length (127.98-149.39 mm), total seeds number per pod (8.98-12), seed weight per pod (1.77-1.98 g), length, width, seed thickness (8.19-9.33, 6.34-7.67 and 3.95-4.5 mm, respectively) and the average seed weight (0.176-0.24 g). On the other hand, width, pod thickness (15.66-19.5 and 5.52-6.9 mm), the weight of fresh pod material (7.86-11.75 g) and pulp (5.96-9.55 g) are lower.

The morphological characteristics of the pods obtained in this study for domesticated trees compared to those of other countries in the Mediterranean basin, namely Spain [62-65], Portugal [66, 67], Turkey [45, 68, 69], Italy [70, 71], Cyprus [72], Egypt [73] and Lebanon [74], show a great diversity, which can be explained by the differences in the edaphoclimatic factors of the different sites, the type of tree (variety), the geographical location, the treatments applied and the agricultural practices [71, 75, 76].

However, similarity is observed for the total number of seeds with a low number of aborted ones per the studied domesticated tree pod (11.04 and 0.75, respectively) compared to those of Turkey (7.27-10.83), Tunisia (10.98-12.26 and 1.38) [32, 67, 77], Spain (10-12.17 and 1.73-3.76) [62-65] and Portugal (11.2-12.17 and 2.06-2.4) [66, 78]. The rate of aborted seeds does not depend on the origin or type of carob trees, but on other factors such as pollination, fertilization or climatic conditions [28, 79].

The abundance of seeds in the pods is a distinctive character between the spontaneous and domesticated types. Indeed, the spontaneous types are known for their large seed/pod productions which are generally small and not fleshy [1, 12, 45, 55]. This may suggest that Moroccan plantations, even domesticated trees, exhibit typically spontaneous traits, what confirms the reports of Ouchkif [6], Batlle and Tous [1] and Gharnit *et al.* [58] for whom the mass production of pods and seeds is ensured in Morocco by the forest populations. Currently, a commercial cultivar will be considered if the average seed yield is at least 15%.

For the morphological characteristics of seeds for the domesticated studied tree, the length, width, thickness and mean seed weight (9.39 mm, 7.22 mm, 4.08 mm and 0.1984 g) are similar to those of Turkey (8.69-9.04 mm, 6.43-6.78 mm, 3.88-3.90 mm) [45, 80], Tunisia (9.11 mm, 6.94 mm, 4.15 mm and 0.197-0.209 g) [70, 71], Spain (9.4-9.8 mm, 7.0-7.25 mm, 3.8- 4.02 mm and 0.19-0.213 g) [62-65], Portugal (9.52-9.59 mm, 7.15-7.32 mm, 4.22-4.29 mm and 0.193-0.221 g) [66, 78], Italy (9.28-9.3 mm, 6.8-6.84 mm, 3.9-3.93 mm) [70, 71] and Cyprus (9.45 mm, 6.92 mm, 3.82 mm and 0.185 g) [72].

The percentage by weight of dry matter of different parts of the seed of the studied carob is 13.55% of tegument, 64.91% of endosperm and 21.54% of germ. In previous studies, average seed tegument, endosperm and seed germ contents range from 20.81 to 39.12; from 43.19 to 57.30 and from 16.59 to 21.10%, respectively [15, 28, 65, 81, 82]. These differences have an undeniable economic impact for industrial use.

The mineralogical analysis of carob pods made it possible to determine the content of various minerals of pulp, seed tegument, endosperm and germ. While the content of organic elements is widely studied by different authors, publications relating to the mineral composition need to be well illustrated for pulp; moreover, the mineral composition of different parts of the seed has not been carried out, hence the interest of such a study.

According to the literature, many factors affect the chemical composition of fruits, as well as mineral salt content, for example temperature, drought [83, 84], irrigation and fertilization [85], salinity [86], the origin of the tree and cultivars, and the fruit harvest period [15, 83]. Analysis and comparison of the mineralogical compositions for the same organ show that the mineral contents can vary substantially according to the genotype of the same species. The variation of the composition of mineral elements also depends on the year of collection and the analyzed organ. Indeed, in the same plant, important variations in the mineral content of the tissues can be remarked according to the analyzed organs (roots, bark, flowers, fruits and leaves) [48]. These are due in particular to internal transfers of mineral elements between different organs of the plant. For example, the mineral composition of leaves may vary considerably depending on the position of the branch along the trunk and that of the leaf on the branch, as has been demonstrated in Congo in *Eucalyptus alba* x *E. urophylla* [87-89].

Short and long irradiation studies show that some elements are more abundant, such as potassium, calcium, chlorine, magnesium, sodium, aluminum and iron. These elements represent the main macro and microelements used by plants. Potassium is the most abundant in pods and its content is especially high in the germ.

The most abundant macro and microelements in the pulp are: K; Ca; Cl; Mg; Na; Fe; Al; Sr; Rb. The contents are higher than those obtained by other authors for potassium (6.40 to 8.64 g/kg DM) [9, 47, 90], calcium (2.07 to 3.70) [11, 16, 44, 45, 47], magnesium (4.10-3 to 0.346) [50, 91], sodium (0.04 to 0.147) [21, 43, 44], iron (4.7 to 23.4) [9, 11, 21, 45, 49] and strontium (3.0 to 7.9) [44]. Potassium is also the main mineral in other fruits: kiwi [92], apple, quince, fig, date [93], medlar [94], apricot [95] and kaki [96].

The results of this study are in agreement with those obtained by several published studies, in particular for potassium (9.7 to 12.30 g/kg DM) [21, 49, 97], magnesium (0.4 to 0.99) [9, 11, 43, 98], sodium (0.17 to 0.30) [99, 100], aluminum (49.56 to 109.45) [42], iron (35.7 to 76.60) [42, 44, 50, 98] and strontium (11.87 to 70.54) [42]. On the other hand, authors have found higher values in mineral composition than our results for potassium (14.86 to 27.20 g/kg DM) [42, 91], calcium (4.40 to 9.60) [9, 42, 51, 98], magnesium (1.44 to 6.90) [42, 48], sodium (0.51 to 2.05) [42, 47, 91] and iron (130.43 to 1390.82) [16, 47, 48].

Short and long irradiation analyzes revealed that the seed and its constituents (seed tegument, endosperm and germ) are characterized by high levels of mineral macroelements: K; Ca; Mg; Cl and Na. Similarly, the mineral microelements are present: Al; Fe; Zn; Mn; Cu; Sr and Rb.

The results described above are in concordance with the results of Fidan and Sapundzhieva [50] for Mn (50.8 mg/kg DM) and Se (1.60 mg/kg DM) in the whole seed. While these results are very different from those of El-Shatnawi and Ereifej [51], Gubbuk *et al.* [45], Oziyci *et al.* [49] and Fidan and Sapundzhieva [50] concerning the K (8.10 to 11.30 g/kg DM), Ca (3.50 to 6.74), Mg (0.02 to 1.70), Fe (29.01 to 82), Zn (19.80 to 30.98), Mn (15.91 to 27.20) and Cu (4.25 to 20.2).

In the present study, the mineral composition of seed constituents (seed tegument and germ) is not in concordance with that of the literature [48, 52]. However, no information regarding the mineral composition of the carob seed endosperm is available to compare it with our results.

5. Conclusions

The use of carob bean as a food ingredient by the pharmaceutical and food industries could be developed. Several studies have shown that carob pulp has beneficial effects on excess weight, diabetes, hyperlipidemia, inflammation and oxidative stress. Thus, the incorporation of carob bean and its by-products in food formulations would not only improve their nutritional values but also their functionality by imparting rheological properties and extending the life of the finished product. Many agro-food manufacturers use carob pulp flour as an additive (E410), as a substitute for cocoa in pastries and ice cream, or as a thickening agent in baby milk powder as a replacement for wheat flour, to avoid gastroesophageal reflux.

Carob pod analyzed is rich in potassium (9.7 g/kg DM, 8.3 g/kg FM and 75.5 mg/pod) and poor in sodium (0.16 g/kg DM, 0.14 g/kg FM and 1.2 mg/pod). Similarly, the seed show high potassium content (6.2 g/kg DM, 4.3 g/kg FM and 0.305 mg/seed) and a very low level of sodium (0.17 mg/kg DM, 0.13 mg/kg FM and 0.01 mg/seed). The richness of the pulp and the carob seeds in mineral elements reflects the importance attributed to this food for a long time for animal and human nutrition.

This study provides information on morphological characteristics and mineral composition of pods from a domesticated tree and furnishes a starting point for programs for planting new carob orchards with more economically and environmentally efficient varieties.

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