



Physico-Chemical, Morphological, Organoleptic and Microscopic Study of Fresh Arbutus Unedo L. Fruits from Morocco

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ABSTRACT

The Arbutus unedo L or Arbutus tree is a leafy tree that represents a large part in the Moroccan ecological ecosystem. However, the poor studies on the fruits of Arbutus unedo in the Mediterranean countries cause poor exploitation and lack in the market. In this sense, the objective of this work focuses on a general description of the fruits of the province of Beni Mellal, such as morphological, organoleptic, microscopic, physico-chemical characteristics (pH, titratable acidity, moisture content, and ash content), lipid, protein and sugar content. For morphological characterization the studied fruit samples were red and red-orange color; With soft consistency and a rough skin; Mean total mass of $4.99 \text{ g} \pm 1.38$, a mean length of $1.99 \text{ cm} \pm 0.28$ and a width of $1.89 \text{ cm} \pm 0.21$. On the basis of physico-chemical analysis, we obtained a moisture content of up to $63.33\% \pm 3.21$, an acid pH of 3.86 ± 0.01 , a titratable acidity of $0.6\% \pm 0.06$ and an ash content of $0.35\% \pm 0.07$. In addition, a spectral analysis using ATR-FTIR spectroscopy shows the identification of wave numbers in the (3010cm^{-1} ; 2920cm^{-1}) for lipids, a (1600cm^{-1} ; 1652cm^{-1}) for proteins and (1035cm^{-1} ; 1011cm^{-1}) for sugar. While according to the organoleptic

parameters, we found that the fruit was moderately acceptable by the consumers. Comparing with several results, the variation of morphological and physicochemical parameters may be due to different geographical, climatic and tree age parameters. In addition, these results clearly show the need to seek ways of conservation and industrial exploitation.

Keywords: *Arbutus unedo* L; Physico-chemical parameters; ATR-FTIR; Morphological and microscopic analysis; Organoleptic analysis; Wild fruit.

INTRODUCTION

Morocco, due to its strategic geographical position, has experienced a series of cultivations that have had an influence not only on the human level but also on the environmental level, making this country a great reservoir of plant and animal resources.

In this sense, Morocco, with its geographical diversity, has a wealth of biodiversity reflected in the multitude of ecological ecosystems, which makes it a rich reference for territorial products. Among these products, a plant more abundant in several regions, but it is less studied; we cite *Arbutus unedo* L which is locally known as "Sasnou". The *Arbutus* is a fruit tree belonging to the Ericaceae family [1], widely distributed in the Mediterranean basin, mainly in North Africa, Western Europe, the Canary Islands and Western Asia [2- 4]. In the Mediterranean it exists in Morocco, Portugal, Spain, France, Italy, Albania, Croatia, Bosnia, Montenegro, Greece and Turkey [5-6]. The *arbutus* tree grows on acid soils and can reach up to 5 meters in height [4-7]. This last one is known as an evergreen plant, with dark green leaves; and characterized by antioxidant, astringent, diuretic, anti-diarrheal and depurative properties [8-9], through these last ones they are used in the treatment of diseases like hypertension, diabetes and inflammatory problems [10-11].

Subsequently, the *Arbutus* among the wild food fruits that is found in forests with an altitude reaches up to 1200 m above sea level [12]. With a color varies in different stages of ripening, at the beginning they are green they turn into yellow, orange and then red when the fruits are ripe [13]. We cannot speak about these fruits without mentioning the nutritional characteristics, in which they are rich in bioactive substances, fats, proteins, energy, dietary fibers and several vitamins A, B1, B2, B6, B9, B12 and C [14-15]. Moreover, they contain different phenolic compounds namely gallic acid [16].

In view of these biological or nutritional characteristics several authors take this tree as a basis for these studies, in which we find research generally on the leaves as a pharmacological toxicological study of *Arbutus unedo* L in Morocco [17]. Also, on the physico-chemical and antioxidant characteristics of the fruits [18-19].

Despite their nutritional and antioxidant richness, the fruits of *Arbutus unedo* L remain less consumable and unknown in some regions of Morocco. Indeed, the present work is interested in the determination of morphological, physicochemical, microscopic and organoleptic characteristics, then a comparison with previous works of *Arbutus unedo* L in several geographical areas; In order to look for adequate conservation techniques for a better exploitation.

MATERIALS AND METHODS

Plant Materials

The study was carried out on the basis of samples of fruits of *Arbutus unedo* L which come from the region of Beni Mellal-Khenifra, their province is Beni Mellal (32°20'22"N 6°21'39"W) which is located geographically between the middle atlas and the plain of Tadla; with an altitude ranging between 400 to 700 m. In addition, characterized by a sub-humid climate with average annual rainfall equal to 493 mm and an annual temperature reaches up to 18,7°C [20].

After receiving the samples, the fruits are drawn and washed, then stored in a refrigerator (2°C) until they are used in the various analyses.

Table 1: pedoclimatic data from the harvesting area of the samples

Harvest zone	Beni Mellal
Region	Beni Mellal khenifra
Geographic region	Middle Atlas
Latitude	32°20'22"N
Longitude	6°21'39"W
Altitude	400 to 700 m
Average annual temperature	493 mm
Average annual rainfall	18,7°C
Bioclimatic zone	Sub-humid

Morphological Characterization of *Arbutus unedo* L

Morphological characteristics were measured on 20 fruits taken at random; for which the following characteristics are determined: form, color and consistency are doing visually, and with the help of an analytical balance the weight is determined [21]. While the length and widths are measured by vernier caliper.

Physicochemical Analysis

pH Value

10g of the fruit cut into small pieces in 100 ml of distilled water and stirred for 5 minutes until a juice is obtained [22]. Then the measurement was carried out using an electrode of a pH meter.

Titrateable Acidity

It is the titration of the acidity of an aqueous solution of fruits of *Arbutus unedo* L with a sodium hydroxide solution in the presence of a color indicator (phenolphthalein) [23].

The acidity was measured by neutralizing the free acids in 25 ml of juice obtained with a NaOH solution (0.1N) until a color change was reached [24]. The latter is expressed in relation to the malic acid content [4].

Moisture Content

Moisture content is determined on the basis of weight loss drying [25]. 2g of the fruit cut into small pieces in empty capsules and dried for 15 minutes; put these capsules in the oven at 100°C±2 and weighed every 2h until constant weight [22].

Dry Matter

The dry matter is determined by the same working condition in the determination of the moisture content, using a drying of 2g of plant material in an oven at 103 ± 2 °C and atmospheric pressure [26].

Determination of Ash Content

The pulp of the fruits of Arbutus unedo L is calcined at 550°C in a calcination oven until a whitish ash of constant weight is obtained. After weighing the empty crucibles, 2g of the sample is placed in the crucibles and then placed in a calcination furnace for 4h at 550°C. After cooling, the cooled crucibles are weighed. The ash content is expressed as a percentage of organic matter [24].

ATR-FTIR Analysis

In order to reveal the compositions of lipids, sugars and proteins in arbutus fruits, mid-infrared spectroscopic analysis was used. In fact, MIR spectroscopy has more applications, as the "fingerprints" of functional groups can be displayed narrowly and intensely in the MIR region ($4000-400\text{cm}^{-1}$). So, the chemical compositions in question can be detected easily at this region. In addition, analysis by infrared spectroscopy was chosen because of its multiple advantages and benefits related to ease of handling, good analytical precision with respect for the environment (green analytical method) [27-28]. ATR-FTIR spectra were obtained using a PerkinElmer spectrum, Version 10.5.1 equipped with an attenuated total reflectance accessory (ATR single reflex ion, Diamond, incident angle 45°) with DTGS detector, Global (MIR) Source and KBr Germanium separator, with a resolution of 4 cm^{-1} at 90 scans. Spectra were scanned in the transmittance mode from 4000 to 600cm^{-1} and the data were handled with PerkinElmer logiciel. A total of three samples of the arbutus fruit in the fully mature state were taken at random, and placed directly on a single ATR Diamant bounce (Pike) and analyzed at room temperature (25°C). Before each sample was measured, the ATR plate was cleaned in situ by washing with an alcohol (or acetone) solution, allowing the ATR to be dried. Then background noise was collected.

For the ATR crystal cleaning procedure mainly includes three steps: (1) removing the sample from the surface of the ATR crystal; (2) clean the ATR crystal thoroughly with a cloth dampened with alcohol or acetone; (3) Dry the ATR crystal with a tissue.

Microscopic Analysis

With the help of an optical microscope which gives an idea of the shape of the plant cells. A finely cut fragment of fruit is placed in a slide, then a drop of distilled water is added, and finally a slide is placed [29].

Observing the set-up at $\times 10$ and then $\times 40$ magnification and taking images at each magnification.

Organoleptic Analysis

It allows the study of the sensory characteristics of fruit by using man as a measuring instrument, based on the five senses: smell, taste, sight, hearing and touch [30].

In fact, the rating scale varies from 1 for bad fruit to 5 for excellent fruit. Then we note the remarks in cards, and the tasters must judge the color, the taste, the residual taste, the sweetness, the acidity, the smell and the general acceptance [31].

RESULTS AND DISCUSSION

Morphological Characterization of *Arbutus unedo* L.

The results of morphological analyses carried out on the fruit of *Arbutus unedo* L from the Beni Mellal region (Morocco) are summarized in Table 2.

Table 2: morphological analysis carried out on the fruit of *arbutus unedo* L.

Parameter	Results
Form	Spherical
Color	Red /Orange Red
Consistent	Soft
Skin	Rough
Total mass (g)	4,699±1,38
Length (cm)	1,99±0,28
Width (cm)	1,89±0,21

In the commercial field the color of all foodstuffs is one of the most attractive characteristics for consumer acceptance. In the present study the sampled fruits are characterized by red and red-orange color; these results give a very clear idea about the maturity status of the fruits. Subsequently, the results found by Halil Ibrahim Sagbas indicate that the red and dark red colored fruits are fruits harvested from a fully ripe field [32].

A soft consistency and a rough skin, the fruits of *Arbutus unedo* L from the Beni Mellal province (Morocco) are characterized by a mean total mass of 4.99 g with a standard deviation of 1.38, a mean length of 1.99 cm and a width of 1.89 cm with a standard deviation of 0.21.

Comparing our results with the values found by Nouredine Boussalahon the fruits taken in Algeria, which are characterized by an average mass equal to 5.87g, a length of 2.28cm and a width of 2.12cm [33].; we notice that these results are higher than the results we obtained, but remain comparable. And according to SedatSerçethe studied fruits of Turkish are characterized by an average mass equal to 8.81g±0.64, a length of 2.54cm±1 and a width equal to 2.54cm with a standard deviation of 0.5 [14]; these results are largely superior to the values we obtained.

It can be concluded that geographical area plays a major role in the morphological characteristics of the fruits of *Arbutus unedo* L.

Physicochemical Parameter

The results of physicochemical parameter carried out on the fruit of *Arbutus unedo* L from the Beni Mellal region (Morocco) are summarized in Table 3.

Table 3: physico-chemical analysis carried out on the fruit of arbutus unedo L.

Parameter	Results
The pH	3,86±0,01
Titratable acidity (%)	0,6±0,06
Moisture content (%)	63,33±3,21
Dry matter	36,67±2,75
Ash content (%)	0,35±0,07

pH Value

The determination of the pH is one of the most important parameters in the quality control of all foodstuffs; thus, it gives information on the state of conservation. On the other hand, a pH of about 3 to 6 is very favorable to the devolution of yeast and mold [34-35].

According to our results, the studied fruits of Arbutus unedo L have a pH equal to 3.86 with a standard deviation of 0.01; this value is slightly higher than the one found by Koula Doukani for the Algerian fruits, namely 3.55 ± 0.05 [36]. In addition, the studied fruits from the Turkish geographical area are characterized by a higher pH with a value equal to 5.00 ± 0.3 [37]; in the same context our results are in the range obtained by Hafida Zitouni having worked on several samples from different regions of Morocco having a pH from 2.44 to 3.92 [38].

The difference between our results and those described can be explained by the distinction of the geographical area. This proposal is confirmed by Messaid and Huberson having related the observable difference to the region, climatic conditions and the state of ripening of the fruit [39-40].

Titratable Acidity

The titratable acidity gives us information on the physical state of the fruit; in other words, it gives an idea of the quantity of organic acids present in the sample, which are intermediaries in metabolic processes [41]. They affect the conservation quality of the products in which they influence the growth of microorganisms.

From table 1, we notice that the titratable acidity of the studied fruits equals 0.6% with a standard deviation of 0.06; our results are lower than those found by Sedat SerÇe and higher than the results found by Murat Şekerto which he results 0.4% and $0.67\% \pm 0.17$ respectively for the same fruit of Turkey [14, 37]. In addition, the value of titratable acidity obtained for the studied fruits from Algeria is close to the one mentioned by Koula Doukani who found a range of $0.63\% \pm 0.001$ to $0.74\% \pm 0.0007$ [36]; while for the fruits taken from different regions of Morocco, Hafida Zitouni manages to find a range from 0.65 to 1.01% [38].

Moisture Content

Water is one of the essential components of the fruit; it has an importance on their quality so it acts on the conservation of all foodstuffs. In which water is one of the most important parameters in the proliferation of microorganisms [35].

The results mentioned in the above table show that the moisture content of the fruit of Arbutus unedo L from Beni Mellal province reaches 63.33% with a standard deviation of 3.21. If we refer

to the work of Koula Doukani who obtained a percentage of water content equal to $57.56\% \pm 0.127$ and $68.18\% \pm 0.01$ for fruits from two different regions of Algeria [36], we can see that they are close to the values we obtained; moreover, for Turkish fruits, Murat Şeker found a lower value than our result is 47.21% with a standard deviation of 4.96 [37].

According to several authors, the water content remains an essential criterion for classifying the fruit as soft, semi-dry or semi-soft [42].

Dry Matter

According to the results presented in table (3), the percentage of dry matter of *Arbutus unedo* L. fruits equal to 36.67% with a standard deviation of 2.75. It is noted that the percentage of dry matter is low, and these results are acceptable due to the large amount of water.

Comparing our results with the values found by Kaoula Doukani and Murat Şeker on the same fruits which are 42.44% and 52.79% respectively. We can see that the latter are much higher than the values we found [36-37].

Ash Content

Like all living things, the development of bacteria requires several factors, namely pH, water content and mineral salts. Indeed, the rate of ash represents the total quantity of mineral salts present in a sample; in addition, it provides information on the nutritional quality of food [43]. We note from Table 1 that the fruits studied have an ash content equal to 0.35% with a standard deviation of 0.07; these results are close to those found by Koula Doukani, which are $0.33\% \pm 0.0027$ for fruits from the region Tlemcen (Algeria) [36]. In addition, our result is lower than the value given by the same author for the fruits of another region Tiaret (Algeria) which is $0.69\% \pm 0.0023$ [36].

According to these results, there is a clear variation in the ash content, and according to Athamena these variations may be due to ecological factors, the age of the plant, the period of the vegetative cycle, or genetic factors [44].

Spectroscopic Measurements by ATR-FTIR

ATR-FTIR spectra represents the lipid, protein and sugar composition of *Arbutus unedo* L. fruit in the 400 to 4000cm^{-1} wavelength range. Figure (3) represents the results obtained.

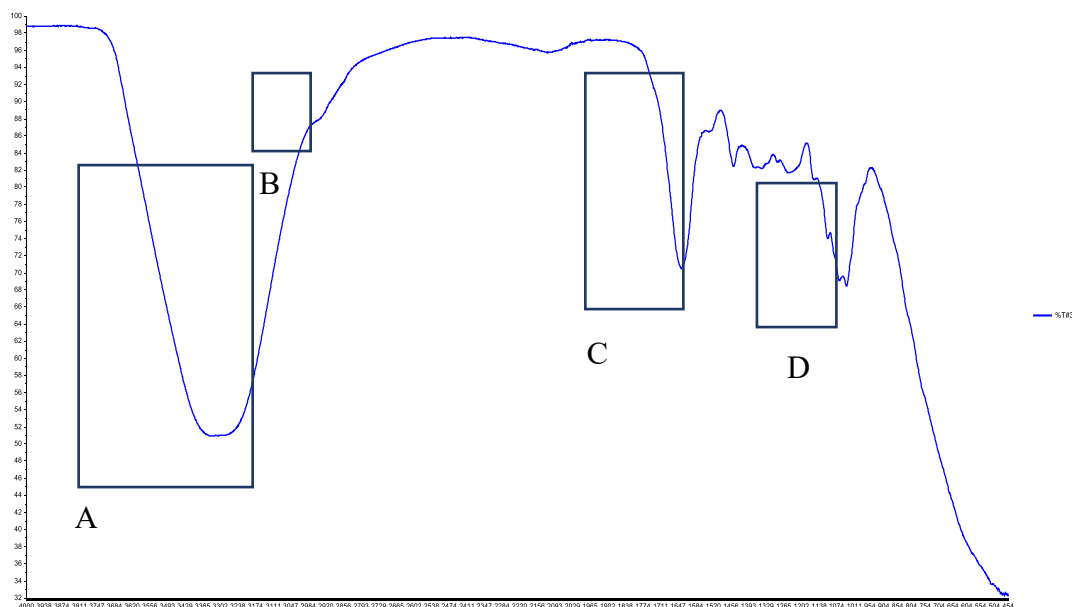


Figure 1: ATR-FTMIR spectra: The mean spectra calculated of studied arbutus unedo L fruit samples, at MIR region of 4000–400 cm⁻¹

The Arbutus unedo L. samples studied show 4 peaks A, B, C and D, the first broad bend located at 3300 to 3238cm⁻¹ is attributed to an O-H grouping. A second weak bend (B) located between 2984 and 2920cm⁻¹ is related to symmetrical and asymmetrical stretching of the CH₃ and CH₂ [45]. We also find a peak located between 1600 and 1652cm⁻¹ (C) related to the vibration of the C=O group. Finally, in the interval from 1035 to 900cm⁻¹ (D) which presents a C-O and C-C group [46].

So, we can conclude that the first peak representing the O-H group is assigned to water. And the second peak (B) that characterizes two groups CH₃ and CH₂, which are associated with the present lipids and fatty acids. Followed by the zone (C) that represents a vibration C=O of the amide absorption (protein). At the end a peak (D) that links the two groups C-O and C-C characterized the deformation of carbohydrates and the glucose region.

Microscopic Analysis

A fruit fragment of Arbutus unedo L observed at two different magnifications, the figure below (Figure 1) represents images for each magnification.

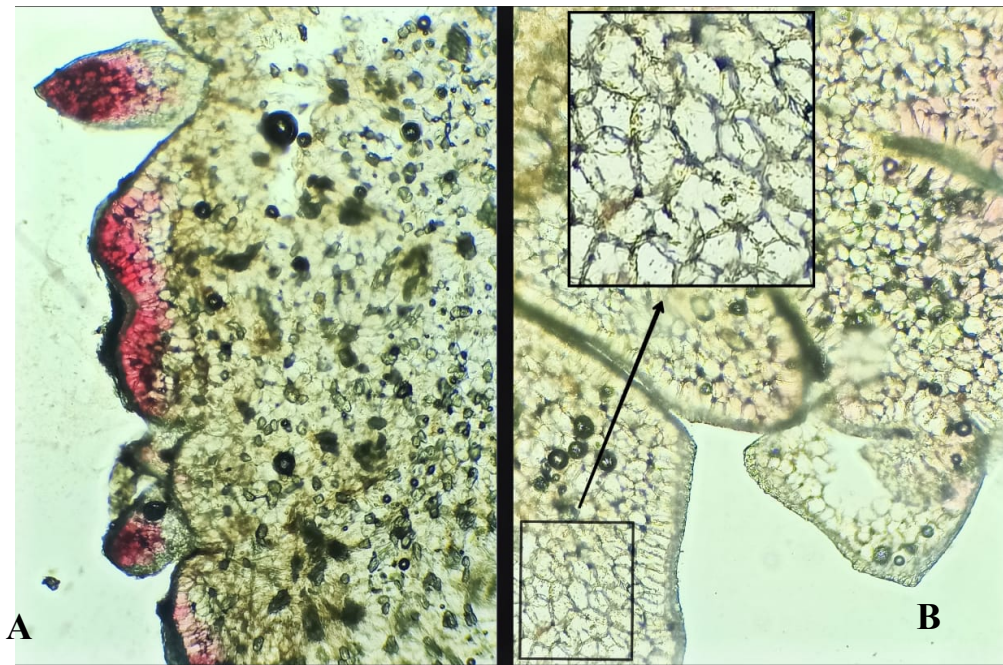


Figure 2: Image of a microscopic examination of arbutus unedo L fruit. A: magnification $\times 10$. B: magnification $\times 40$

To highlight the cellular exchanges in the plant cells of *Arbutus unedo* L fruits, and to get an idea about the shape of the cells and the color intensity as well as the cellular physiological states; from figure 1 we notice that the red color intensity of *Arbutus unedo* fruits is located in the cells which are in the tip especially in the rough part of the fruit as can be clearly seen in the picture (A).

In the second image (B) of the same figure but with a magnification of $\times 40$ we can see that the turgid state of the cells is due to the presence of water in the cells, so this observation is adequate with the high moisture content found.

Organoleptic Analysis

A jury of 20 randomly selected adult students of different sexes (boys and girls) tasted the fruit. The organoleptic characteristics include all the information gathered from the sensory organs; the results obtained are shown in the graph below (Figure 2).

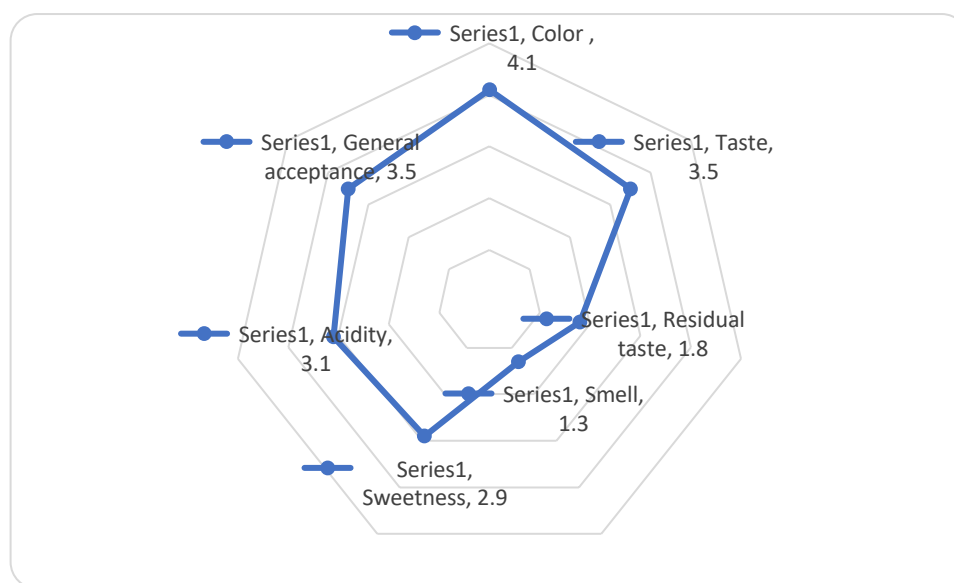


Figure 3: Organoleptic test results of *Arbutus unedo* L fruit

For many foods, color and general shape play an essential role in the commercial rating, in which they represent the customer's first contact with the product ²⁷.

Before starting the tasting, the number of tasters judged the intensity of the color and the smell of the fruit studied. In our study and according to the results of figure 1, we notice that the average note given by the tasters is equal to 4.1 which expresses a very important intensity of the color; we can explain it by the level of maturity of the fruits.

For the smell, we note that the average score given is 1.3; the latter remains low if we base ourselves on a scale of 1 to 5 with 1 for a weak smell and 5 for strong.

A very interesting step is the tasting, in which the number of jury members give a point of view on the taste and the residual taste; in first time we notice that the average note of the taste equals 3.5, the latter expresses a good taste. On the other hand, we found a low value for the residual taste with 1.8.

In the same context and with the parameters of flavors figure 2 shows that the studied fruits are moderately weak for the sweetness with a value of 2.9 on 5; and a value of 3.5 for the acidity. This could indicate the presence of correlations between these two parameters with the taste of the fruits in general.

In this experiment, we obtained an idea about the general acceptance of the fruit by the jury numbers, in which we manage to find a good score equal to 3.5. The receivers can link this parameter to the possibility of consuming this product again.

In the light of all the results obtained during the organoleptic and sensory analyses, we can deduce that the fruits of the Beni Mellal province are characterized by an important intensity of color due to the degree of maturity of the fruits, a smell and a very weak residual taste, a

medium acidity and sweetness that affects a good taste and as a consequence the studied fruits are moderately acceptable by the consumers.

CONCLUSIONS

The *Arbutus* or strawberry tree is among the wild fruits poorly exploited because of the scientific neglect of these types of food products. By the present work, we arrived to make a description of morphological parameters such as length, width, and total mass; as well as physico-chemical characteristics, in which we manage to find a pH value, titratable acidity, moisture content, the ash content, lipid, protein and sugar content. Without forgetting a microscopic analysis that shows the shape of the plant cell of *Arbutus unedo* L, and an organoleptic analysis that gives us an idea about the acceptance of these fruits by the consumers.

This study is part of a scientific database for these fruits, and a prescriptive vision to seek conservation techniques in order to exploit this product and expand its use in all regions of Morocco and other countries.

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