Journal of Food and Nutrition Research, 2017, Vol. 5, No. 4, 253-261
Available online at http://pubs.sciepub.com/jfnr/5/4/7

DOI:10.12691/jfnr-5-4-7

Physicochemical Properties of White (*Morus alba*) and Black (*Morus nigra*) Mulberry Leaves, a New Food Supplement

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Abstract Mulberry foliage is used to feed silkworms and others herbivorous animals due to it is highly nutritious and palatable. A large number of studies have shown the phenolic profiles of mulberry leaves. This study investigated chemical and morphological characterisation of mulberry clones leaves of *Morus alba* and *Morus nigra*. Fresh leaves samples of seven mulberry clones, four white (*Morus alba*) three black (*Morus nigra*) clones were manually collected, after collection, the samples were washed with distilled water and lyophilized. Morphological characterisation generally showed the entire leaf, almada type, base was emarginated, coined and retasa, leaf margin was acutado-serrated. Leaf weight of mulberry species ranged from 2 to 3.4 g, and peduncle length, from 37.7 to 55.9 mm. The main mineral elements in both species were Ca followed by N, K and Mg, but the in leaves of *M. alba* had slightly higher content than *M. nigra*, and all clones showed high concentrations of Fe, while sodium was 0.01 g/100 g dw in all the clones. The protein, and crude fiber, contents ranged between 13.4 (MN3) – 19.4 % dw (MA3), and 3.6 (MA3) - 8.4 g/100 g dw (MN3), respectively; *M. alba* generally presented had higher protein content than *M. nigra*, however *M. nigra* in general showed higher contents fiber than *M. alba*. Moisture ranged from 51.1 (MN3) to 66.9% (MA3) fw. Organic acids quantified, were citric (from 32.2 to 105.5 mg/100 g fw), and malic (from 43.7 to 72.5 mg/100 g fw). Mulberry leaves might be a source of new food supplements or functional foods and pharmaceutical products.

Keywords: *fiber, mineral content, moisture, morphological characterisation, organic acids*

Cite This Article: Eva María Sánchez-Salcedo, Asunción Amorós, Francisca Hernández, and Juan José Martínez, "Physicochemical Properties of White (*Morus alba*) and Black (*Morus nigra*) Mulberry Leaves, a New Food Supplement." *Journal of Food and Nutrition Research*, vol. 5, no. 4 (2017): 253-261. doi: 10.12691/jfnr-5-4-7.

1. Introduction

(*Morus* spp. L., Moraceae) has been domesticated over thousands of years ago and adapted to conditions of a wide area of tropical, subtropical and different temperature zones of Asia, Europe, North and South America, and Africa [1]. Plant species with a wide range of environmental adaptations like mulberry; have been found to exhibit numerous morphological and physiological characteristics [2]. Morphological variability in mulberry is said to have contributed to its growth and survival under various disruptive environmental conditions [3]. Mulberry is valued species for landscaping, gardening in urban conditions, street shade and city embellishment, because it withstands high levels of air pollution. It is also used for the stabilization of sandstone [4]. Mulberry plant is one of the conventional herbs used in medicine since time immemorial due to its chemical composition and pharmacological function. Most parts of mulberry plants are used as medicine in Chinese and Indian medicine [5]. Mulberry fruits are also eaten fresh as well as in dried forms and consumed as marmalades, juices, liquors, natural dyes and cosmetic industries [6]. Previous studies have reported that mulberry fruits, contain several bioactive compounds, like organic acids, phenolic compounds (benzoic acid derivatives, cinnamic acid derivatives, flavonols anthocyanins) and sugars, among others [1,7,8,9,10].

In most mulberry-growing countries, mulberry foliage is used to feed silkworms [11]. Its leaf is also used for feeding cattle, goat and other animals as it is highly nutritious and palatable to herbivorous animals [12].

A large number of studies have shown the phenolic profiles of mulberry leaves [13,14,15].

Furthermore the mulberry leaves are used as infusion in Asian countries beeing most common in Japan and Korea due to the presence of steroids, flavonoids, amino acids, vitamins, triterpenes and other trace elements that show valuable effects [16]. The consumption of mulberry-leaf tea has increased over the past decades because of its hypoglycemic, antidepressant, antioxidant and hepatoprotective effects [17,18]. Mulberry leaves are rich in alkaloids including 1-deoxynojirimycin (DNJ), the most potent glycosidase inhibitor that decreases blood-sugar

levels [19,20]. *Morus* plant species has also started to gain an important position in the food industry due to the increasing findings of its health benefits such as reduced risk of certain types of cancer, coronary heart disease, stroke, high blood glucose, and ageing [1,21,22]. Recently, various other food-grade mulberry products (i.e., drink powders, snacks and tablets) have been developed and marketed in Asian regions, including China, Korea, Japan and Thailand [23].

Although numerous studies have been published on mulberry in the last years, few studies focused on the morphological and chemical characteristics of leaves. The objective of this study was to evaluate, for the first time, the some of the principal morphological and chemical characteristics of leaves of seven mulberry clones from Spain. These results will be important in order to select those clones that have better characteristics and besides, strengthen value-added use of leaf morus as a source edible with nutritional, industrial and pharmaceutical importance.

2. Material and Methods

2.1. Plant Materials

Fresh leaves samples of seven mulberry clones, four white (*Morus alba*) clones: MA1, MA2, MA3, and MA4, and three black (*Morus nigra*) clones: MN1, MN2, and MN3, were manually collected in Orihuela (latitude 38º04'08''N x longitude 0º58'58''W, 27 m above sea level, Alicante, SE Spain) in September 2014. After collection, the samples were washed with distilled water and lyophilized. The dried materials were ground into powder with a blender and stored in airtight container until use.

2.2. Morphological Characterisation

Leaf characterisation was done following Cifuentes and Kee-Wook [24] guidelines. The parameters assessed were leaf shape, leaf type, leaf base, and leaf margin (Table 1). Other physical leaf parameters were studied: leaf weight (Wl) expressed in g, leaf width (w) expressed in mm, leaf length (L) expressed in mm and peduncle length (Lp) expressed in mm (Table 2). The leaf width, leaf length and peduncle length were measured with an electronic digital slide gauge Mitutoyo (model CD-15 DC, Telford, UK, 0.01 mm accuracy). The leaf weights were taken using a digital scale Sartorius (model BL-600, with an accuracy of 0.01 g).

A sample size of 60 adult leaves per clone was characterised. All of them were taken from all tree orientations and middle parts of shoots, and only healthy and undamaged ones were selected.

2.3. Determination of Mineral Elements

The leaves were briefly rinsed with deionised water, were oven-dried at 60°C for at least 48 h, weighed and ground to a fine powder. Tissue N and C concentration was measured using a Thermo-Finnigan 1112 EA elemental analyzer (Thermo-Finnigan, Milan, Italy). Tissue Na, K, Mg, Ca, P, S, B, Cu, Fe, Mn, Zn, Cu, Mo concentrations were determined by inductively coupled plasma emission optical spectrometry (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA) after previous acid digestion in HNO3:H2O2 (5:3, v/v) in a microwave reaching 200°C in 20 min and holding at this temperature during 2 h (CEM Mars Xpress, North Carolina, USA).

2.4. Determination of Protein Contents

Protein content was calculated from the nitrogen content (%N × 6.25) analyzed by the Kjeldahl method.

2.5. Determination of Moisture and Crude Fiber

The moisture percentage of leaf was determined by drying the material in a hot air oven at 60°C until reaching constant weight. The crude fibre (CF) contents were determined by a digester , an Ankon fiber analyzer (model A220, Macedon, NY, USA), following the official methodology established by the Spanish Ministry of Agriculture, Fisheries and Food [25]. Results were expressed as g/100 g dw (dry weight). Analyses were conducted in triplicate.

2.6. Determination of Profile of Organic Acids of Leaf

The analysis of organic acids was determined by freeze-dried the material according to Sánchez et al. [26]. Standards of organic acids (phytic, ascorbic, citric, malic, tartaric, quinic, shikimic, and oxalic acids) were obtained from Sigma (Poole, Dorset, UK). Calibration curves, obtained by triplicate injection of standard solutions, were used for quantification purposes and showed good linearity (R2>0.999; Figure 1). Results were expressed in mg/100 g fw (fresh weight).

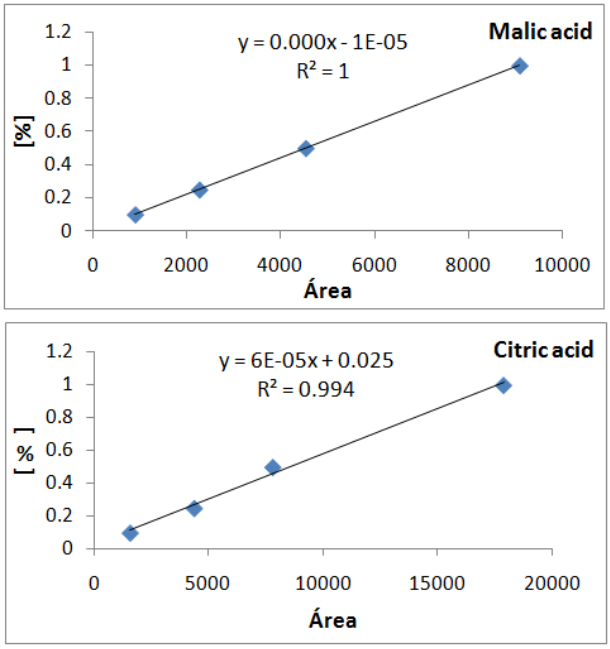


Figure 1. Calibration curves of the malic acid and citric acid

Table 1. Mean values of mulberry leaves morphological characters

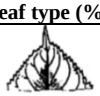
	Clones						
	White mulberry (*Morus alba*)				**Black mulberry (*Morus nigra*)**		
Leaf shape (%)	**MA1**	**MA2**	**MA3**	**MA4**	**MN1**	**MN2**	**MN3**
 1 Entire	100	33.3	100.0	85.0	100.0	100.0	100.0
 Oval	55.0	61.7	70.0	76.7	91.7	66.7	31.7
 Eliptic	45.0	28.3	6.7	8.3	5.0	8.3	65.0
 Roped	0.0	10.0	23.3	0.0	3.3	25.0	0.0
 2 Lobed	0.0	66.7	0.0	15.0	0.0	0.0	0.0
Leaf type (%)							
 Caudate	26.7	25.0	45.0	23.3	41.7	16.7	15.0
 Almada	66.7	33.3	43.3	73.3	48.3	70.0	85.0
 Obtuse	6.7	33.3	11.7	3.3	10.0	13.3	0.0
 Dual	0.0	8.3	0.0	0.0	0.0	0.0	0.0
Leaf base (%)							
 Retasa	80.0	6.7	5.0	3.3	3.3	5.0	21.7
 Emarginate	20.0	83.3	11.7	41.7	8.3	6.7	65.0
 Coined	0.0	10.0	88.3	33.3	55.0	60.0	13.3
 Linear	0.0	0.0	11.7	21.7	33.3	28.3	0.0
Leaf margin (%)							
 Acutado-serrated	66.7	100.0	100.0	75.0	100.0	100.0	91.7
 Serrated mastoid	33.3	0.0	0.0	25.0	0.0	0.0	8.3
 Double serrated	0.0	0.0	0.0	0.0	0.0	0.0	0.0
 Serrated waist	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. Physical parameters mean values of mulberry leaves

	Clones						
	White mulberry (*Morus alba*)				Black mulberry (*Morus nigra*)		
Parameters	**MA1**	**MA2**	**MA3**	**MA4**	**MN1**	**MN2**	**MN3**
Leaf weight (g)	1.6±0.1 a	2.9±0.1 d	3.4±0.14 e	2.4±0.1 c	2.8±0.1 d	2.8±0.1 d	2.0±0.1 b
Leaf width (mm) (w)	71.2±1.5 a	93.8±1.5 c	132.8±2.8 e	88.2±2.7 bc	101.9±2.4 d	103.9±1.9 d	85.6±2.4 b
Leaf length (mm) (L)	102.1±1.8 a	127.5±1.4 b	170.7±3.1 d	131.5±2.3 b	147.7±2.6 c	143.6±2.8 c	125.1±3.4 b
Length/ width ratio L w^{-1}	1.4	1.3	1.3	1.5	1.4	1.4	1.4
Peduncle length (mm)	38.8±1.2 ab	54.4±1.6 c	55.9±1.4 c	41.3±0.6 b	53.9±0.8 c	41.3±1.0 b	37.6±1.1a

Values (means ± SE) followed by the same letter, within the same row, are not significantly different according to Fisher´s least significant difference (LSD) procedure at 5% significance level. L/w ratio=3:1Lanceolate; ratio=2:1Narrow ovate; ratio=1,5:1Ovate; ratio=1,2:1Wide ovate; ratio=<1:1Cordate.

2.7. Statistical Analysis

Statistical analyses were performed using SPSS 20.0 for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an variance analysis (ANOVA) test for mean comparisons. The method used to discriminate among the means (Multiple Range Test) was Fisher's Least Significant Difference (LSD) procedure at a 95.0 % confidence level. Pearson correlation and principal component analyses (PCA) were also performed. Cluster analysis was applied to the standardized data for hierarchical associations employing Ward´s method for agglomeration and the spuared Euclidean distance as dissimilarity measure.

3. Results and Discussion

3.1. Morphological Characterisation

Average values of leaf morphological parameters according to Cifuentes and Kee-Wook [24] guidelines are included in Table 1. The results showed that the entire leaf predominated in all clones except MA2 clone had more of lobed leaf (66.67%). The main leaf type was almada, while that MA2 had the same almada and obtuse, and MA3 had slightly more caudate. The leaf base of MA2, MA4 and MN3 clones were emarginated, while those of MA3 and MN2 were coined, and finally of MA1 was retasa. The leaf margin acutado-serrated was the dominated in all clones. Similar to results found Nderitu et al. [27] in a study of genetic divergence of five mulberry (*M. indica*) accessions grown in Kenya, these authors have noted that three accessions were dentated and two serrated. This might be attributed to differences in environmental conditions and water stress experience [27], and a variation among genotypes used. Many studies have shown that leaves can present adaptations in their morphological and photochemical characteristics in places subject to different temperatures [28,29]. Drought was also found to reduce leaf area, and vary mulberry morphological characters [30,31,32].

3.2. Physical Parameters

Physical parameters of leaves are shown on Table 2. Regarding leaves weight, significant differences were found ($p < 0.05$). Whereas MA1 yielded the lowest leaf weight, the other clones showed higher leaf weights ranging from 2 g (MN3) to 3.4 g (MA3). Moreover, MA1 significantly showed the lowest leaf width and leaf length, the other clones ranging from 85.6 mm (MN3) to 132.8 mm (MA3) and from 125.1 mm (MN3) to 170.7 mm (MA3) respectively. Similar results were reported by Nderitu et al. [27] and Chang et al. [33] in leaf width and length. Peduncle length ranged from 37.6 mm (MN3) to 55.9 mm (MA3). This result agrees with Chang et al. [33] (petiole length mean 3.3 cm). However, Nderitu et al. [27] showed lower values of peduncle length (from 2.8 cm to 3.5 cm).

3.3. Mineral Composition of Mulberry Leaves

Table 3 shows the mineral composition of mulberry leaves. The macro-elements N, P, K, Ca, Mg, and S, ranged from 2.1 g/100 g dw (MN3) to 3.1 g/100 g dw (MA3), from 0.1 g/100 g dw (MN1) to 0.2 g/100 g dw (MA3), from 1.2 g/100 g dw (MA1) to 3.9 g/100 g dw (MA3), from 1.7 g/100 g dw (MN2) to 3.9 g/100 g dw (MA1), from 0.5 g/100 g dw (MA3) to 1.4 g/100 g dw (MA1), from 0.2 g/100 g dw (MA2) to 0.3 g/100 g dw (MN3), respectively. The Na was 0.01 g/100 g dw in all the clones. Sodium is also an essential element, but is only necessary in small amounts. High intakes of this element are associated with increased blood pressure and risk of cardiovascular disease [34].

Our results were slightly higher that obtained by Srivastava, Kapoor, Thathola, and Srivastava et al. [35] in leaves of *M. alba*. Another study on mulberry leaves grown in Serbia reported the Ca, Mg content also were lower (Ca 1399 µg/g *M. alba* and 1548 µg/g *M. nigra* dry extract and Mg 1641 µg/g M. alba and 1942 µg/ g *M. nigra* dry extract) than Spanish mulberries [36]. The most abundant macro-element was Ca (average content of 2.7 g/100 g dw in the *M. alba* leaves, and 2.6 g/100 g dw in the *M. nigra* leaves), followed by N (average content of 2.6 g/100 g dw in *M. alba* and 2.5 g/100 g dw *in M. nigra*), K (average content of 2.4 g/100 g dw in *M. alba* and 2.2 g/100 g dw in *M. nigra*) and Mg (average content of 0.8 g/100 g dw in *M. alba* and 0.7 g/100 g dw in *M. nigra*). Radojković et al. [36] found that the first macro-element was Mg and the second was Ca in the extract of the *M. alba* and *M. nigra* leaves.

Regarding the micro-elements, (Fe, Cu, Mn, Zn and Ni), all clones showed high concentrations of Fe ranged from 119.3 mg/kg dw (MN1) to 241.8 mg/kg dw (MN3), but the concentration in MN3 clone was significantly higher than the other clones. Fe was more than two times higher in MN3 than in MN1. According to the [37] the

recommended dietary intakes (RDI) of Fe for adults, are 18 mg/day for women and 8 mg/day for men; thus 100 g of dried mulberry leaves, of Spanish clones, contain more than 100% of the dietary needs for men and between 134.33 and 66.27 for woman of the RDI. The content of Cu, Mn, Zn and Ni, varied from 4.2 mg/kg dw (MA2) to 5.9 mg/kg dw (MA4), varied from 35.8 mg/kg dw (MA3) to 90.5 mg/kg dw (MA1), varied from 23.9 mg/kg dw (MN1) to 39.5 mg/kg dw (MN3) and varied from 1.7 mg/kg dw (MA4) to 5.4 mg/kg dw (MA1), respectively. Similar results have been obtained by Radojković et al. [36] in the content of Fe of *M. Nigra* (143 µg/g) but lower of *M. alba* (2.6 µg/g). However, Srivastava et al. [35] showed higher content of Fe in *M. alba* (19-35.7 mg/100 g dw). Radojković et al [36] reported content of Cu higher in both species (17.4 µg/g *M. alba* and 19.5 µg/g *M. nigra* dry extract).

Several factors may contribute to these variations as, genotype, growing region, climate, cultivation conditions, degree of ripeness, and soil nutrient content [38].

3.4. Protein Content

The protein content in mulberry leaves, varied ranging from 13.4% dw (MN3) to 19.4% dw (MA3) (Table 3). *M. alba* generally had higher protein content than *M. nigra*. This result agrees with those previously observed by Güven, [39] and Srivastava et al. [35]. However, Iqbal et al. [40] found slightly higher levels (18.4 of *M. alba* and 19.7 of *M. nigra*) but Jyothy et al. [41] reported lower contents than ours. Proteins of mulberry leaves are of high quality and used with wheat flour to make parathas in the sub-continent. Supplementation of mulberry powder also improves storage stability of wheat up to two months [42].

3.5. Moisture and Crude Fiber

Moisture content in fresh mulberry leaves, ranged from 51.1% fw (MN3) to 66.9% fw (MA3). Srivastava et al. [35] reported higher values from 71.1 to 76.6% fw in *M. alba*. Regarding crude fiber, ranged from 3.6 g/100g dw (MA3) to 8.4 g/100g dw MN3). The *M. nigra* clones in general showed higher contents than *M. alba* clones, this agree with Iqbal et al. [40] but they found higher values (10.1 of *M. alba* and 12.3 of *M. nigra*).

3.6. Organic Acids Content

The organic acids quantified in mulberry leaves were citric acid and malic acid (Table 4). Citric acid was the most abundant organic acid whose concentratrion ranged between 32.2 mg/100 g fw (MA4) and 105.5 mg/100 g fw (MN3). Malic acid, ranged from 43.7 mg/100 g fw (MN3) and 72.6 mg/100 g fw (MN2). Other study [43] in aqueous extracts made from the two kinds of dietary suplements, 1) containing 100% of *M. alba* leaf and 2) containing a mixture of mulberry leaves and other medicinal herbs. The dietary suplements 100% of *M. alba* leaf had values 14.1 citric 4.8 malic mg/100 mL.

Table 3. Mineral content (macroelement composition (g 100 g^{-1} dw)) (microelement composition (mg kg^{-1} dw)) and protein (%) in mulberry leaves

	Clones						
	White mulberry (*Morus alba*)				Black mulberry (*Morus nigra*)		
Mineral content	**MA1**	**MA2**	**MA3**	**MA4**	**MN1**	**MN2**	**MN3**
Nitrogen (g 100 g^{-1})	2.2±0.1 ab	2.5±0.05 bc	3.1±0.1 e	2.7±0.1 cd	2.3±0.05 ab	2.9±0.1 de	2.1±0.05 a
Phosphorus (g 100 g^{-1})	0.2±0.02 b	0.2±0.01 c	0.2±0.01 c	0.2±0.01 b	0.1±0 a	0.1±0.01 ab	0.1±0 ab
Potassium (g 100 g^{-1})	1.2±0.05 a	2.2±0.03 b	3.9±0.04 e	2.5±0.05 d	2.1±0.1 b	2.2±0.12 bc	2.4±0.02 cd
Calcium (g 100 g^{-1})	3.9±0.3 d	2.8±0.03 bc	1.7±0.2 a	2.2±0.1 ab	3.2±0.1 c	1.7±0.05 a	2.9±0.3 c
Sodium (g 100 g^{-1})	0.01±0.0 ab	0.01±0 a	0.01±0 b	0.01±0 b	0.01± 0c	0.01±0.00 b	0.01±0 b
Magnesium (g 100 g^{-1})	1.4±0.1 d	0.8±0.03 bc	0.5±0.03 a	0.6±0.02 ab	0.7±0.03 abc	0.5±0.05 a	0.9±0.08 c
Sulfur (g 100 g^{-1})	0.2±0.02 b	0.2±0 a	0.2±0 b	0.2±0.01 a	0.2±0 a	0.2±0.01 a	0.3±0.01 c
Iron (mg kg^{-1})	124.8±6.1 a	124.7±10.2 a	197.8±8.6 c	159.2±7.4 b	119.3±14.4 a	149.3±8 ab	241.8±16 d
Zinc (mg kg^{-1})	31.7±4.1 abc	34.2±2.2 bc	29.5±1.7 ab	33.9±0.1 bc	23.9±2.4 a	24.1±1.4 a	39.5±3. c
Manganese (mg kg^{-1})	90.5±7.5 d	53.6±2.5 b	35.8±2.4 a	53.6±2.3 b	48.9±2.3 b	48±2.6 ab	74±5.4 c
Boron (mg kg^{-1})	548.3±34 c	459.7±20.2 b	277.4±4.1 a	432.6±8.1 b	253.5±12 a	256.4±4.6 a	825.3±31.6 d
Copper (mg kg^{-1})	4.7±0.2 a	4.2±0.1 a	5.8±0.1 b	5.9±0.3 b	4.4±0.9 a	5.7±0.4 b	5.8±0.1 b
Molybdenum(mg kg^{-1})	2.3±0.2 c	1.4±0.05 b	1.1±0.05 ab	2.2±0.2 c	0.8±0.7 a	1.3±0.1 ab	1.2±0.1 ab
Nickel (mg kg^{-1})	5.4±0.4 d	1.9±0.2 a	3.4±0.1 c	1.7±0.1 a	2.1±0.2 a	2.9±0.2 bc	2.3±0.2 ab
Lead (mg kg^{-1})	0.3±0.04 a	0.3±0.05 a	0.6±0.04 bc	0.5±0.1 b	0.4±0.02 a	0.5±0.05 b	0.8±0.1 c
Carbon (g 100 g^{-1})	37.4±0.3 a	39.5±0.3 bc	40.5±0.5 cd	41.3±0.5 d	39.7±0.1 bc	41.4±0.4 d	39±0.1 b
Lithium (mg kg^{-1})	17.2±1.03 e	5.3±1 b	2.4±0.4 a	1.9±0.2 a	3.1±0.1 a	7.4±0.3 c	10.1±0.5 d
Titanium (mg kg^{-1})	5.6±0.2 ab	5.4±0.5 a	8.1±0.3 c	6.9±0.5 bc	5.5±0.8 ab	6.5±0.3 ab	10.8±0.6 d
Protein (%)	14.1±0.4 ab	15.6±0.3 bc	19.4±0.7 e	16.9±0.9 cd	14.8±0.3 ab	18.7±0.7 de	13.4±0.3 a

Values (means± SE) followed by the same letter, within the same row, are not significantly different according to Fisher´s least significant differences (LSD) procedure at 5% significance level.

Table 4. Organic acids (mg 100 g^{-1} dw), crude fiber (g 100 g^{-1} dw) and moisture (%) in mulberry leaves

	Clones						
	White mulberry (*Morus alba*)				Black mulberry (*Morus nigra*)		
	MA1	MA2	MA3	MA4	MN1	MN2	MN3
Acids							
Citric	74.1±11.2 bcd	103.7±25.4 cd	63.9±9 ab	32.2±15.2 a	68±7.7 abc	57.6±5.6 ab	105.5±2.7 d
Malic	56.3±6.7 abc	66.7±3.2 bc	56.6±0.9 abc	51.5±1.8 ab	57.4±5.6 abc	72.6±10.1 c	43.7±2.7 a
Fiber	7.1±0.2 cd	5.6±0.2 bc	3.6±0.2 a	5.3±0.2 ab	7.6±0.1 d	5.1±0.3 ab	8.4±1 d
Moisture	51.3±0.5 a	58.7±0.5 b	66.9±1.8 c	57.3±1.8 b	55.5±1.9 b	59.7±1.4 b	51.1±0.1 a

Values (means ± SE) followed by the same letter, within the same row, are not significantly different according to Fisher´s least significant difference (LSD) procedure at 5% significance level.

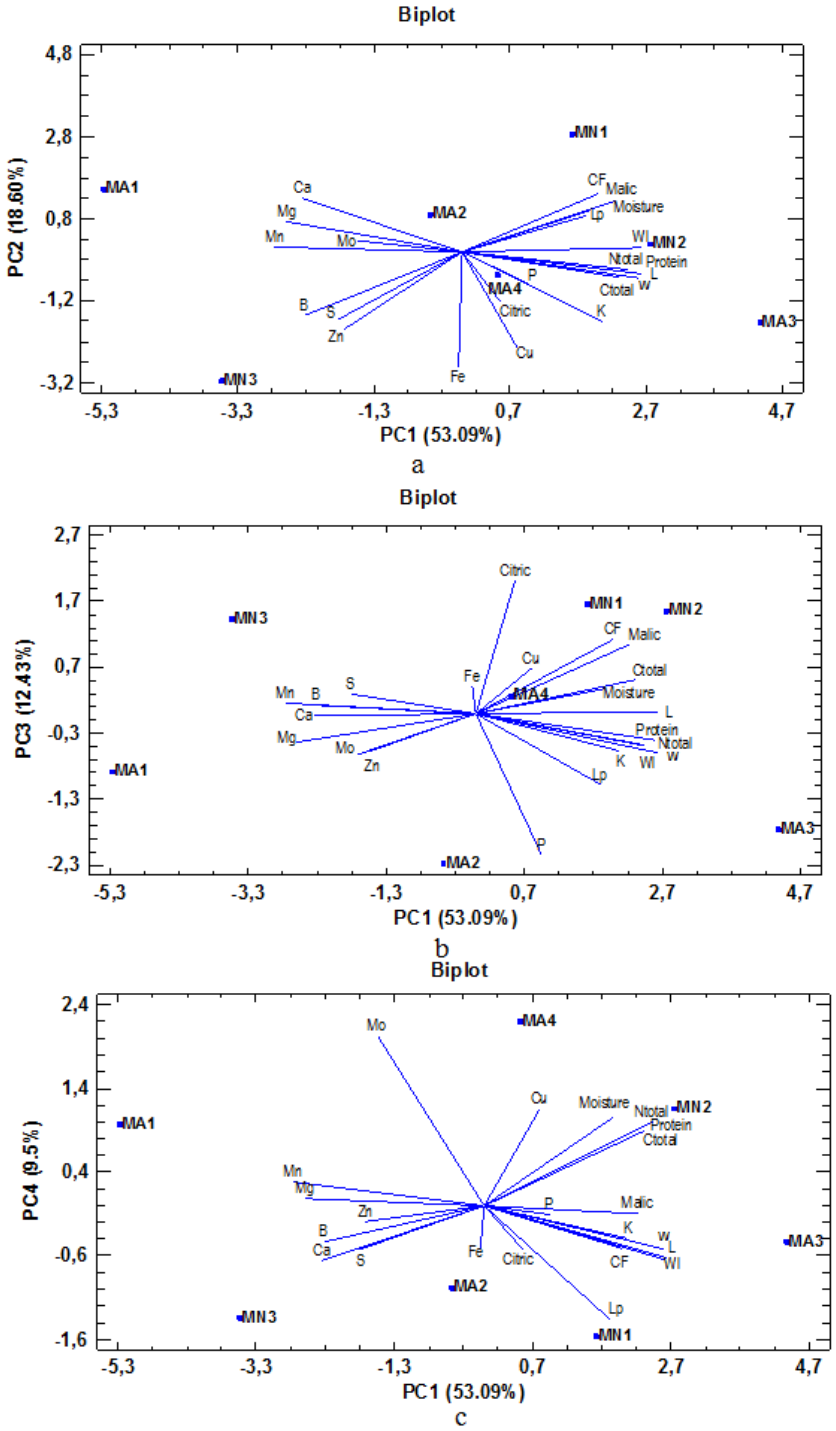


Figure 2. Principal component analysis (PC1 vs PC2, PC1 vs PC3 and PC1 vs PC4) of mulberry leaves: loading plot and distribution of samples in the consensus space

3.7. Principal Components Analysis

To achieve a better understanding of the trends and relationships among the many studied variables (22) for the different mulberry samples (7 clones), principal component analysis (PCA) was applied. The first four principal components (PCs) explained >93.62% of the total variation. Nearly 71.69% of the observed variability was explained by the first two PCs. The first component (PC1), representing 53.10% of total variance, was positively linked to the content of certain physical parameters (except to peduncle length, Lp), moisture, total nitrogen, total carbon, protein and negatively correlated to calcium, magnesium and manganese (Figure 2a). PC2 accounted for the 18.59% of the total variance. It was negatively correlated with boron, copper, iron, potassium, sulfur and zinc (Figure 2a). PC3 accounted for the 12.44% of observed variability and was negatively correlated to fiber, citric acid and malic acid (Figure 2b). PC4 only accounted for 9.49% of the total variance and was negatively correlated peduncle length (Figure 2c).

The results of PCA showed that PC1 axis separated into four groups to the mulberry clones. First group consisting of MA1 and MN3 clones, disposed on the left side, another group of the MA2 and MA4 clones disposed from the center, and the group of the MN1, MN2 and the last group MA3 of clones on the right side (Figure 2a).

Clones MA1 and MN3 had large negative scores on the PC1, characterised by low leaf weight, leaf width, leaf length, moisture, nitrogen, carbon, and protein. MA1 showed positive scores on the PC2, accounting for its high content of the Ca, Mg, Mo and Mn, while MN3 showed negative scores on the PC2 accounting for its high content of the Zn, S, B.

MA3 clone showed the highest positive value in PC1 (Figure 2a). This was clearly separated from the other mulberry clones. MA3 clone is characterised by a high content of total N, C, protein, moisture, leaf weight, leaf width and leaf length.

MN1 and MN2 clones exhibited similar PC1 and PC3 values, but they differed in their values in PC2 and PC4. These clones presented positive values in PC1, PC2, PC3 and MN1 negative values and MN2 positive values in PC4 (Figure 2a). MN1 clone showed higher values of iron, copper and peduncle length than MN2.

MA2 and MA4 shared similar values in PC1 and PC2, displayed scores around 0. They can thus be considered as clones with intermediate characteristics compared to the rest of the clones aforementioned.

The results obtained from hierarchical cluster analysis with Euclidean distance, using linkage method between groups, are shown as a dendrogram (Figure3). The Spanish mulberry clones were separated into three main groups. The first group consisted of two clones (MA1 and MN3), the second one included four clones (MA2, MN1, MA4, and MN2) in this group clones MA4 and MN2 were close so, showing very similar characteristics. The third and last group included only one (MA3).

4. Conclusions

In this study, for the first time, morphological parameters, mineral content, organic acids, protein, and fiber of mulberry leaves grown in Spain were examined. The present data indicated that the entire leaf predominated in clones. The main leaf type was almada. The leaf base were found emarginate, coined and retasa. The leaf margin was acutado-serrated dominated in all clones. The most abundant macro-element was Ca followed by N, K and Mg. All clones showed high concentrations of Fe. It is worth noting that the Na was 0.01 g/100 g dw in all the clones, so they are suitable for low-sodium diets. The protein content in mulberry leaves, *M. alba* generally had higher protein content than *M. nigra*. The organic acids quantified in mulberry leaves, were citric acid followed by malic acid. The results reported showed that the Spanish mulberry leaves clones could be an inexpensive source of mineral elements, protein and crude fiber. Therefore, it can be said that mulberry leaves could be used as additives or extracts in functional foods and for medicinal purposes.

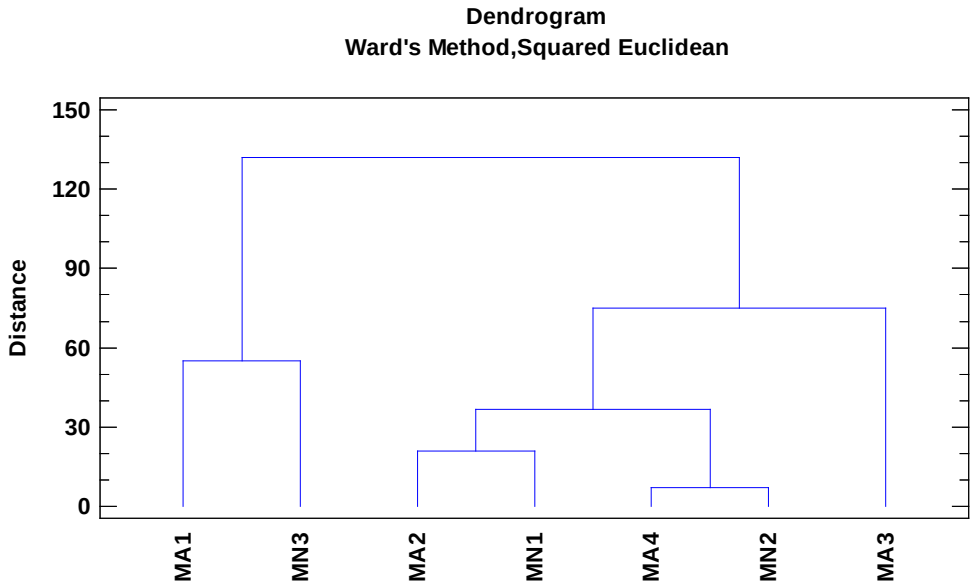


Figure 3. Dendrogram of the seven mulberry leaf clones using Ward's method based on squared Euclidean distance from physical and chemical parameters

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