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ORIGINAL ARTICLE

NUTRITIONAL COMPOSITION AND BIOACTIVE COMPOUNDS IN A LOCAL GRAPE VARIETY *VITIS VINIFERA* L. CULTIVATED IN MOROCCO

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ABSTRACT

Background. The vine is considered one of the most important and popular fruits in the world thanks to its high nutritional value and high fiber content.

Objective. This study aim was to evaluate the nutritional composition in a local variety 'Doukkali' grape (*Vitis vinifera* L.) in the perspective of their suitability for pharmaceutical and agri-food use.

Material and methods. The proximate composition and minerals were determined using AOAC methods, the total sugar by HPLC method. The total phenolic compounds using the *Folin-Ciocalteu* reagent, the total flavonoids using aluminum chloride colorimetric method and tannins by the method of vanillin.

Results. The analysis of the raisin of this variety revealed a high carbohydrate content of 61% with high levels of glucose (31.6%), fructose (30.4%), dietary fiber (13.92%) and minerals including potassium (445.50 mg/100 g DM), calcium (193.26 mg/100 g DM), sulfur (171.63 mg/100 g DM), sodium (137.50 mg/100 g DM), phosphorus (99.79 mg/100 g DM), magnesium (54.74 mg/100 g DM) and iron (2.39mg/ g DM). Analysis of bioactive compounds showed significant content of polyphenols varying from 43 to 309.1 mg GAE/g DM, flavonoids from 1.0 to 23.8 mg CEQ/g DM and tannins from 2.5 to 50.45 mg TAE/g DM.

Conclusions. The study shows that the local variety of Doukkali grape has an important nutritional potential that can contribute to the nutritional needs and the fight against malnutrition as well as to the dietary diversity of the local population.

Key words: *Vitis vinifera, Doukkali grape, sugars, minerals, fibers, polyphenols, flavonoids, tannins*

INTRODUCTION

The vine (*Vitis vinifera* L.) is a species of great economic interest with a world production of 78 million tons in 2018, of which China is the world's leading producer, followed by Italy, the United States, Spain and France [34].

In Morocco, the area occupied by vines is approximately 43,106 ha, including 36,178 ha of table vineyard and 6,928 ha of vat vineyard, representing respectively 84% and 16% of the total area. The average production of 357,000 T/year which generates more than 200 million dirhams per year [30], is mainly located in the regions of Casablanca-Settat (41%), Marrakech-Safi (24%), Rabat- Salé-Kenitra (11%), Fez-Meknes (10%) and the Oriental region (7%). The most popular grape varieties introduced and available on the Moroccan market are Valency and Muscat from Italy, Muscat from Alexandria, Superior, Early Sweet and Flame. However, there are still some local varieties which are among the most cultivated varieties for commercial purposes, in particular the 'Doukkali' and 'Abbou' varieties which cover an area of 18,555 Ha [21]. The composition and properties of vine different parts, have been widely studied, qualifying the vine as a potential source of phenolic compounds, mainly flavonoids including catechins [8, 25, 36]. Because of these compounds multiple biological activities were

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conferred on the vine, including antioxidant activity [42], antimicrobial activity [44], antiproliferative activity [28] and antiobesity activity [52]. Moreover, grapes are considered one of the most important and popular fruits in the world due to their high nutritional value and high fiber content [15]. The local variety grapes, of protected geographical indication "Raisin Doukkali" is a mid-season table grape whose cultivation extends over two Moroccan provinces, El Jadida and Sidi Bennour encompassing 20 rural territorial communes. The fruit is characterized by ellipsoid-shaped red to purple berries with a predominant sweet taste and a muscat flavor when ripe [29].

Compared to imported raisins, the Doukkali variety raisin is often prepared by traditional methods and its consumption remains low and limited to some traditional recipes.

Studies on the physico-chemical composition of raisins have received little attention by the scientific community. To our knowledge no study has been published on the chemical composition of the Doukkali local raisin variety. Therefore, the aim of this study is to evaluate the nutritional composition and phytochemical profile of raisins of this variety in the perspective of their suitability for pharmaceutical and agri-food use.

MATERIALS AND METHODS

Plant material

Mature bunches of grapes (*Vitis vinifera* L.) (Figure 1) were harvested in September 2018 in 3 communes (Jabria, Bni Hilal and Bouhmame) in the province of Sidi Bennour in Morocco. The bunches were thoroughly washed with tap water to remove attached dust particles and stalks. Then, the berries obtained were washed several times successively with distilled water and deionized water. They were then drained and then dried in an oven at 45°C to a humidity of 16.56±0.85%, after which they are stored in airtight containers until time of analysis.

Instrumentation

A Spectrophotometer-UV/Visible (Jenway 6300, USA), oven (SM400, Memmert, Germany), pH meter (AD1000, Adwa instruments Kft., Szeged, Hungary), high performance liquid chromatography (Hewlett-Packard 1100), refractive index detector (Hewlett-Packard, series 1100, G1362A, Wilmington Del., USA), analytical column Agilent Zorbax NH2- 5µm, 4.6×250mm (Agilent Technologies, Wilmington, USA), inductively coupled plasma (ICP-AES ultima 2C Horiba) were used.

Chemical reagents

Vanillin, quercetin, gallic acid, tannic acid and HPLC standards ethanol, acetonitrile, fructose, glucose, sucrose and maltose (purity >99.0%) were obtained from Sigma – Aldrich (Germany). Other chemicals and solvents were of analytical grade.

Preparation of extracts

The extracts were obtained by maceration for 72 hours with methanol (MeOH, 100%), methanol/water (MeOH/H2O, 70%/30%), dichloromethane/methanol (DCM/MeOH, 50/50) and dichloromethane (DMC, 100%). Then, the extracts were filtered and the solvent was removed by rotary evaporation under pressure at a temperature of 45°C and then stored in the freezer at -4°C until analysis [22].

Biochemical characterization

Moisture and ash content was determined according to AOAC method [2], protein by *Kjeldahl* AOAC procedure [3] and lipid by Soxhlet extraction technique using petroleum ether [4]. Soluble and insoluble dietary fibers were determined according to the AOAC enzymatic-gravimetric method [37] by enzymatic incubation with protease, α-amylase and

(a) (b)

Figure 1. Bunch of grapes (a) and raisins (b) of the Doukkali variety (*Vitis vinifera* L.)

amyloglucosidase, carbohydrates were estimated by the difference method [4] and total energy was calculated according to the following equation:

Total Energy (kJ) = 17 x (Crude Protein (g) + Total Carbohydrates (g)) + 37 x (Crude Fat (g))

The pH value was determined according to the method described in the literature [14, 18] on 3 grams of raisins dispersed in a flask with 200 ml of boiling water. After cooling, the flask was completed to volume with distilled water. The pH was determined in this solution using a pH meter.

Carbohydrate composition analysis

The simple sugar composition was determined by high performance liquid chromatography, according to the protocol described in the literature [7, 18]. After the obtained extract filtration using a 0.45 µm filter, a volume of 20 µl of the final extract was injected into the HPLC system by an automatic sampler. The detection is carried out by a refractive index detector with an analytical column. The mobile phase consisting of acetonitrile and ultrapure water (80:20, v/v) was delivered at a flow rate of 1ml/min.

Quantification of sugars was performed from the integrated peak areas of the sample against the corresponding standard graph, and the data analysis was performed using Chemstation software (Hewlett Packard). The results were expressed as a percentage of dry matter. Analysis of the ash solution was conducted in accordance with the AOAC. [2] method and the mineral and heavy metal content of the samples was determined using an inductively coupled plasma with a radio frequency generator of 1200 Watt, Optics: I: 160 - 800 nm, a high dynamic detector (HDD), a Meinhard type CI nebulizer, Plasma gas 14 l/min, an auxiliary gas flow of 3 l/min, a rinsing time of 30 sec and an integration time of 5 sec).

Determination of total polyphenols

The dosage of phenolic compounds was carried out according to the method using the *Folin-Ciocalteu* reagent [27] with a slight modification. The absorbance of all samples was measured at 760 nm using a spectrophotometer and the results were expressed in milligrams of gallic acid equivalent per gram of dry matter (mg GAE/g DM).

Determination of total flavonoids

Total flavonoids were assayed by the method described by *Dehpour* et al. [13]. The absorbance was measured at 415 nm using a spectrophotometer and the results are expressed in milligrams of catechin equivalent per gram of dry matter (mg CEQ/g DM).

Determination of tannins

Tannins were determined by the acid medium vanillin method described by *Ba* et al. [5] the absorbance is measured at 500 nm by a spectrophotometer against a blank and the results were expressed as equivalent of tannic acid in milligrams per gram of dry matter (mg TAE/g DM).

Statistical analyzes

Results were subjected to analysis of variance (ANOVA) and *Tukey's* test (5% probability) was used for comparisons of means using SPSS version 26 software.

RESULTS

Biochemical characterization

The biochemical composition of the grapes samples analyzed is presented in Table 1. The results obtained show that the moisture content of the grape fruit is 83±0.01% while that of the raisin prepared in the laboratory was found to be 16.56± 0.85%. The tested raisin samples contain 2.31±0.22 g/100 g of ash, 2.91±0.11g/100 g of protein, 1.3±0.04g/100 g of lipids, 65.91 g/100 g of sugars, 13.92±0.05g/100 g of fibers and 1218.04 (kJ) of energy, with a pH value of 3.84±0.01.

Table 1. Biochemical composition of the Doukkali raisins variety (g/100 g DM)

Parameters	Content
Moisture of fresh grape (%)	83±0.01
Raisin moisture (%)	16.56±0.85
Ash	2.31±0.22
Protein	2.91 ±0.11
Total lipids	1.3 ± 0.04
Total simple sugars	65.91 ±0.00
Total fibers	13.92±0.05
Energy (kJ)	1218.04
pH	3.84±0.01

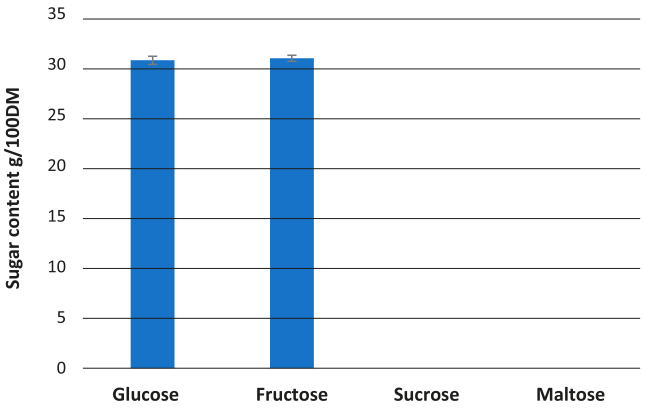

Figure 2. Sugars content in raisin (*Vitis vinifera L.*) extract

Carbohydrate analysis by HPLC

The HPLC analysis results, indicate that glucose and fructose are the most present sugars (30.4±0.4 and 30.6±0.30g/100MS, respectively) while sucrose and maltose are not detected in the grape variety samples studied.

Mineral analysis

The analysis of the mineral composition show that 'Doukkali' raisin variety have a high content in particular of potassium (445.50 mg/100 g), calcium (193.26 mg/100 g), followed by that of sulfur (171.63 mg/100 g DM), sodium (137.50 mg/100 g DM), phosphorus (99.79 mg/100 g DM), magnesium (54.74 mg/100 g DM) and iron (2.39mg/100 g DM). The contents of chromium, copper, cadmium were lower than 1 mg/100 g (Table 2).

Table 2. Minerals and heavy metals in Doukkali raisins variety (mg/100 g DM)

Elements	Content
Ca	193.26±2.25
P	99.79±3.14
K	445.50±4.94
Mg	54.74±2.07
Na	137.50±5.73
S	171.63±7.75
Cr	0.25±0.01
Fe	2.39±0.97
Cu	0.6±0.07
Cd	<0.004

Content of total polyphenols, flavonoids and tannins

According to the results obtained, the total content of polyphenols varied from 43 to 309.1 mg GAE/g DM (Table 3). The highest content of total polyphenols was observed in the hydromethanolic extract and the lowest content was found in the dichloromethanol extract ($P < 0.05$). The results of this study also show a considerable content of flavonoids in the local grape variety examined, revealing values ranging from 1.0 to 23.8 mg CEQ/g DM, the highest content being that observed in the methanolic extract ($P< 0.05$). The tannin content found in the grapes analyzed reveals a predominance in the methanolic extract, with a content of 50.45 mg TAE/g DM, followed by the dichloromethanol extract and the dichloromethane extract containing 32.7 and 9.0 mg TAE/gMS, respectively.

DISCUSSION

The humidity value of less than 17% found in the raisin makes it possible to meet the recommended storage conditions [50]. Also, the pH average of the raisin studied was 3.84 ± 0.01. This value is included in the range of values described by *Al Askari* et al. [1] for varieties grown in or imported to Morocco, as well as for the values found for Tunisian raisin varieties [18]. In addition, it has been shown that a low pH value of raisins together with its low humidity, are among the factors that provide stability against the microorganisms growth and development [46].

The total ash content evaluated in the local grape variety was 2.31±0.22% and this value is lower than that found in the Apyrena variety. The contents of protein (2.91±0.11%) and total lipids (1.3±0.04%) are however, higher than those found by *Nikolidaki* et al. [33] in the same variety (2.2±0.4% and 0.43±0.06%, respectively). The total sugar content was 65.91±0.00 g/100 g DM. This content is lower than the value found by *Sabanis* et al. [43] in Greek grape juice concentrate. In the present study the results found concerning the proximate as determined in the local grape variety are in general, almost similar to the results found in the Tunisian raisin varieties [18].

Fibers that play many important roles in the protection against several chronic diseases including cardiovascular diseases, diabetes and certain cancers [12]. Also have a prebiotic effect protecting the health of the digestive system [48]. The determination of the fiber content in the present study shows that the local variety Doukkali studied contains 13.92±0.05 g/100 g DM. This high content corresponds to 3 times that found in the American Food Data Base, which is 4.5 g/100 g [16]. The richness of this local variety of grapes in fibers can be explained by the presence of grains in the Doukkali variety.

Table 3. Contents of polyphenols, flavonoids and tannins in the different grape extracts

Extract	Total phenolic compounds (mg GAE/g DM)	Total flavonoids (mg CEQ/g DM)	Tannins (mg TAE/g DM)
MeOH/water	309.1±14.2*	1.1 ±0.1*	2.5±0.4*
MeOH	184.7±6.9	23.8± 3.3	50.45±2.83
MeOH/DCM	59.2±9.9*	1.8±0.1*	32.7±0.4*
DCM	43.0±9.7*	1.0±0.1*	9.0±2.3*

Data presented as means ± standard deviation from the triplicate analysis.
*Significant when MeOH was compared to the other extracts in the same column at $P < 0.05$.

Carbohydrate analysis by HPLC

The carbohydrate contents obtained by HPLC are different from those reported by *Lee* et al. [26] but relatively similar to those found for glucose content (32.37±0.70 g/100 g DM) in the Meski [45] and for fructose (31.21±0.29 g/100 g DM) in the Chriha variety [18]. The predominance of these two simple sugars could be explained by the decomposition of sucrose following the high drying temperature [18]. However, in the present study, the drying being carried out at a low temperature of 45°C, the results obtained concerning the presence of those simple sugars, might be linked to the influence of the grape variety type or to the different environmental and experimental conditions [18, 51]. In addition, ingested glucose and fructose pass easily into the blood without digestion, and may constitute a crucial nutritional source for children and patients with celiac disease, thus generating immediate energy, especially for athletes [6, 43].

Data on minerals in the different parts of grapes, determined in several studies conducted in different countries of the world, show low contents of K, Na, Ca, Fe, P and Mg [38, 46, 47]. The results of the mineral composition of the local grape variety analyzed in this study are show that Potassium is the most abundant mineral in this grape with a content of 445.50±4.94 mg/100 g DM. This content is lower than that found in Tunisian varieties [18].In second position comes calcium, the content of which found in this study is 193.26 ± 2.25 mg/100 g DM followed by that of sulfur (171.63 ± 7.75 mg/100 g DM), sodium (137.50±5.73 mg/100 g DM), phosphorus (99.79±3.14 mg/100 g DM), magnesium (54.74±2.07 mg/100 g DM) and iron (2.39±0.97 mg/100 g DM). On the other hand, the contents found for the elements Cr, Cu, Cd were lower than 1 mg/100 g. The Na, Ca, Fe and Mg contents of the samples analyzed in this study are higher than those of Tunisian raisin varieties [18].

Given the essential role of minerals in the physiological functions and the maintenance of the proper functioning of the ogranism, their contents in the studied grapes reveal the importance of the potential contribution of this local variety to the nutritional intake of the local population. Indeed, it is known that potassium is involved in lowering blood pressure and reducing mortality from cardiovascular disease [19]. It is also reported that potasium plays an important role in kidney function, the management of calcium balance and level, the reduction of its urinary excretion and the risk of osteoporosis [32, 35].

Calcium is also a nutrient known for its contribution in the formation of the bone skeleton and teeth and has a role in the regulation of endo- and exo-enzymes [10]. It has also been reported to be helpful in maintaining blood pressure for normal brain function [17]. The present study also reveals a significant content of this mineral in the variety of grapes studied.

Another nutritional value revealed by the analysis of this variety of grape is its Magnesium content, the role of which is known in many biological reactions such as the synthesis of DNA and RNA and the production of ATP energy [39].

Furthermore, although the iron content found here was lower than that reported by *Fang* et al. [15] in the seedless variety Thompson (46.6-70.3 mg/kg), the data of the present study reveal that both Fe and Na, Ca and Mg contents are higher in the samples studied than in the varieties of Tunisian raisin [18].

As for iron, cadmium content was lower than that reported in Thompson seedless grape variety (0.004-0.01 mg/kg) [15]. This cadmium content is also lower than the value of the provisional WHO guideline, which is 0.003 mg/kg [49]. The chromium and copper levels found in this study are below the US EPA health criteria recommending 8 mg/kg and 30 mg/kg, respectively [15]. All these results confirm that the 'Doukkali' variety can meet a significant part of the daily mineral needs by consuming recommended amounts of this commodity. In addition, its metal content respects the levels recommended for health safety.

Content of total polyphenols, flavonoids and tannins

The total content of polyphenols obtained in this study is lower than those found in the work of *Mnari* et al. [31] on Tunisian raisin varieties, which were 401.5 to 534.2 mg GAE/g DM. However, the content reported in the variety studied remains higher than that found in the study by J*ediyi* et al. [21] on two other local Moroccan varieties, the Abbou variety with 37.91 ± 2.97 mg/g FW and the Adari variety which contained 22.67±1.73 mg/g FW. Other studies carried out in other countries had also found contents between 1.8 and 294.5±2.5 mg GAE/g in extracts of different varieties [20, 23, 24, 41]. This result suggests that the Doukkali variety grape can be highlighted as a variety extremely rich in phenolic compounds.

The flavonoid contents are higher than those obtained in aqueous extracts of 11 varieties in India which had flavonoid contents between 0.324 and 1.503 mg EQ.g-1 fresh weight [24], but they are lower than the reported levels of 66.5-93 CEQ/g DM by *Mnari* et al. [31].

On the other hand, the tannin contents observed in the present study are lower than the condensed tannin contents of the methanolic extracts of four Tunisian varieties of grapes which had contents varying from 189.3 and 208.6 CEQ/g of DM [31].

This observed difference in bioactive compounds, can be partly explained by different factors including genotype, geographical location, growing conditions

including, soil composition, irrigation, light intensity, infection by pathogenic organisms etc. [9, 11, 20, 40].

CONCLUSIONS

The results of this study demonstrate that the local variety of Doukkali grapes is of great value on health, since it constitutes a real source of macro and micronutrients and polyphenols. This nutritional potential can be used as a food fortifier (yogurt, cake, biscuit) or as an ingredient in several culinary preparations. It can also be a good snack food for children by contributing, through its consumption, to protecting the population against malnutrition and emerging diseases. This study also draws attention to the interest of completing research on this local variety by determining its polyphenol constituents and their antioxidant, antimicrobial and anticancer activities.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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