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Biochemical Diversity of Wild Carob Tree Populations and Its Economic Value

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1. Introduction

Carob tree *Ceratonia siliqua* L. has been included in a national list of priority forest genetic resources for conservation and management in Tunisia (Bouzouita et al., 2007). The reduction of the biochemical diversity of this species in this region is a real risk. The species belongs to the Cesalpinaceae sub-family of the family Leguminoseae (syn Fabaceae).

It is an evergreen tree that is widespread in the Mediterranean basin. The species is mainly used for animal feeding and pharmaceutical and food industries (Yousif & Alghzawi, 2000; Tous et al., 2006; Silanikove et al., 2006).

Carob pods provide two important products: carob kernels from which carob or locust bean gum (LBG) is extracted, and carob kibbles or the remaining pulp obtained after the removal of the kernels. This can be used directly in animal and human nutrition (Mhaisen, 1991; Silanikove et al., 2006) or as a raw material for industrial processing (Carlson, 1986).

Pods are characterized by a high sugar content with about 75% of those sugars are sucrose (Biner et al., 2007). Carob kernels (10-20% of the fruit weight) contain principally galactomannans (Egli, 1969; McCleary & Matheson, 1976). The Locust bean gum is the ground endosperm of seeds; owing to its remarkable water-binding properties, it is widely used to improve food texture (Imeson, 1997). Gum-aqueous solutions have a high viscosity and are used as a substitute for pectins, agar or other mucilagenous substances (Fulgancio et al., 1982). The purification of polysaccharides improves this situation; unacceptable flavours of the crude gums are removed and the purified gums give clear and more stable solutions due to the elimination of impurities and endogenous enzymes. There are several methods for purificated crude galactomannan samples. Precipitation with ethanol has been largely used (Doublier, 1975). Purification by methanol (Rafique & Smith, 1950) and by copper

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(McCleary & Matheson, 1976) or barium complexes (Kapoor, 1972) has also been used; isopropanol is the best method for industrial processes (Bouzouita et al., 2007).

The aim of this study is to assess the variation of physical and chemical traits (moisture, ash, pH, protein, acid-insoluble matter, fibres, total phenols, sugars contents and mannose/galactose ratio of crude samples) among Tunisian natural populations. The rheological behaviour of the purified and crude bean gum is also assessed. Our study is an extension of those performed to assess the genetic diversity of Tunisian carob populations (Afif et al., 2006) (via izozymes and molecular markers) in order to elaborate conservation and product improvement strategies.

2. Methods

2.1 Analysed populations and sampling

Twelve Tunisian wild carob populations were analysed (Figure 1).

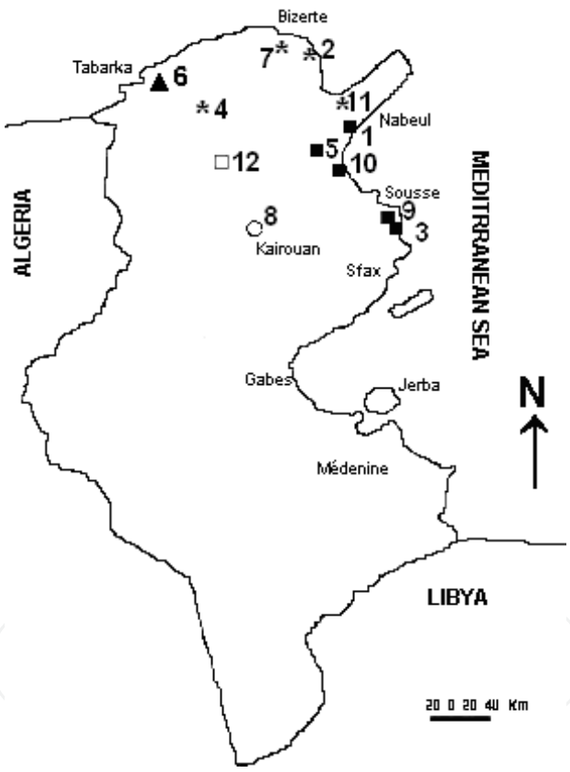


Fig. 1. Location of Tunisian carob populations analysed in this study.
Numbers indicate populations: **1:** Hammamet**; 2:** Gharelmelh**; 3:** Sayada**; 4:** Slouguia**; 5:** Jradou**; 6:** Ainessobh; **7:** Menzel Bourguiba**; 8:** Chbika**; 9:** Khnis; **10:** Enfidha**; 11:** Slimen**; 12:** Bargou**.**
▲: Low humid; *: Sub humid; □: Upper Semi arid; ■: Low Semi arid; ○: Upper arid.

Populations belong to the upper arid, low semi arid, sub humid and low humid bioclimates according to Emberger's (1966) pluviothermic coefficient (Q2) 17. Pods were collected during the period of July-September 2009. Five to 10 trees per population sampled at random were considered, and 25 randomly selected mature pods per tree were analysed.

2.2 Experimental methods

2.2.1 Chemical analysis

Mature pods collected in each population were air dried for 4 weeks and kept at 4°C before analyses. Pod pulp and kernels were ground in particles less than 500µm and 180µm respectively. The resulting powder: pulp (p) and non purified gum (g) powder was analysed for moisture (Mp, Mg), ash contents (Ap, Ag) and pH (pHp, pHg) according to Food Chemical Codex. The total nitrogen was evaluated by the Kjeldahl's method. Crude protein (Prp, Prg) contents were calculated by multiplying the nitrogen content by 6.25 (Anderson, 1986). Total phenols (Php, Phg) in pulp and non purified locust bean gum (LBG) were determined by Folin-Ciacolteu's method. Neutral Detergent Fibre (NDFp, NDFg) and Acid Detergent Fibre (ADFp, NDFg) were estimated according to AOAC method.

D-glucose (Gp), saccharose (Sp) and D-fructose (Fp) in pods pulp were determined by enzymatic kits and the determination of Optic Density at 750nm with spectrophotometer (UV/Vis).

To assess mannose and galactose ratios (M/G), twenty millilitres of H_2SO_4 (1M) were added to 400 mg of carob bean gum (purified and crude gum). After boiling, the mixture was cooled and treated with $BaCO_3$ and adjusted to pH 7, filtered and evaporated (30°C and 50°C) to obtain crystalline residue or syrup. The precipitate was dissolved in 2ml of desionised water and analysed by HPLC using an Agilent HP chromatograph, series 1100 with waters differential refraction detector and equipped with a Pb^{2+} column at 80°C.

The contents of galactomannan were deduced from a calibration curve obtained from five synthetic mixtures of β-D-galactose and β-D-mannose in known proportions.

2.2.2 Purification of the gum

The required amount of the powdered gum was gradually added to highly stirred distilled water. The precipitation is initiated at 13g.l-1 concentrated aqueous solutions. The solution was moderately stirred at different temperatures (from 25°C to 80°C) for 90 minutes. The resulting solution was allowed to cool and then centrifuged at 21875 g at 19°C. The obtained visquous supernatant corresponding to the crude LBG solution was separated for its use in the purification process. The solubilized galactomannan was precipitated from crude LBG solution by pouring into a two-volume excess of isopropanol. The precipitate was collected by filtration and lyophilisated for 5h at 40°C, 3 h at 5°C and for 3 days at 20°C, respectively.

2.2.3 Viscosity determination

The rheological properties were assessed by the measurment of the viscosity of the crude and purified LBG solutions, using an Ares Advanced Rheometric Scientific, with a coaxial cylindrical system rheometer which works under both dynamic and static regimes. The samples were sheared in the gap between the fixed inner cylinder (U = 10.1 mm, h = 61.4 mm) and the rotated mobile outer cylinder (U = 11.5 mm) and sample volume is 12.0cm3. To prepare 2% and 1% aqueous solutions of crude and purified gum, we add to 360ml distilled water, 8g and 4g respectively of crude and purified gum. The solution was mixed at 25°C for 15 minutes and at 90°C for 15 minutes and then remained at ambient temperature. The viscosity was measured at different shear rates.

2.3 Statistical analysis

The heterogeneity for each measured parameter among populations was tested using variance analysis with one effect (population or bioclimate effect) using the program SAS, procedure ANOVA (SAS, 1990). Averages of parameters were compared using Duncan's multiple range test (at $P<0.05$).

A principal component analysis (PCA) based on all considered parameters was performed to assess the divergence among populations, using the program MVSP version 3.1 (Kovach, 1999). Cluster analysis was performed on the matrix of Euclidean distances for the estimation of the physico-chemical variability distribution among populations and ecological groups. The divergence among populations and their locations (bioclimatic zones) was assessed by Euclidean distances calculated between pairs of populations and by the construction of a dendrogram generated from these distances using the UPGMA (Unweighted Pair Group Method with Arithmetic Averages) method. The distances matrix determined by cluster analysis was correlated with those of geographic distances.

3. Results

3.1 Variation of pulp parameters

Averages of the analysed parameters are presented in table 1. A significant variation ($P<0.001$) was observed among populations for all examined parameters.

Pods had low moisture (158 g kg $^{-1}$) and values of Mp varied from 93 g kg $^{-1}$ (population 9) to 234 g kg $^{-1}$ (population 5). The crude protein contents ranged from 2.8 g kg $^{-1}$ (population 4) to 7.1 g kg $^{-1}$ (population 5). Acid and Neutral Detergent Fibres contents showed high proportions, they ranged from 2.4 g kg $^{-1}$ to 3.7 g kg $^{-1}$ and from 2.8 to 3.9 g kg $^{-1}$, respectively. Population 8 from the low semi-arid bioclimate showed the lowest values of NDF and ADF, while the upper arid population 7 and the low semi-arid population 11 showed the highest ones.

The average of Ash equalled 0.21 g kg $^{-1}$. Populations were subdivided into two groups without apparent correlation to geographic or bioclimatic locations of populations. The first group showing low values (0.15 to 0.18 g kg $^{-1}$) included populations 2, 3, 7 and 8 and the second group included the other populations which were characterized by high Ash values.

The amounts of phenols varied highly among populations. Populations 5 (2.3 g kg $^{-1}$), 4 (2.1 g kg $^{-1}$), 12 (2.1 g kg $^{-1}$) and 11 (2.0 g kg $^{-1}$) had the highest amount of phenols while the group of populations 7, 1, 9, 3 and 8 showed the lowest values.

Regarding sugar content, it is obvious that carob pulp contained high levels of sugar: 2.8 g kg $^{-1}$ sucrose, 0.36 g kg $^{-1}$ D-glucose and 0.46 g kg $^{-1}$ D-fructose. The level variation of sugars contents among populations was higher than that for the other constituents. In fact, 8 groups of populations were observed.

3.2 Variation of locust bean gum parameters

The crude locust bean gum has an average of 0.27 g kg $^{-1}$ Ash (Ag). A high variation among populations was revealed (table 2), with populations 11, 6 and 12 showing the highest values (0.28 to 0.31 g kg $^{-1}$).

Population code	Mp		AIMp		Ap		Prp		php		Fibre				Sugar					
											ADFp		NDFp		Gp		Sp		Fp	
1	109	e	2.0	bc	0.24	a	3.5	c	1.1	e	3.1	c	3.8	b	0.44	c	4.4	a	0.46	e
2	187	cd	2.1	ab	0.18	b	4.4	b	1.9	cd	2.8	e	3.4	e	0.47	a	2.7	h	0.55	d
3	94	e	1.6	de	0.17	b	5.1	b	0.9	e	3.0	cd	3.5	d	0.37	e	2.9	f	0.38	i
4	212	b	1.6	e	0.22	a	2.8	c	2.1	b	2.8	e	3.5	c	0.35	f	3.3	b	0.66	a
5	234	a	1.6	de	0.23	a	7.1	a	2.3	a	3.5	b	3.6	c	0.44	c	3.0	d	0.45	f
6	171	d	1.9	bcd	0.25	a	6.5	a	1.7	d	2.6	f	3.6	c	0.13	c	3.2	c	0.28	k
7	108	e	1.7	de	0.16	b	4.9	b	1.1	e	2.5	g	3.0	i	0.24	i	1.9	l	0.56	b
8	108	e	1.8	cde	0.15	b	3.4	c	1.1	e	2.4	g	2.8	j	0.46	b	2.3	j	0.44	f
9	93	e	1.8	cde	0.22	a	6.4	a	0.9	e	3.1	cd	3.2	g	0.34	g	2.8	g	0.36	j
10	174	d	2.0	b	0.21	a	3.5	c	1.7	d	3.7	a	3.1	h	0.31	h	3.0	e	0.39	h
11	195	bc	1.9	bcd	0.23	a	3.7	c	2.0	bc	3.0	d	3.3	f	0.37	e	2.5	i	0.54	c
12	209	b	2.3	a	0.22	a	4.6	b	2.1	b	3.4	b	3.9	a	0.42	d	2.3	k	0.43	g
Over all popuations	**15.8*** **		**1.9*** **		**0.21*** **		**4.7*** **		**1.6*** **		**3*** **		**3.4*** **		**0.36*** **		**2.9*** **		**0.46*** **	

Table 1. Average of the pulp measured parameters (g kg $^{-1}$) for populations.
Values with different letters in the same trial column differ significantly (P<0.05), *** Highly significant at p<0.001.
Mp: Moisture, pHp: pH, AIMp: Acid insoluble matter, Ap: Ash, Prp: Protein, ADFp: Acid Detergent Fibre, NDFp: Neutral Detergent Fibre, php: Total phenols, Gp: D -Glucose, Sp: Sucrose, Fp: D-Fructose.

The content of crude bean gum protein (Prg) varied from 1.6 g kg $^{-1}$ (population 3) to 1.9 g kg $^{-1}$ (population 5) with an average of 1.7 g kg $^{-1}$, and an average of 1.1 g kg $^{-1}$ acid insoluble matter (AIM)g.

Six and eight groups of population were distinguished respectively for crude bean gum ADF and NDF. The ADFg values (1 g kg $^{-1}$) varied between 1.3 g kg $^{-1}$ (population 3) and 0.8 g kg $^{-1}$ (population 7), while those NDFg (3.5 g kg $^{-1}$) ranged from 3.9 g kg $^{-1}$ (population 8) and 2.8 g kg $^{-1}$ (population 3).

The average of moisture content of crude LBG (80 g kg -1) was lower than that of carob pulp, the pH pulp value (5.2) was less than the pH of LBG (6.0). Both pulp and locust bean gum were remarkably rich in NDF (3.4 and 3.5 g kg $^{-1}$ respectively).

The mannose/galactose ratios (M/Gg) varied among gum samples (Table 2). Crude LBG samples showed higher M/Gg ratios. The highest ratio (M/Gg= 4.2) was observed for

Population code	Mg		AIMg		Ag		Prg		phg		Fibre				Yg		M/G ratio			
											ADFg		NDFg				M/Gg		M/Gpg	
1	90	a	0.9	c	0.23	f	1.8	ab	2.1	b	0.9	g	3.4	C	389.9	abc	4.2	a	3.9	abc
2	79	cd	1.1	b	0.27	c	1.7	ab	1.3	e	1	d	3.5	f	388.8	abc	3.3	de	3.9	abc
3	78	d	1.2	a	0.27	c	1.6	c	1.2	e	1.3	a	2.8	f	340.2	bc	4	b	3.4	bc
4	78	d	1.1	ab	0.25	d	1.7	bc	2.1	b	1.1	b	3.6	g	337.9	bc	4	b	3.4	bc
5	78	d	0.8	d	0.25	de	1.9	a	1.9	c	0.8	h	3.2	e	435.3	a	4	b	4.4	a
6	83	c	1.1	b	0.29	b	1.8	a	1.7	d	0.9	e	3.8	f	412.0	ab	3.2	de	4.1	ab
7	87	b	1.1	ab	0.24	e	1.7	ab	1.2	e	0.8	h	3.5	b	453.7	a	3.5	c	4.5	a
8	71	e	1.1	ab	0.26	c	1.8	a	2.3	a	1.1	b	3.9	b	382.7	abc	3.9	b	3.8	abc
9	81	cd	0.9	c	0.26	c	1.6	c	1.9	c	0.9	ef	3.1	d	406.0	ab	3.3	d	4.1	ab
10	79	cd	1.1	b	0.27	c	1.7	bc	1.6	d	1.1	c	3.3	c	323.5	c	3.5	c	3.2	c
11	81	cd	1.1	ab	0.28	b	1.7	bc	1.5	d	0.9	fg	3.6	a	385.3	abc	3.9	b	3.9	abc
12	80	cd	1.1	b	0.31	a	1.7	abc	1.4	e	1.1	b	3	h	344.7	bc	3.1	e	3.5	bc
Over all populations	**80*** **		**1.1*** **		**0.27*** **		**1.7*** **		**1.7*** **		**1*** **		**3.5*** **		**383.4*** **		**3.67*** **		**3.83*** **	

Table 2. Average of the locust bean gum measured parameters (g kg $^{-1}$) for populations
Values followed by different letters in the same trial column differed significantly (P<0.05), *** Highly significant at p<0.001.
Mg: Moisture, pHg: pH, AIMg: Acid insoluble matter, Ag: Ash, Prg: Protein, ADFg: Acid Detergent Fibre, NDFg: Neutral Detergent Fibre, phg: Total phenols, Yg: Yield (% total gum. dry weight basis), Mannose/Galactose ratio of crude LBG: M/Gg, Mannose/Galactose ratio of purified LBG: M/Gpg.

population 1 (from the low humid climate). Populations 3, 4 and 5 (from sub humid) with 8 and 11 (from the low semi arid) showed also high M/Gg ratios but did not exhibit significant differences. Populations 7 and 10 had intermediate values. Populations 2, 6, 9 and 12 showed the lowest M/Gg ratios.

Purified LBG showed also higher M/Gpg ratio (3.83). Low and non significant differences among populations were observed, the population 10 showed the lowest value (3.2) and the populations 7 and 5 showed the highest values (4.5 and 4.4 respectively).

The level of ANOVA, which considered the twenty populations as five groups (according to bioclimate) indicated that all constituents (except protein Prg) differed significantly ($P < 0.05$). Variation observed between populations belonging to the same bioclimatic zone was not significant for all characters.

Populations from the sub-humid bioclimate had shown significant differences in all characters except ADFp pulp and ADFg gum contents (p=0.89 and p=0.26 respectively), gum moisture (p=0.38) and Mannose/Galactose ratio of pure gum (p=0.2). Within the Lower semi arid ecological group, the major parameters (13/24 parameters) were significantly different at $p<0.05$.

The principal component analysis showed that the first three axes explain 80.72% of the total variation. A specific meaning could be variables as follows:

- The first axis (48.35%) is correlated to Acid insoluble matter (AIMp), Neutral Detergent fibre (NDFp, NDFg), yield of gum (Yg) and Mannose/Galactose (M/Gp) of purified gum.
- The second axis explained 20% of total inertia and correlated to pH and phenols of pulp and gum (pHp, pHg, Php, Phg), glucose pulp content (Gp), acid insoluble matter gum amount (AIMg) and protein locust bean gum content (Prg).
- The third component, instead, explained 12.37% of total variance. Loaded variables were moisture, ash and acid detergent fibre contents of pulp and gum (Mp, Mg, Ap, Ag, ADFp, ADFg), saccharose (Sp), fructose (Fp), protein (Prp), and Mannose/Galactose (M/Gg) of crude gum and viscosity (Vg, Vp) of crude and purified gum

The plot according to axes 1-2 and axes 1-3 (Figure 2 and 3) showed a high dispersal of populations.

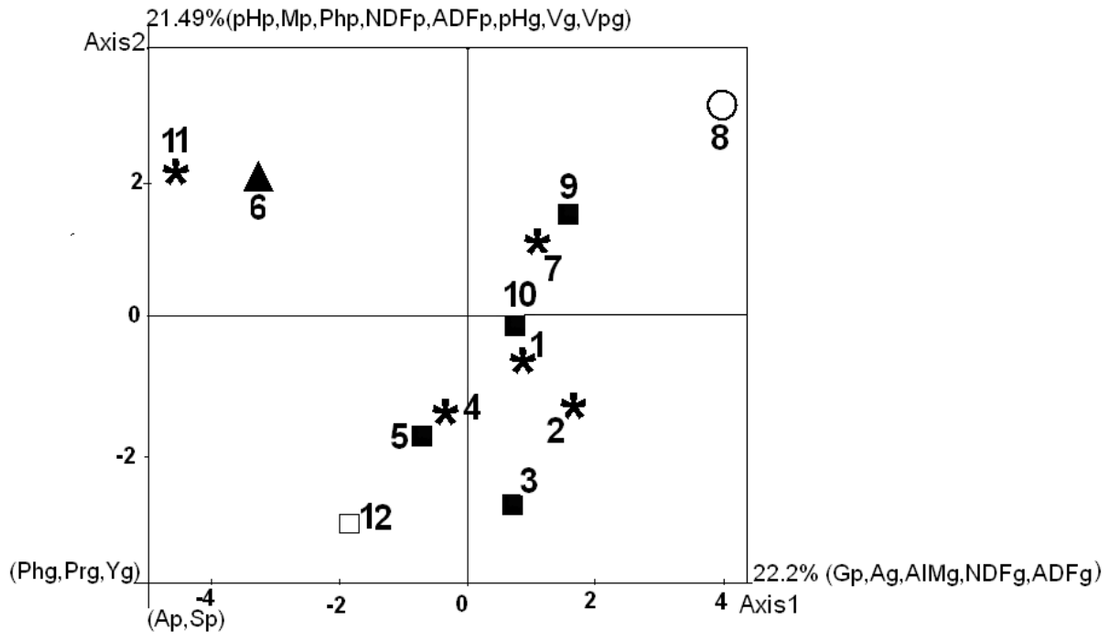


Fig. 2. PCA: Principal component analysis. Plot of the populations according to the first two components. Codes indicate populations. 1: Hammamet; 2: Gharelmelh; 3: Sayada; 4: Slouguia; 5: Jradou; 6: Ainessobh; 7: Menzel Bourguiba; 8: Chbika; 9: Khnis; 10: Enfidha; 11: Slimen; 12: Bargou.
▲: Low humid; *: Sub humid; ■: Low Semi arid; □: Upper Semi arid; ○: Upper arid.

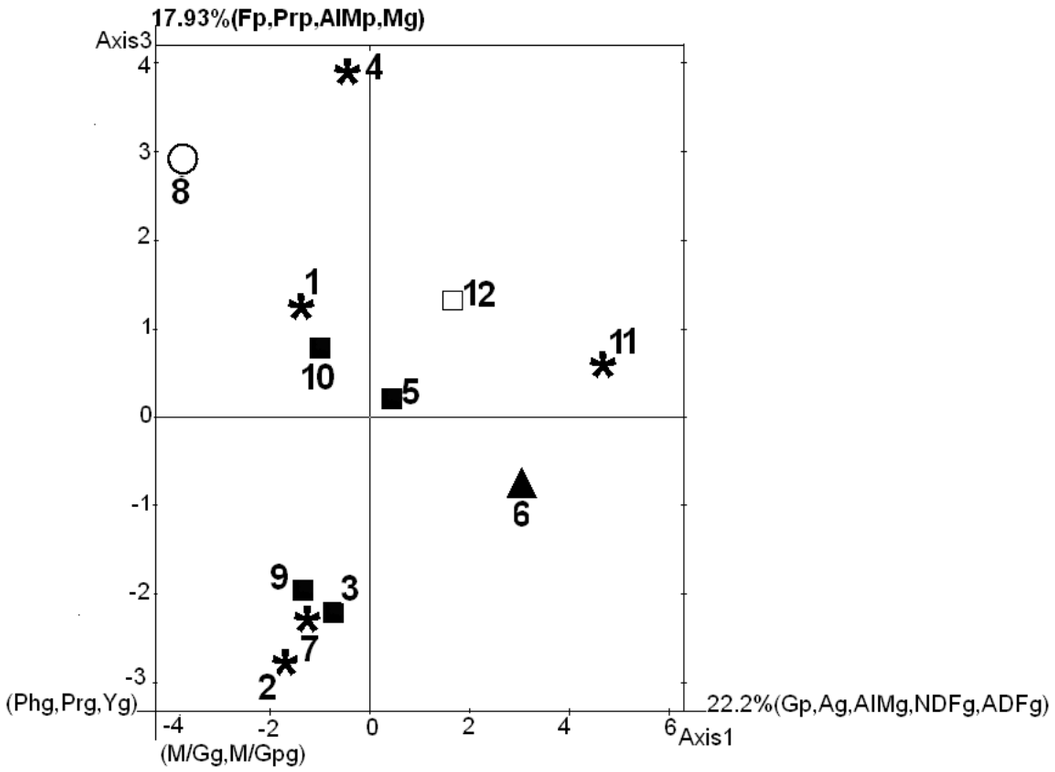


Fig. 3. PCA: Principal component analysis. Plot of the populations according to the first and the third components. Codes indicate populations. 1: Hammamet; 2: Gharelmelh; 3: Sayada; 4: Slouguia; 5: Jradou; 6: Ainessobh; 7: Menzel Bourguiba; 8: Chbika; 9: Khnis; 10: Enfidha; 11: Slimen; 12: Bargou.
▲: Low humid; *: Sub humid; ■: Low Semi arid; □: Upper Semi arid; ○: Upper arid.

According to the axis 1, two major groups can be distinguished: the first includes populations from Low semi arid and populations from Upper semi arid zones, populations 7 and 6 from (the same bioclimate) were separated according to axis 2; the second on negative side of axis 1 is constituted by populations from Sub humid and upper arid zones, population 12 is well isolated from the others.

The dendrogram constructed using Euclidean distances (Fig. 4) showed four groups of populations:

- The first group is constituted by two populations (7 and 9) respectively from Upper and Low semi arid bioclimates. This group is located in the lower-right of the graph (Fig. 2) with positive values of the second and third components.
- The second group included 8 populations belonging to all bioclimates, two aggregates can be distinguished in this group; the first aggregate is made up of five populations (2, 3, 4, 5 and 6), all the populations clustered together according to their geographic proximity (Sub-humid bioclimate) except for the population 6 that belongs to the Upper semi arid bioclimate, the second aggregate included three populations (1, 10 and 11). In this aggregate, the population 1, belonging to the Lower humid bioclimate, was separated from populations 10 and 11 that belongs to the Low semi arid bioclimate.

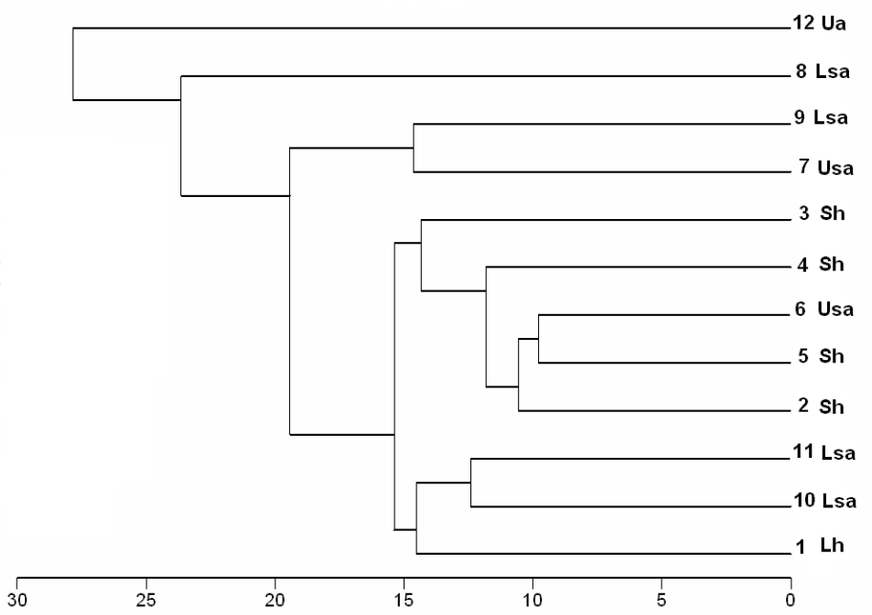


Fig. 4. Cluster analysis of the 12 populations of Carob based on Euclidean distance.
Codes indicate populations. 1: Ainessobh; 2: Hammamet; 3: Gharelmelh; 4: Menzel Bourguiba; 5: Slimen; 6: Slouguia; 7: Bargou; 8: Sayada; 9: Jradou; 10: Khnis; 11: Enfidha; 12: Chbika.
Bioclimatic zones: Lh: Low humid; Sh: Sub humid; Lsa: Low semi arid; Usa: Upper semi arid; Ua: Upper arid.

Populations 12 (from the Upper arid) was alone considered as group. Contrary to population 8, population 12 was located in the negative part of axis 3 with high and negative values of the second and third components (Figure3).

The plot of the Principal Component Analysis (PCA) of the *Ceratonia siliqua* individuals sampled showed that the first three axes described 79.7% of the total variation; The first, second and third axis explained respectively 52.22%, 16.36% and 11.11% of total inertia (Fig. 5 and 6).

Euclidean distances (D) between populations ranged from 9.78 to 41.78. Mean D value for all population was 20.97. The highest D-value was observed between populations 8 and 12 belonging respectively to the lower semi-arid and the upper arid bioclimatic zones and 139 km distant from each other. The lowest genetic distance (9.78) was recorded between populations 5 and 6 which are geographically close (10 km). However the correlation between geographic and Euclidean distance is not significant for all carob populations ($r^2 = 0.033$, $p = 0.168$) (Fig.5).

3.3 Yields of the gum

The extraction and purification processings were used to obtain the purified galactomannans with efficiency varying from 323.5 g kg^{-1} (population 10) to 453.7 g kg^{-1} (population 7). The average value was 383.4 g kg^{-1}. Populations 5, 2, 6, 8, 9 and 1 presented high yields (from 382.7 to 435.3 g kg^{-1}) but the values did not differ significantly.

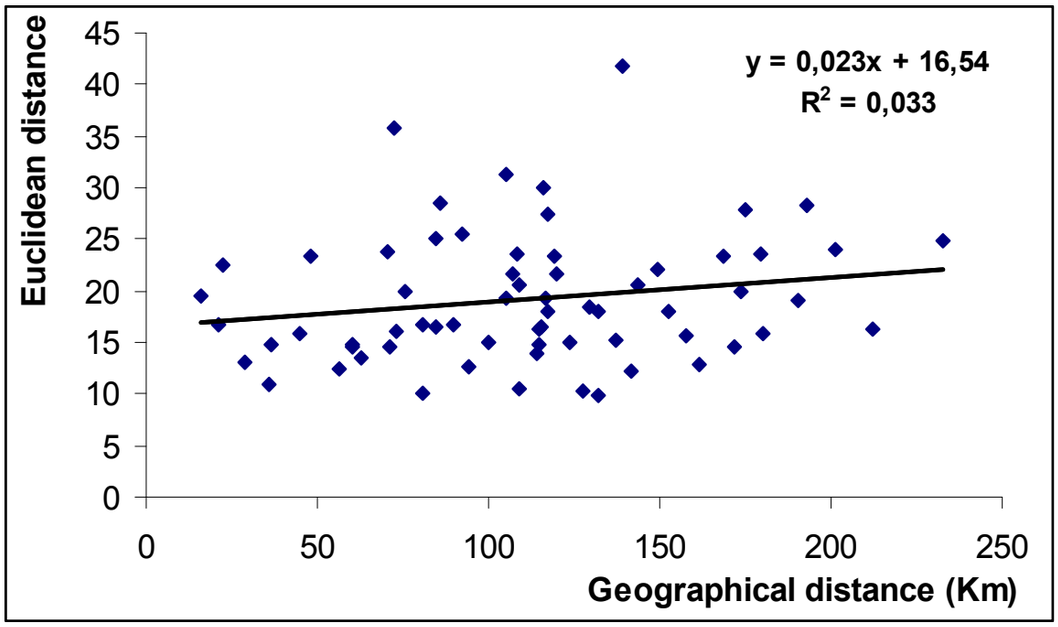


Fig. 5. Correlation between Euclidean distances and geographical distances (km) between all pair of populations.

3.4 Rheological characteristics

The flow curves of crude and purified LBG samples reported in figures 8 and 9 showed that all solutions had a shear-thinning behaviour showing that apparent viscosity decreased when the shear rate increased. The apparent viscosity at 6s-1 varied significantly from 0.04 to 4.78 Pa.s for crude LBG and from 0.07 to 4.83 Pa.s for purified LBG (Table 3), according to populations (P<0.001).

Population code	Crude Locust Bean Gum		Purified Locust Bean Gum	
1	4.37	b	4.6	a
2	1.55	f	1.8	d
3	1.65	f	1.78	d
4	2.95	d	3	c
5	4.78	a	4.82	a
6	0.04	h	0.07	f
7	1.25	f	1.45	d
8	2.17	e	2.7	c
9	0.81	g	0.9	e
10	3.5	c	3.8	b
11	3.42	h	4	f
12	1.45	f	1.72	d
Over all populations	**2.33*** **		**2.55*** **	

Table 3. Apparent viscosity (Pa.s) of crude and purified locust bean gum for the analysed populations. Values with different letters in the same trial column differ significantly (P<0.05)
*** Highly significant at p<0.001.

The best rheological properties were observed for populations 5 (4.78 Pa.s and 4.83 Pa.s respectively for crude and purified LBG) and 1 (4.38 Pa.s and 4.6 Pa.s respectively for crude and purified LBG). The lowest apparent viscosity at 6s-1 was obtained for population 6 with 0.04 and 0.07 Pa.s for crude and purified LBG respectively. Figures 6 and 7 show that purified LBG solutions had a higher shear-thinning (response of a fluid's viscosity to a shearing stress, that is, a force tending to make part of the fluid slide past another part) than the crude LBG solutions.

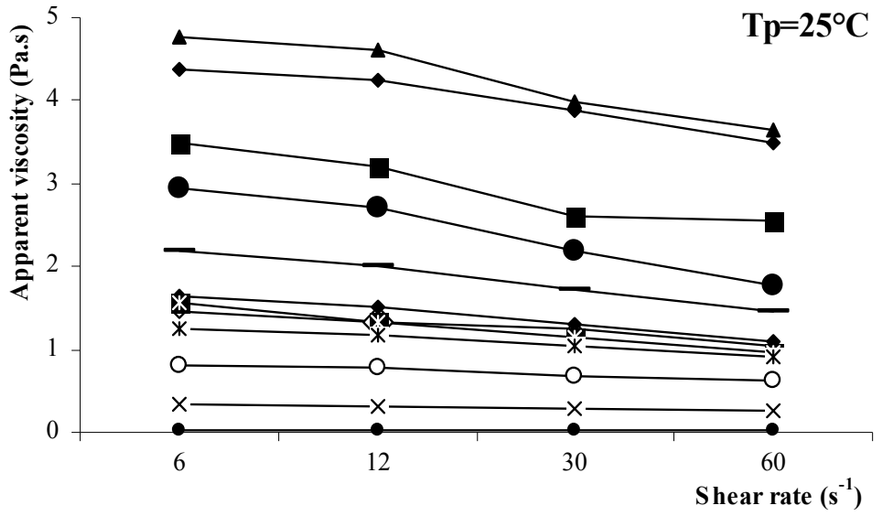


Fig. 6. Apparent viscosity of crude locust bean gum at different values of shear rate for samples from all populations.

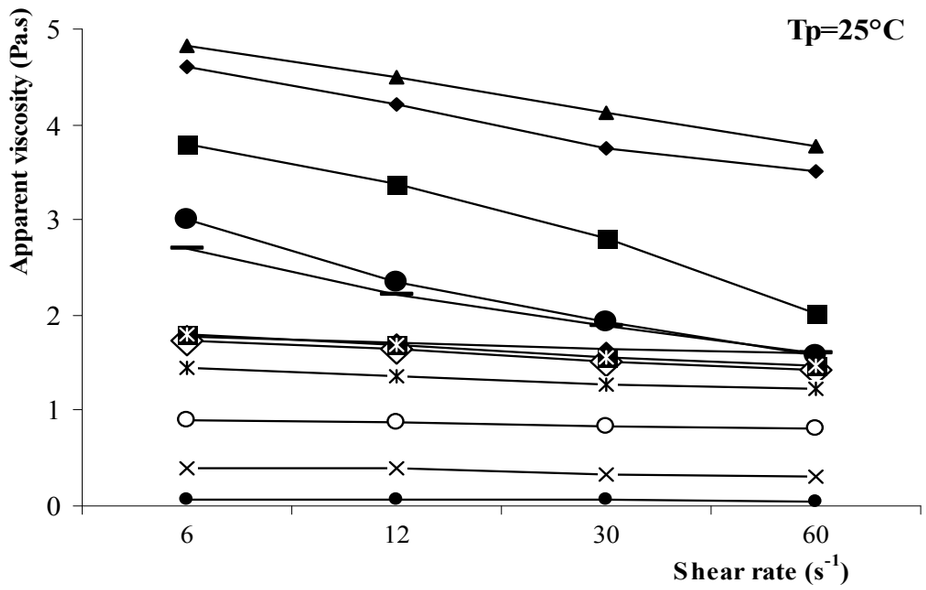
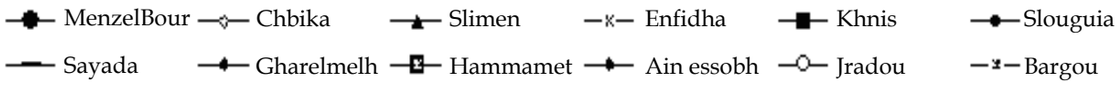


Fig. 7. Apparent viscosity of purified locust bean gum at different values of shear rate for samples from all populations.

Each symbol indicates a population:



4. Discussion

4.1 Biochemical properties

The moisture values of carob pulp from Tunisian natural populations were higher than those previously reported for carob collected from Portugal, Spain and Jordan (Blenford, 1979; Brandt, 1984; Albanell et al., 1991). Our results confirmed that, as reported by Lopes da Silva & Gonçalves (1990), the differences may be attributed to the difficulties experienced during the milling of carob pods. In our experiments, the level of variation differed according to population and bioclimate. The pH of carob pulp was lower than that reported in the literature, due probably to caramelisation during the powdering process and to the formation of by-products and other intermediates such as pyruvic acid (Lee et al., 1990).

Ash and protein contents showed significant differences among populations, yet the obtained values were similar to the FAO standard ones. The ash, phenol and protein values of carob pulp are lower than those of kernels farina or LBG. These results are consistent with earlier findings (Blenford, 1979; Youssif & Alghzawi, 2000). The low protein content may support the assumption that carob could be considered a natural healthy food (Bengoechea et *al*., 2008). On the other hand, carob pulp is free from the two anti-nutrients caffeine and theobromine (Craig and Nguyen, 1984) and has higher rates of crude fibre. Absence of caffeine might be considered an advantage, from a nutritional point of view, qualifying pulp as a good replacer or extender for cocoa powder in many food items. The moderate amount of phenols in pulp favored for its use as ingredient in animal feed (Tamir & Nachtomi, 1973) and dietetic products, or as a cocoa and sugar replacer in human food free from caffeine or theobromine. Individuals from populations such as populations 11, 7 and 8 could be selected to promote low phenol varieties.

Sucrose, fructose and glucose contents of pulp were high and found to differ significantly among populations. Sucrose is the dominant sugar found in carob. Our results are similar than those found by Biner *et al*. (2007).

The high yield or gum percentage is one of the important required parameters for carob industry, because locust bean gum was the first galactomannan used both industrially (paper, textile, pharmaceutical, cosmetic and other indus-tries) and in food products (ice cream and other preparations). In our experiments, the yield oscillated between 323.5 g kg^{-1} and 453.7 g kg^{-1} and showed significant differences between populations. Bargou followed by Slimen, Slouguia and Jradou were the populations with higher amounts of gum. In the literature, the yields were approximately between 290 g kg^{-1} and 460 g kg^{-1} (Andrade *et al*., 1999).

The apparent viscosity varied with shear rate. The behaviour is pseudoplastic at every shear rate, in agreement with other studies (Garcia & Casas, 1992). The rheological properties of locust bean gum did not depend only upon protein, but the most important factor was the galactomannan. The purification of the samples of crude locust gum eliminated practically all fat and fibre; the ash and protein contents may drastically be reduced (Bouzouita et al.,

2007). This diversity in biochemical composition of pulp and kernel and the physical propriety of the gum may allow plant breeders to develop improved varieties of carob which can economically benefit commercial cultivation.

The significant biochemical differences observed provide a basis for the selection of plant material with desired traits from different populations.

Biochemical characterisation of fruit quality offers new opportunities to evaluate important fruit postharvest traits (Rudell, 2010). Standardized trait evaluation among breeding programs and, most importantly, germplasm collections is expected to allow more precise comparison between populations, expediting integration of economically important fruit quality traits into commercial populations (Rudell, 2010).

The principal components analysis showed interesting relationships between pulp and locust bean gum characters. The group of populations 7 and 9 from Upper and Low semi arid bioclimates, respectively, was characterized by high pulp moisture, Ash, protein, phenols, acid insoluble matter and neutral and acid detergent fibre contents. The locust bean gum in these populations was rich on moisture, protein, acid insoluble matter and phenols contents and had higher rate of mannose /galactose. They also had a good quality of gum that is characterized by a high viscosity. The second heterogenic group contains populations 2, 3, 4 and 5 from the sub humid zone and the population 6 from the Upper semi arid bioclimate. This group showed fruits and gum with moderate characteristics.

Populations 1, 10 and 11, clustered together, were characterized by a high quality of gum with high yield and viscosity. Contrary to this group, population 12 (from the Upper arid) was characterized by fruits with higher contents of saccharose, fructose, glucose and protein and a gum rich only on ash and acid detergent fibre. The quality and viscosity of gum is not good, but population 8 (from the Lower semi arid zone) had a good quality of gum with high viscosity and mannose /galactose ratio. The pulp was characterized by very high pulp moisture, ash, protein, phenols, acid insoluble matter and neutral and acid detergent fibre contents. The locust bean gum was very rich on moisture, protein, acid insoluble matter and phenols contents.

The structuring of populations according to their biochemical characteristics depends on bioclimate rather than geographic distance between sites. The UPGMA clustering established for all populations through Euclidean distances did not clearly show that, for the majority of populations, grouping had resulted from geographic location.

The biochemical variation among populations could result both from genetic and environmental factors. However, the variation observed among populations from the same bioclimatic zone, for example populations from sub humid, suggests that at least some of this variation could be related to genetic factors. The cultivation of individuals (via cuttings or grafting) in the same conditions (clonal orchard) with mixed individuals (male and female) could help the interpretation of results and facilitate selection of select economically important cultivars.

4.2 Conservation

Fragmentation of carob habitats in Tunisia adds to the spatial isolation of populations, which may reduce gene flowand increase differentiation between them (Afif et al., 2006).

The decline of populations stemming from habitat fragmentation induced genetic bottlenecks and increased genetic drift (Booy et al., 2000) may lead, particularly in disturbed sites, to gradual reduction of individual fitness (Clegg, 1995) and further population decline

Analysis of biochemical diversity and the level of differentiation between Tunisian carob populations from different geographic and bioclimatic zones is the first step in developing in-situ and ex-situ conservation strategies. It also helps provide insight into the evolutionary and demographic history of the species, and identifies potential genotypes for industrial and pharmaceutical use.

In this study, biochemical analysis has been used to detect variation between populations, and to assess their differentiation level. We suggesttargeting for conservation and for intensive cultivation and industrial use the populations with high biochemical pulp and kernels quality (populations 7 and 9) and high yield of seeds and LBG (populations 1, 10 and 11). Populations 1 and 5 should be better protected because they have a good quality of gum.

Smalland isolated populations such as populations 8 and 12 are likely the most threatened.oWe suggest that 1) these carob populations be fully protected by land use authorities, and 2) that additional populations be established through transplants to nearby suitable habitat areas.

In-situ conservation strategies should strive to preserve high biochemical diversity found in wild populations. Conservation strategies should be implemented that take into account the varied environmental conditions of each bioclimatic zone.

Our research is based not only on biochemical analysis, but also on observed high phenotypic variability within populations (size and number of seeds per pod, pod size, etc.). However, correlation between these quantitative traits or quantitative traits and molecular markers should be investigated. The joint examination of all these traits is highly recommended for the conservation of genetic resource species (Sagnard et al. 2002). Further genetic diversity analyses combining biochemical and adaptive traits are needed to assist in developing more fully conservation and management strategies for the species.

The analysis of biochemical diversity of Tunisian carob populations has led to useful information which could help preserve the genetic diversity of the species. Ex-situ preservation should be based on a maximum number of individuals collected within populations in each ecological group and their propagation in different bioclimates by means of cuttings.

5. Conclusion

The nutritive value of different fractions of carob pods and kernels was evaluated by their chemical composition. There were significant biochemical differences among the fractions of carob pods in relationship with bioclimatic location of populations.

Carob pulp was characterised by high sugar content, relatively moderate protein content compared with kernels farina. Additionally, it was established by Craig et al. (1984) that carob is free of the two anti-nutrients found in cocoa (theobromine and caffeine).

The data obtained in this study show the high variability of the carob pods and kernels collected in different areas of Tunisia. These results suggest the importance of preserving the

genetic resources of carob to elaborate improvement programs, with the aim of the clonal selection with highest yield of seeds and LBG for intensive cultivation and industrial use.

Development of varieties of carob trees better suited to actual and future demands of industry must take in consideration a strategy to prevent genetic erosion in the wild. Evaluation of biodiversity in non-grafted or wild carob trees throughout the region is fundamental to successful conservation.

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7. References

Afif, M., Benfadhl, N., Khouja, M.L. & Boussaid, M. (2006). Genetic diversity in Tunisian C*eratonia siliqua* L. (*Cesalpinioideae*) natural populations. *Genetic Resources and Crop Evolution,* 53, 1501-1511.

Albanell, E., Caja, G. & Plaixatas, J. (1991). Characteristics of Spanish carob pods and nutritive value of carob kibbles. *Options méditerranéennes,* 16, 135-136.

Anderson, D.M.W. 1986. Food Additives and Contaminants 3: 231-234.

Andrade, C.T., Azero, E.G., Luciano, L. & Gonçalves, M. P. (1999). Solution properties of the galactomannans extracted from the seeds of *Caesalpinia pulcherrima* and *Cassia javanica*: comparison with locust bean gum. *International Journal of Biological Macromolecules,* 26, 181-185.

AOAC. (1984). Official Methods of Analysis (13th edition). Association of Official Analytical Chemists, Washington DC. USA.

AOAC. (1990). Official Methods of Analysis (15th edition). Association of Official Analytical Chemists, Washington DC. USA.

Bengoechea, C., Romero, A., Villanueva, A., Moreno, G., Guerrero, A., Puppo, M.C. (2008). Composition and structure of carob (*Ceratonia siliqua* L.) germ proteins. . *Food chem*., 107:675- 683

Biner, B., Gubbuk, H., Karhan, M., Aksu, M. & Pekmezci, M. (2007). Sugar profiles of the pods of cultivated and wild types of carob bean (*Ceratonia siliqua* L.) in Turkey. *Food Chem, 100*, 1453-1455.

Blenford, D.E. 1979. Toasted carob powder. *Confectionery Manufacture and Marketing, 16*, 15-19.

Booy, G., Hendriks, R.J.J., Smulders, M.J.M., Van Groenendal, & D. Vosman B. (2000). Gene diversity and the survival of populations. *Plant Biol*. 2: 379–395.

Bouzouita, N., Khaldi, A., Zgoulli, S., Chebil, L., Chaabouni, M. & Thonart, P. (2007). The analysis of crude and purified locust bean gum: A comparison of samples from different carob tree populations in Tunisia. *Food chem*., 101, 1508-1515.

Brandt, E. (1984). Carob. *Nutr and food Sc, 91*, 22-24.

Carlson, W.A. (1986). The carob: evaluation of trees, pods and kernels. Intl. *Tree Crops J*, 3,281-290.

Clegg, M.T. (1995). Conserving biological diversity: an evolutionary perspective. *Calif. Agric*. 49: 34–39.

Craig, W.J. & Nguyen, T.T. (1984). Caffeine and theobromine levels in cocoa and carob products. *Journal of Food Sc*, 49, 302-306

Doublier, J. L. (1975). Propriétés rhéologiques et caractéristiques macromoléculaires des solutions aqueuses de galactomannanes. *PhDThesis*. Université Paris VI France.

Egli, W. (1969). Origin, characteristics, and possible applications of plant hydrocolloids, particularly galactomannan. *Schweizerusche Zeitschriftfuer Obst-und Weinbau, 105*, 470- 477.

Emberger, L. (1966). *Une classification biogéographique des climats*. Recherches et Travaux de Laboratoires de Géologie, Botanique et Zoologie. Faculté des Sciences de Montpelier (France), *7*, 1-43.

Food and Nutrition Board-National Research Council. (1981). *Food chemicals codex*. Washington, DC: National Academy Press.

Fulgancio, S., Calixto, F.S. & Canellas, J. (1982). Components of nutritional interest in carob pods *Ceratonia siliqua*. *Journal of the Science of Food Agr, 33*, 1319-1323.

Imeson, A. (1997). *Thickening and gelling agents for food* (2nd ed.). New York: Culinary and Hospitality Industry Publication Services.

Kapoor, V.P. (1972). *Phytochem, 11*, 1129- 1132.

Lee, C.Y., Kagan, V., Jawarski, A.W. & Brown, S.K. (1990). Enzymatic browning in relation to phenolic compounds and polyphenol oxidase activity among various peach cultivars. *Journal of Agricultural and Food Chem, 88,* 99-101.

Lopes da Silva, J. A. & Gonçalves, M. P. (1990). Food Hydrocolloid, *4*, 277–287.

McCleary, B. V. & Matheson, N. K. (1976). Galactomannan utilization in germinating legume seeds. *Phytochem, 15*, 43–47.

Mhaisen, A. (1991). Cocoa tree. *Agric. Eng*. 43, 90–91.

Rafique, C.M. & Smith, F. (1950). Processing and characterization of carob powder. *Food Chemistry, 69*, 283-287.

Rudell, D. (2010). Standardizing postharvest quality and biochemical phenotyping for precise population comparison. *Hortsc*, 9, 1307-1309

Sagnard, F., Barberot, C. & Fady, B. (2002). Structure of genetic diversity in Abies alba Mill. From southwestern Alps: multivariate analysis of adaptive and non-adaptive traits for conservation in France. *Forest Ecol. Manage*, 157: 175–189.

Silanikove, N., Landau, S., Or, D., Kababya, D. & Nitsan, Z. (2006). Analytical approach and effects of condensed tannins in carob pods (*Ceratonia siliqua*) on feed intake, digestive and metabolic responses of kids. *Livestock* Sc, *99*, 29-38

Tamir, M. & Nachtomi, E. (1973). Urinary phenolic metabolites of rates fed carobs (*ceratonia siliqua*) and carob fractions. *Int J Biochem*, 3, 123-124.

Tous, J., Rovira, M., Romer, A., Afif, M., Khouja, M.L., Naghmouchi, S. & Boussaid, M. (2006). Carob tree germplasm in Tunisia. FAO-CIHEAM. *Nucis newsletter,*13, 55-59.

Tous, J., Rovira, M., Romer, A., Afif, M., Khouja, M.L., Naghmouchi, S. & Boussaid, M. (2006). *Estudio de poblaciones de algarrobo en Tunez*. III congreso de mejora genetica de plantas Valencia. *Actas de horticultura, 45*, 233-236.

Yousif, A.K. & Alghzawi, H.M. (2000). Processing and characterization of carob powder. *Food Chem, 69*, 283–287.

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Conservation biology is called a "crisis discipline." In a world undergoing rapid change, this science informs us about research, technologies, management practices, and policies that can help protect the earth's naturally-occurring biological diversity. The six chapters of this book provide insightful analysis on managing protected areas (Middle East), conserving biochemical and genetic diversity of carob tree (Tunisia) and wild pear (Japan), determining the health status of Amazon manatee, manipulating sex ratios to benefit wildlife, and narrowing the gap between religion and conservation. The authors approach threats to biological diversity from varied angles, reflecting the interdisciplinary nature of the field. This book offers room for reflection on the definition and utility of the word 'natural' on a planet now overwhelmingly dominated by people.

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