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Comparative study of the mineral composition of carob pulp (*Ceratonia siliqua L.*) from various regions in Morocco

K. EL Oumlouki [(a,d)*], ***G. Salih*** [(b)], ***A. Jilal*** [(C)], ***H. Dakak*** [(d)], ***M. EL Amrani*** [(a)] ***and A. Zouahri*** [(d)]

[(a)] *Ibn Tofail University, Faculty of Sciences, Advanced Materials and Process Engineering Laboratory, Kenitra, Morocco.*
[(b)] *National Institute of Agricultural Research (INRA), Regional Centre of Rabat, Department of Food Technology, Rabat, Morocco.*
[(c)] *National Institute of Agricultural Research (INRA), Regional Centre of Rabat, Department of Plant Breeding, Conservation and Valorization of Plant Genetic Resources, Rabat, Morocco*
[(d)] *National Institute of Agricultural Research (INRA), Regional Centre of Rabat, Research Unit on Environment and Conservation of Natural Resources, Rabat, Morocco.*

* Corresponding author:
Kaoutar18@gmail.com
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Abstract

Ceratonia siliqua L. or the carob is a species agro-forestry-pastoral with enormous socio-economic and ecological interests. It has been long cultivated for food and feed. Currently, it is increasingly used by the food, pharmaceutical and dietary. This is a typical Mediterranean and widely distributed species in Morocco. In order to characterize and enhance the carob of Morocco, a comparative study of the mineral composition of the carob pulp from four regions in Morocco (Beni-Mellal, Chaoun, Tafraoute and Taounate) for the identification of the region elite for each mineral element, the results of physico-chemical and statistical analyzes shows a significant difference between the variables and regions studied. The Characterization has showed a variation in terms of the moisture content, the ash content and of the mineral composition for different populations of carob, the results obtained were as follows: the moisture content (Mc=8973.5-10419.2 mg/100g), the ash content (Ac= 2950.3-3836.2 mg/100g) and the mineral composition (K^+=1121,1-1300,2 mg/100g, P=41,9-57.,2 mg /100g, Ca^{2+}=270,4-305,3 mg /100g, Mg^{2+}=40,4-144 ,8 mg /100g et Na^+=31,6-81,7 mg /100g, Fe^{2+}=5,26-6,66 mg /100g, Zn^{2+}=1,80-2,61 mg /100g, Mn^{2+}=0.,60-0,75 mg /100g, Cu^{2+}=0,42-0,56 mg/100g).

Keywords: *Ceratonia siliqua L, carob pulp, region, moisture content, ash content, mineral composition*

1. Introduction

The forest and trees provide food in addition to natural and traditional agricultural sources. Yet until recently, foresters and nutritionists paid little attention to forest foods. In many parts of the world, forest products, defined as "products of organic origin" play a lasting biological and social role, also an important significant element in local food systems. They can make a real contribution to nutrition as part of the diet or as an asset for families' food security or to prevent nutritional deficiencies. They serve health through their role in the prevention and treatment of disease as well as maintaining a balanced diet. In Morocco, malnutrition and the development of food products occupy a considerable interest of several research programs, among them, our theme which is based on "the valorization of the Moroccan Carob", which is a natural resource with high added value. The carob tree (*Ceratonia siliqua L.)* is a dioecious tree ranging in size from 8 to 15 m and can live up to 500 years, native to the Mediterranean regions [1,2]. It has been cultivated since antiquity for human and animal consumption, it is an almost endemic species [3], it tolerates poor, sandy, heavy loamy, rocky, limestone, schist and sandstone soils, with pH values ranging from 6.2 to 8.6 ; but it fears acidic and very humid soils [4,5]. Currently it is found in several countries of the world such as Europe and North Africa in the wild in association with oleaster, thuja, pine, holm oak and lichens [6,7,8]. At Morocco level, it covers an area of around 10144 hectares, more than half of w nghich is in the Moroccan forest estate. Morocco ranks third after Italy and Portugal with world production of 21,974 tonnes [9]. The carob production areas in Morocco are the regions of Chefchaouen, Chaouan, Taounate, Ouezzane, Fes, Beni-Mellal, Marrakech, Agadir, Essaouira, Taza, El Hoceima and Khénifra, one would also find between Tiznit and Tafraout. [7, 10, 11]. It has enormous socio-economic and ecological interests, such as the important role in protecting the soil against degradation and erosion and in the fight against desertification, as well as in agricultural landscapes and forest ecosystems [1, 3, 4, 12]. Its use is beginning to gain momentum in the pharmaceutical, dietetic and food industries [7, 13, 14, 15], and also as heavy metal biosorption [16]. The purpose of this study is to enhance the carob which is an endemic species in order to study the feasibility of its use as a nutritive source by determining the mineral compositions of their different populations from four regions of Morocco (Beni-Mellal, Chaoun , Tafraout and Taounate), as well as physico-chemical and statistical analyzes aimed at comparing the regions between them for the identification of the elite region (Figure 1).

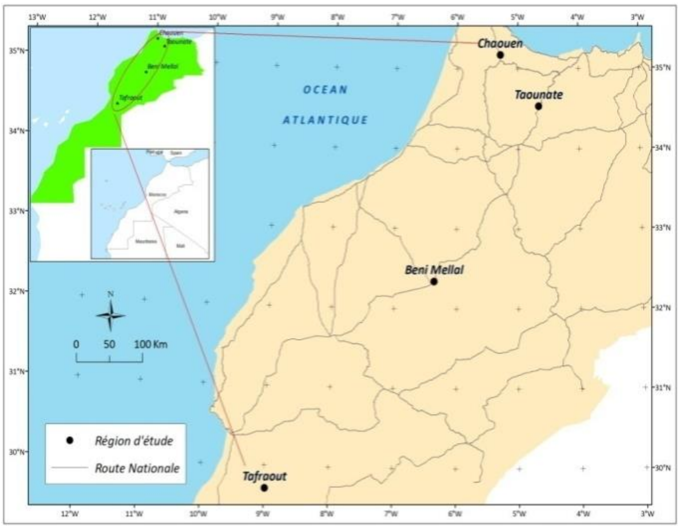

Figure 1. Geographical location for collecting carob samples

2. Materials and methods

2.1. Plant Material

The carob fruits have been collected from four regions of Morocco. The choice of regions has not been made at random because we take into account the difference in climate, the size, the shape and the color of carob pods. Two

northern regions were chosen (Taounate and Chaouen are located in the Rif mountains, characterised by a Mediterranean climate, cold and humid in winter and warm temperate in summer) and the other two regions belonged to the South of Morocco (Beni Mellal region located in the centre of the country, it is characterized by a continental climate, with high temperatures ,that can be very hot in summer and relatively mild in winter, then the region of Tafraout located in the Anti-Atlas mountains, nestled in the Ameln valley, it has an arid climate, hot and dry in summer and the winter is cold and dry too). Fifteen carob fruit samples were collected for each of the four regions of Morocco, during the 2011 campaign. The samples were stored at a temperature not exceeding 4°C. In the total 60 samples are analyzed in the laboratory (Figure 2).

Figure 2. Carob pods collected from different regions: Beni-Mellal (1), Taounate (2), Tafroute (3), Chaoun(4)

2.2. Preparation of plant material

The carob pulp and seeds is separated manual and the pulp is finely ground. After crushing and sieving, the flour of the carob pulp of each sample is put in a kraft paper on which is marked and identified by region.

2.3. Physico-chemical analyzes

2.3.1. Moisture contents (Mc)

The moisture content was determined according to the official method AOAC 931.04, who it's based a Gravimetry [17], we take 2g of each sample of the carob pulp powder crushed and placed in a ventilated oven at a temperature of 70 °C for 24 hours. The samples are dried to stable weight. After cooling the samples are placed in a desiccator. Weighs are done using a precision balance. Each sample was repeated three times.

The moisture content is given by the following formula (1):

$$\textit{Moisture contents (Mc)} = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100 \text{ in } (\%) \qquad (1)$$

Or: m_0 : mass of the empty crucible in (g). m_1: mass of the crucible containing the test sample before drying in (g). m_2 : mass of the crucible containing the test sample after drying in (g). ***2.3.2. Ash content (Ac)***

The ash content was determined according to the official method AOAC 972.15, who it's based a Gravimetry [18]. We take 0.5g of the dried carob pulp powder is introduced into porcelain crucibles which are placed in an oven. Incineration is at a temperature of 600 °C and which are thus maintained for 5 hours. A level is carried out around 200

°C until the end of smoke generation. Then they are cooled in the desiccator for 40 minutes, and weighed with a precision balance [19]. Three repetitions are established for each sample.

The ash contents (Ac), expressed by the following formula (2):

$$\textit{Ash content (Ac)} = \frac{(m_2 - m_0)}{(m_1 - m_0)} \times 100 \text{ in } (\%) \quad (2)$$

Or: m_0 : mass of the empty crucible in (g). m_1 : mass of the crucible containing the test sample before incineration in (g). m_2 : mass of the crucible containing the test sample after incineration in (g).

2.4. Minerals Contents

2.4.1. Mineral composition

The white ash obtained after incineration is moistened with a few drops of distilled water and then 2 ml of concentrated HCl are added. It is left in contact for 10 minutes and filtered through 100 ml volumetric flasks [19].

2.4.2. Determination of mineral elements

- **Phosphorus :** was determined according by Colorimetry (molybdovanadate) using the Visible UV spectrophotometer at a wavelength of 825 nm [19].
- **Sodium and Potassium :** are determined by emission spectrophotometry of flames [19].
- **Calcium and Magnesium :** are determined by a Complexometric titrimetry [19].
- **Trace elements (Iron, Copper, Zinc and Manganese):** are determined by atomic absorption spectrophotometer [20, 21].

2.5. Statistical analysis

Statistical analyses used are multivariate analysis of variance MANOVA, Univariate analysis ANOVA, Correlation analysis, principal component analysis and hierarchical classification [22].

3. Results and Discussion

The results of the analyzes carried show a wide diversity in terms of the different studied variables such as the moisture content, the ash content, the mineral composition and statistical analysis of the carob populations from the four regions in Morocco, which are presented by the following symbols : (Beni-Mellal (BM), Chaoun (CH), Tafraout (TF) and Taounate (TA)).

3.1. Moisture content (Mc)

Determining the nutritional value of foods requires that the moisture content must be known [23]. According to the literature, the moisture content of carob pods varies between 3.6% -18.0%. In the fresh state the carob pods generally have moisture content between 10 and 20%, they are reduced to less than 10% by drying under shelter in dry places and by ventilation to prevent the pods from rotting during storage before the treatment [7,24]. The figure 3 represents a significant difference in the moisture content of studied carob pulp. The Tafraout region has the highest average moisture content (Mc = 10419.2 mg /100g = 10.41%) followed by the Chaoun region of (Mc = 9222 mg/100g = 9.22 %) and finally Beni-Mellal region of (Mc = 9917.2 mg /100g = 9.91%) and Taounate region of (Mc = 8973.5 mg /100g = 8.97 %).

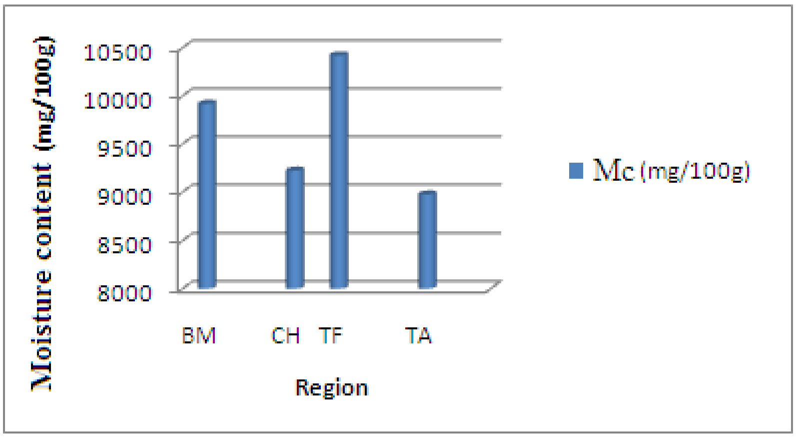

Figure 3. Moisture content of the carob pulp from the four regions

These results are comparable a study in South Africa about five carob cultivars (Tylliria, Sfax, Aaronsohn, SantaFe and "unknown" cultivar) that it shows a the average proximate composition of raw carob pods was 8.17-9.56% moisture [25], and another study shows also the moisture content of roasted carob powder (9.00%) was lower than that of the non-roasted carob powder (11.1%) [26]. Other researchers explain this difference in moisture content by environmental conditions (rain, humidity and temperature), carob cultivars, ripening time, harvest time and storage time and Carob pods size will produce different types of carob powder [7, 24, 26].

3.2. Ash content (Ac)

The ash content attributed to inorganic residues remaining after ignition or complete oxidation of organic matter in food products gives an indication of the total mineral content of food products and the nutritional quality of the sample to be analyzed [23]. For carob powder, the ash content normally varies between 2 and 6% [26]. The results obtained from the ash content of the carob pulp represented in the figure 4 shows a significant difference between the different regions studied. The Taounate region has the highest average ash content (Ac = 3836.2 mg /100g) followed by the Tafraout region of (Ac = 3601.7 mg /100g) and lately two regions Beni-Mellal and Chaoun ash content respectively are: (Ac= 3479.6 mg /100g and Ac = 2950.3 mg /100g).

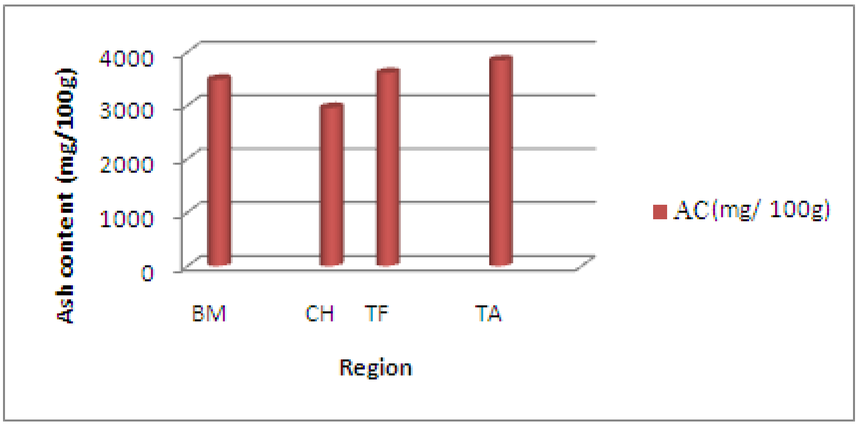

Figure 4. Ash content of carob pulp from the four regions

Ash content for carob pulp was determined between 2.95% -3.83%, as also showed by other researchers [27, 28 , 29]. Second study in jordan about two varieties of carob shows that the siliqua variety had higher ash contents than the macrocarpa variety [30]. Another study shows no significant difference between carob pulp or for the carob seeds from three regions in Algeria, those of Tlemcen (Ac= 1.83%), Blida (Ac= 2.25%) and Jijel (Ac= 2.67%) [31]. Finaly

the ash contents of Carob pulp in 7 studied provenances in Morocco ranged between 2.44 and 3.89 g/100g and Taza differed significantly ($P<0.05$) from that of Agadir, Marrakesh and Al Hoceima [32].

3.3. Mineral composition

The mineral composition considered as one of the criteria used to assess the nutritional value of a food. Minerals are essential support substances for the proper functioning of our body. More than 100 µg of macro-minerals (P, Na, K, Mg, Cl and S) are required daily, while less than 10 µg of nutrients from trace minerals (Fe, Zn, Cu, Cr, Mn, Fr, Se and Si) are required in the human diet daily. The results obtained for the different mineral compositions of studied carob pulp indicate a significant difference between the regions studied. They varied in these contents for the major elements we find the following contents in the figure 5: (K^{+}= 1121.1-1300.2 mg/100g, P = 41.9-57.2 mg/100g) for the secondary elements have shown in the figure 6 (Ca^{2+} = 270.4-305.3 mg / 100g, Mg^{2+}= 40.4-144.8 mg/100g and Na^{+}= 31.6-81.7 mg /100g). And finally for the trace elements we find in the figure 7 (Fe^{2+}= 5.26-6.66 mg / 100g, Zn^{2+}= 1.80-2.61 mg / 100g, Mn^{2+}= 0.60-0.75 mg / 100g and Cu^{2+}= 0.42-0.56 mg /100g).

These results show a significant difference between the mineral compositions analyzed, there are comparable to two studies ; the first study about four major minerals (Ca, P, K and Mg) of Anatolian carob pod , they found that pods were richer on potassium (K^{+} =970 mg/100g) and (Ca^{2+}=300 mg/100g) but the levels of (P=71 mg/100g) and (Mg^{2+}=60 mg/100g) were considerably lower, and for the trace minerals, (Fe^{2+}=1.88 mg/100g and Mn^{2+}=1.29 mg/100g) are predominated, although substantial amounts of (Zn^{2+}= 0.75 mg/100g and Cu^{2+}= 0.85 mg/100g) were also present [33]. Second study about the compositional properties and mineral contents of carob fruit, flour and syrup shows that the Calcium, potassium, magnesium, sodium and phosphorus also the trace minerals (iron, copper ,zinc and Manganese) were the most abundant elements had the highest values, in carob flour and carob fruits [34]. The mineral composition of carob pulp varies according to cultivars, geographical origin, harvest time, environmental factors, soil type and and seasonal climate [33, 35]. The Taounate region is our elite region in terms of element contents (K, P, Na), because it has the highest contents.

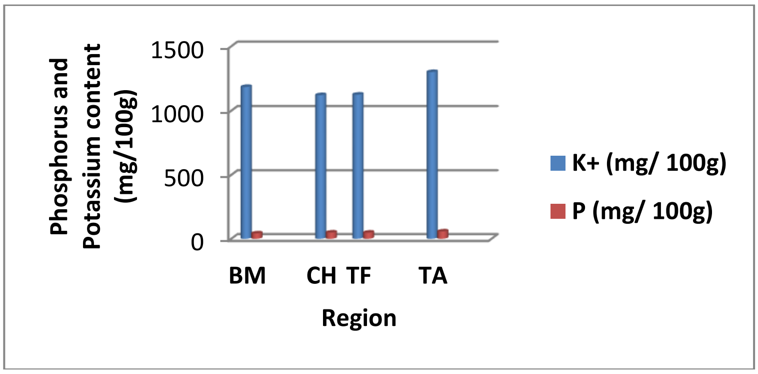

Figure 5. Contents of the major elements (phosphorus and potassium) in the carob pulp from the four regions

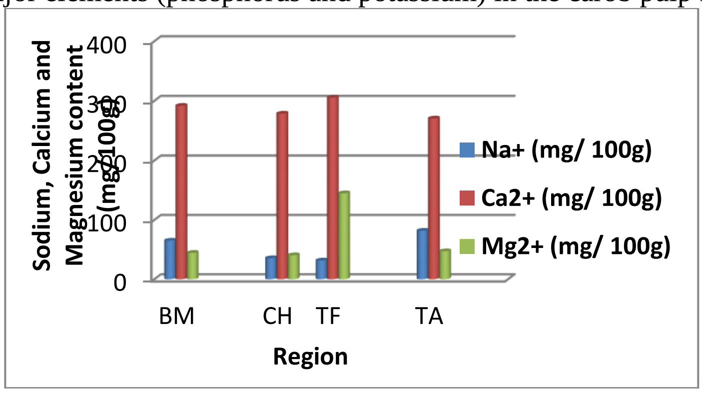

Figure 6. Contents of secondary elements (sodium, calcium and magnesium) in the carob pulp from the four regions

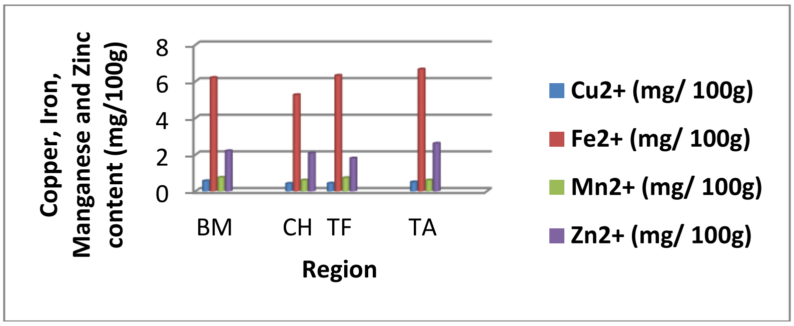

Figure 7. Contents of trace elements (copper, iron, manganese and zinc) in the carob pulp from the four regions

3.4. Statistical analysis

3.4.1. Multivariate variance Analysis (MANOVA) and of the different factors and their interaction

The multivariate variance analysis result of the different factors studied such as the region, the sample, as well as the repetition and their interaction represented by table 1:

Table 1. Multivariate variance analysis (MANOVA) of different factors studied

Effect	*Value*	*F*	*Hypothesis dl*	*Mistake dl*	*Sig.*
Region	0.000	138.271	33.000	94.982	0.000***
Sample	0.000	7.081	154.000	298.865	0.000***
Repetition	0.094	28.076	11.000	32.000	0.000***
Region*Sample	0.000	6.257	462.000	377.343	0.000***
Region * Repetition	0.026	7.069	33.000	94.982	0.000***
Sample * Repetition	0.019	1.112	154.000	298.865	0.220 **NS**

******* : Very Highly Significant ($P<0.001$) ; ****** : Highly Significant ($P<0.01$)

***** :Significant ($P<0.05$) ; **NS** : Not significant ($P>0.05$)

According to Table 1, there is a very highly significant effect for the various factors studied such as the region, sample and the repetition and their interactions. This shows that there is a difference between the regions and an intra-region difference with regard to the parameters studied.

3.4.2. Comparison analysis of means using the Duncan method

The result of comparison analysis of the means of the various variable factors such as the moisture content, the ash content and the mineral compositions via the Duncan method is represented by Table 2:

Table 2. Analysis of the comparison of means using the Duncan method

Region	*Mc* *(mg/100g)*	*Ac* *(mg/100g)*	*K^+* *(mg/100g)*	*P* *(mg/100g)*	*Na^+* *(mg/100g)*	*Ca^{2+}* *(mg/100g)*	*Mg^{2+}* *(mg/100g)*	*Cu^{2+}* *(mg/100g)*	*Fe^{2+}* *(mg/100g)*	*Mn^{2+}* *(mg/100g)*	*Zn^{2+}* *(mg/100g)*
Beni- Mellal	9917.2^d	3479.6^c	1184.9^c	41.9^b	64.9^b	291.7^b	44.4^c	**0.56^a**	6.20^c	**0.75^a**	2.20^c
Chaoun	9222.0^c	2950.3^b	1121.1^b	48.6^c	35.4^c	278.6^c	40.4^b	0.42^b	5.26^b	0.60^b	2.08^c
Tafraoute	**10419.2^a**	3601.7^c	1124.8^b	47.7^c	31.6^c	**$305,3^a$**	**144.8^a**	0.43^b	6.32^a 6.32^c	0.73^a	1.80^b
Taounate	8973.5^b	**3836.2^a**	**1300.2^a**	**57.2^a**	**81.7^a**	270.4^d	47.2^d	0.50^c	**6.66^a**	0.60^b	**2.61^a**

(a)= 1st group; **(b)** = 2nd group; **(c)** = 3rd group; **(d)** = 4th group

The processing of the Duncan analysis results shows that the means of the following studied variables: moisture content, calcium and magnesium content formed four groups for the four regions studied, because they present different means. And the Tafraoute region has the highest content of these successive variables (Mc= 10419.2 mg/ 100g, Ca^{2+}= 305.3 mg/100g, Mg^{2+}= 144.8 mg/100g). And about the means of the following studied variables: the ash content, the potassium, the phosphorus, the sodium, the iron and the zinc content are classified into three different groups for these four regions. Consequently, the Taouante region has the highest content of these successive variables (Mc= 3836.2 mg/100g, K^+ = 1300 mg/100g, P = 57.2 mg/100g, Na^+ = 81.7 mg/100g, Fe^{2+} = 6.66 mg/100g and Zn^{2+}= 2.61 mg/100g). The highest content of these successive variables (Ac = 3836.2 mg / 100g, K^+ = 1300 mg / 100g, P = 57.2 mg / 100g, Na^+ = 81.7 mg / 100g, Fe^{2+} = 6.66 mg / 100g and Zn^{2+} = 2.61 mg / 100g). In the same way, the means of the following studied variables: the copper content and the manganese content formed respectively three groups and two groups for all these four studied regions and the Beni-Mellal region contains the highest content (Cu^{2+} = 0. 56 mg /100g, Mn^{2+} = 0.73 mg /100g).

3.4.3. Correlation analysis

Table 3 presents the result of the correlation analysis of the studied variables such as the moisture content, the ash content and the mineral composition. Analysis of the correlation matrices of the studied variables such as the moisture content, the ash content and the mineral compositions shows that there are higher positive and negative correlations that are highly significant, namely:

- The ash content is strongly positively correlated with the Potassium content.
- A strong negative correlation observed between the sodium content and the calcium content on the one hand and on the other hand between the sodium content and the copper content a strong positive correlation.

3.4.4. *Principal component analysis (PCA) of the regions and samples studied*

3.4.4.1. Principal component analysis of the studied regions

The principal component analysis makes it possible to present the 11 studied parameters in two axes PCA1 and PCA2. The minimum spanning tree (lines connecting the regions) makes it possible to increase the degree of closeness between the different regions. The principal component analysis for the studied regions represented by Figure 8:

Table 3. Correlation matrix of the studied variables

Variable	*Mc*	*Ac*	*K^+*	*P*	*Na^+*	*Ca^{2+}*	*Mg^{2+}*	*Cu^{2+}*	*Fe^{2+}*	*Mn^{2+}*	*Zn^{2+}*
Mc	1										
Ac	0.152	1									
K^+	-0.090	**0.539** (0,000)**	1								
P	-0.306*	0.063	0.406**	1							
Na^+	0.231	0.082	0.055	-0.339**	1						
Ca^{2+}	0.025	0.200	-0.039	0.178	**-0.657** (0,000)**	1					
Mg^{2+}	0.118	0.224	0.236	0.149	-0.007	0.243	1				
Cu^{2+}	0.043	0.202	0.205	-0.077	**0.552** (0,000)**	-0.369**	0.073	1			
Fe^{2+}	-0.044	0.233	0.270*	0.135	0.097	0.001	0.054	0.165	1		
Mn^{2+}	0.444**	0.060	0.012	-0.084	0.368**	-0.150	0.030	0.174	0.130	1	
Zn^{2+}	-0.267*	0.089	0.347**	0.090	0.027	-0.150	-0.068	0.034	0.173	0.069	1

** Highly significant correlation <0.01 ; * Significant correlation <0.05

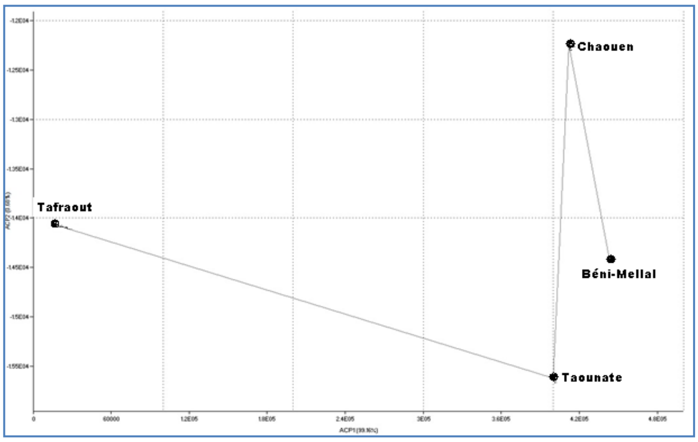

Figure 8. Graphical representation of principal component analysis of the studied regions

Figure 8 shows that there is approximately 99.84% of total variability grouped on the two successive axes (PCA1 and PCA2) and the minimum associated principal component analysis spanning tree, we can observe that:

✓ The Beni-Mellal region is very close to the Chaoun region for the parameters studied.

✓ The Chaoun region is close to the Taounate region for the parameters studied.

✓ The 3rd region is Tafraout is very far from the Beni-Mellal, Chaoun and Taounate regions for the studied parameters.

3.4.4.2. Principal component analysis of the studied samples

The principal component analysis result for the samples studied of carob pulp from four regions (BM, CH, TAF and TAO) represented by Figure 9. This figure shows that there is 93.27% of variability explained by the two axes (PCA1 and PCA2). The intra-region samples are associated in distinct groups (A, B, C) with a group D comprising samples of the different regions overlapping each other.

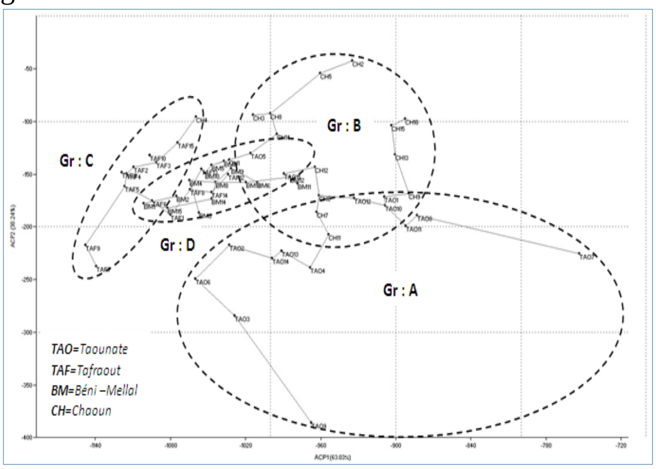

Figure 9. Graphic representation of Principal component analysis of the studied samples

3.4.5. Dendrogram of the regions, samples and variables studied

3.4.5.1. Dendrogram of the studied regions

The figure 10 represents the dendrogram of the studied regions Beni-Mellal, Chaoun, Tafraout and Taounate, it shows there are approximately 94% and 75% of similarity respectively between region Beni-Mellal and Chaoun, and between Taounate and Tafraout. This confirms the result of the previous analysis which explains the grouping of the regions and the samples studied.

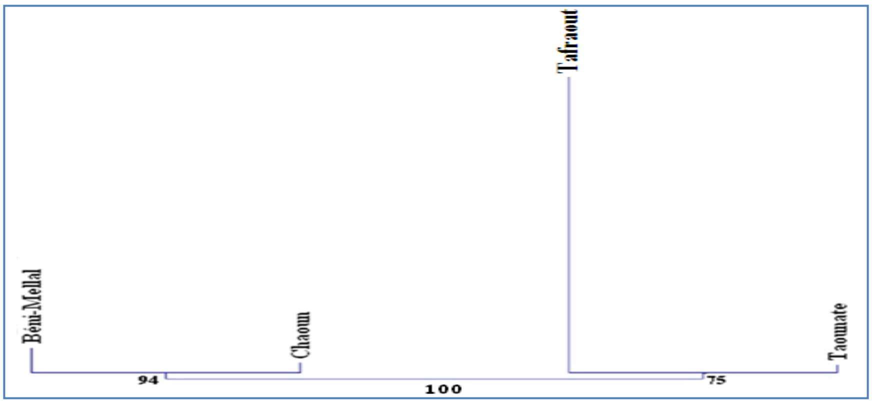

Figure 10. Dendrogram of the studied regions

3.4.5.2. Dendrogram of the studied samples

The figure 11 represents the dendrogram of the studied samples of the carob pulp from four studied regions. We obtained inter-mixed associative groupings, which are composed of three clusters: first cluster is subdivided into two sub-groups which contain samples from the Taouante, Chaoun and the Beni-Mellal region with different similarities. The second cluster made up of Tafraout and Beni-Mellal region samples who are correlated with each other with different resemblances. The last cluster also subdivided into two sub-groups which contain samples from the Taouante, Tafraout region and the Beni-Mellal region with different similarities. This order of correlation was confirmed by the result of the previous PCA analysis of the samples studied.

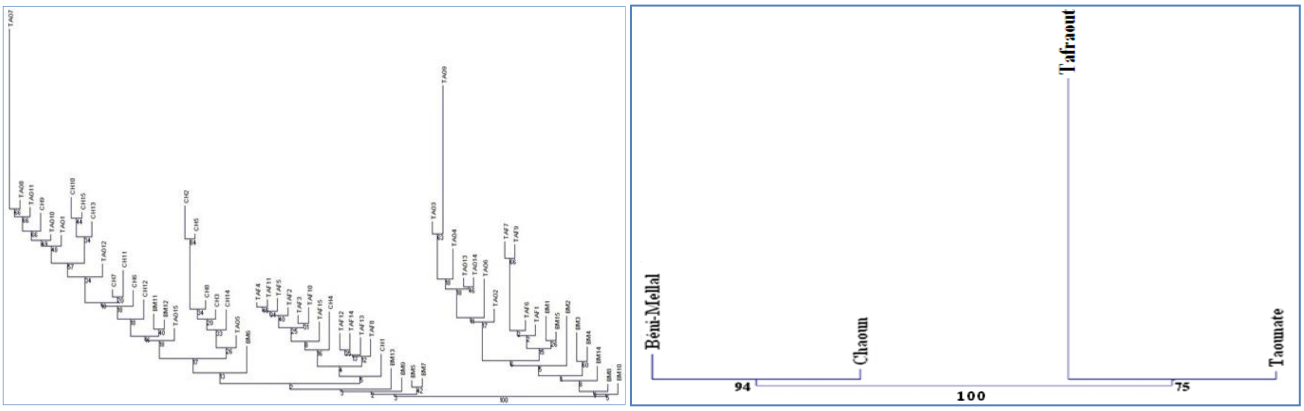

Figure 11. Dendrogram of the studied samples

3.4.5.3. Dendrogram of the studied variables

The figure 12 represents the dendrogram of the studied variables such as the moisture content, the ash content and the mineral composition, it presents the hierarchical classification of the different variables studied. Two main classes can be distinguished:

- The first is composed only of the moisture content.
- All the other variables (Ac, K^+, Ca^{2+} and Na^+) are gathered in the second class.

The Principal component analysis and dendrograms show that the carob populations from four regions studied in Morocco are grouped independently and far from their geographic origin (Beni-Mellal, Chaoun) and (Taounate, Tafraout). These results are similar to those observed in two studies on the Tunisian and Moroccan carob populations [36, 37]. This result can be explained either by the presence of a common genetic base between the Moroccan carob populations or by the influence of ecological factors or by the exchange of plant material between regions by farmers [37].

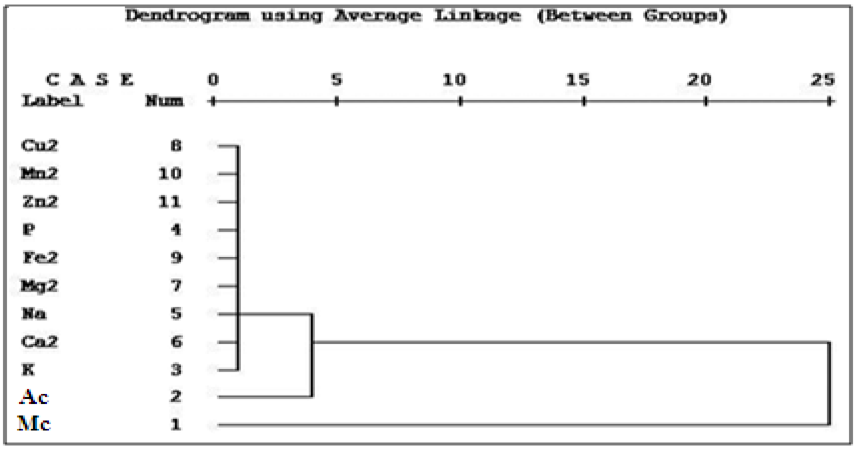

Figure 12. Hierarchical representation of the studied variables

Conclusion

The study of the evaluation of the content, the ash content and the mineral composition of the carob populations from four regions (Beni-Mellal, Chaoun, Tafraout and Taounate), revealed that there are differences statistically significant in these contents. The contents of mineral elements (P, K^+, Na^+, Ca^{2+} and Mg^{2+}) and the trace elements (Fe^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+}) obtained in our study are within the ranges of values found by several researchers in other countries.
The results of this contribution show that the Taounate region is the elite region, since their carob pulp is rich in minerals such as potassium, phosphorus, sodium, iron and zinc. On the other hand, the Tafraout region is also rich in calcium and magnesium, while the Beni-Mellal region is rich in copper and manganese, as well as the Chaouen region has average contents of the studied mineral elements. This research confirms that Moroccan carob has good nutritional value and can be an alternative source, in the food industry and in certain foods such as cocoa or jam, molasses and ice cream or used in medicine as an antioxidant.

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