

PHYSICO-CHEMICAL PROPERTIES OF MOROCCAN *OPUNTIA DILLENII* FRUIT, EXTRACTION OF BETACYANINS AND STUDY OF ITS STABILITY AND ANTIOXIDANT ACTIVITY

Malak Rehioui*¹, Bouchra Benzidia¹, Said Abbout¹, Mohammed Barbouchi², Hamid Erramli¹, Najat Hajjaji¹

Address(es): Dr. Malak Rehioui,

¹Laboratory of Organic Chemistry, Catalysis and Environment, Department of Chemistry, Faculty of Sciences, Ibn Tofail University, BP 133, 14000, Kenitra, Morocco.

²Laboratory of Molecular Chemistry and Natural Substances, Faculty of Sciences, Moulay Ismail University, B.P.11201, Zitoune, Meknes, Morocco.

*Corresponding author: rehioui.malak0@gmail.com

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ABSTRACT

The *Opuntia dillenii* (*O. dillenii*) belongs to the Cactaceae family and usually grows in arid and semi-arid zones worldwide. This succulent plant is known for its multiple health benefits and virtues. In practical terms, the current study aims to investigate the morphological and the physicochemical characteristics of *Opuntia dillenii* fruit collected from Morocco, as well as the determination of flavonoids, polyphenols and betacyanins contents in order to characterize, identify and facilitate the valorization of this species. The obtained results are compared with existing literature and thoroughly discussed. In addition, the pigment of betacyanins was extracted from the fruit pulp and characterized by UV-visible spectrophotometry. The study of the stability of betacyanins was carried out in the presence of certain parameters such as the change of pH, temperature, light and the addition of salt. The antioxidant activity of the extracted betacyanins was further examined using the DPPH free radical scavenging assay and the total antioxidant capacity method.

Keywords: *Opuntia dillenii*, physicochemical characteristics, betacyanins, stability, antioxidant activity

INTRODUCTION

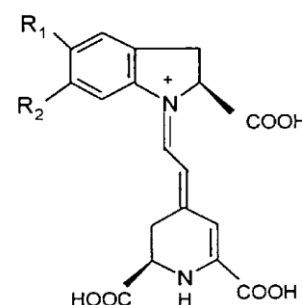
Opuntia is a tropical and subtropical shrub which supplies both edible stems and fruits; Processing this plant leads to massive accumulation of byproducts that can be a vital source of both bioactive and pigmented agents. Herein, the fruit of *O. dillenii* is originated from the southeastern parts of North America, the east coast of Mexico and the north of South America, and it develops in arid and semi-arid climate (Shirazinia *et al.*, 2019). This species has recently generated considerable attention in developed countries as valuable compounds with acceptable efficacy and safety in treating various diseases. Several investigations have highlighted its high efficacy in the treatment of diabetes, gastric ulcers and inflammations. It has also analgesic and anti-hyperglycemia actions. Its capacity to protect nerve cells from Alzheimer's, Parkinson's, and cerebral vascular diseases has also been reported. Additionally, it constitutes a great important food source, exhibiting high nutritional and fitness properties (Shirazinia *et al.*, 2019; Bastola *et al.*, 2023). Figure 1 presents the plant of *O. dillenii*.



Figure 1 *Opuntia dillenii* fruit

The prickly pears of *O. dillenii* are berries composing of a thick pericarp containing a few small clefts of prickles, and reddish-purple in color. The pulp is intermixed with numerous small rounded seeds, and has an acidic taste (Embaby *et al.*, 2016). Despite there are multiple studies on the physical and chemical characteristics of several species of cactus pear, the investigations on *O. dillenii* species are scarce. There is also insufficient data on the physico-chemical properties of fruit growing

in Morocco. The identification of these characteristics would assist in the characterization, valorization, and promotion of future use of this species. The exceptional red-purple hue of the *O. dillenii* pulp is due to the high betacyanins content. In fact, betacyanins are the most characteristic substances of *O. dillenii* that have recently received a great deal of attention. The chemical structure of betacyanins is presented in Figure 2.



Betanidin: R₁ and R₂ = OH

Betanin: R₁ = glucose ; R₂ = OH

Figure 2 Chemical structure of betacyanins

It's a water-soluble nitrogen compounds that are accumulated abundantly in the vacuole of the fruit pulp (Azeredo, 2009; Meena *et al.*, 2022). This natural pigment is a new target that is gaining importance in industrial applications and is replacing several artificial pigments that have been barred from the market for safety issues, as they are carcinogenic and harmful to the consumer's health, unlike betacyanins pigment, which are nontoxic and can help prevent several diseases (Cejudo-Bastante *et al.*, 2014; Gandia-Herrero *et al.*, 2014; Madadi *et al.*, 2020). In addition, there is clearly a rising interest in their antioxidant activity and their free radicals scavenging ability; and therefore, has a strong influence in the consumer acceptance. However, natural colorants and pigments cannot be always satisfied because there are some limitations such as the ease of degradation by some environmental factors, especially high temperature, light and changes in pH (Azeredo, 2009). Therefore, the use of a natural colorant requires detailed research of its stability against possible deterioration processes because such knowledge enables the optimization of industrial production, packing, and storage of the colored products.

Several authors have attempted to assess the extraction of betacyanins from *O. dillenii* and to study its stability, for instance by using ascorbic acid to ameliorate its stability (Azeredo, 2009). Nevertheless, the use of salt has not yet been tested on betacyanins stability. For this, we will evaluate the effects of adding different concentrations of sodium chloride on the stability of betacyanins. In this way, the pivotal objective of this investigation was, firstly, the determination of the physicochemical characteristics of *O. dillenii* fruits grown in Morocco, then the extraction and the evaluation of its stability and its antioxidant activity of betacyanins. This investigation was performed for the purpose of enlarging the knowledge of this species and fostering its cultivation which is very important for future applications.

MATERIAL AND METHODS

Plant material

As raw material, fresh matured *O. dillenii* fruits from the region of Essaouira in Morocco that were harvested and collected in December 2020, were used in this study. The involved purple fruits were immediately washed with running tap water, drained and left to dry for 15 min at room temperature and then stored at 4 °C to prevent degradation until use.

Physico-chemical characterization

Morphological characterization. For this determination, 25 intact pieces of ripe *O. dillenii* fruit were selected; each was weighed, then the peel was separated from the pulp manually, to obtain the weights of the pulp and the peel separately. We used a precision balance model RADWAG (type AS 220.R2, max = 220g, min = 10 mg ± 0.1 mg). Then, the seeds were washed under running water many times on a sieve to separate the seeds from the fruit pulp, and then drained and dried, after that the weight and the number of seeds per each fruit were recorded. Linear dimensions (length and width) were also measured. The length of the fruit was taken from the point of the attachment of the fruit to the paddle until the tip of the fruit.

Moisture and dry mass. Moisture and dry mass were assigned on three replicates by desiccation of samples at 105 ± 2 °C.

Ash. Ash was determined on three replicates by ashing for the residue of moisture determination at 550 °C for 24 hours.

pH. The pH of the fruit pulp was determined by potentiometric measurement at 20 °C with a pH meter model HANNA Instruments pH211, previously calibrated with standard regulating solutions.

Titrateable acidity. The acidity was determined by means of titration with sodium hydroxide (0.1 N), expressing the results in grams of anhydrous citric acid per 100 g fresh weight.

Total soluble solids. Total soluble solids (°Brix) were obtained in triplicate by refractometric measurement at 20°C in pulp juice using the Brix scale (AOAC, 2006).

Total polyphenols content. The total polyphenols content in the fruit pulp samples was determined spectrophotometrically after the colorimetric reaction with the Folin–Ciocalteu reagent by following the previously described method of Barbouchi et al., (2019). Typically, an aliquot volume of 0.3 mL of the sample (1 mg/mL) was mixed to 1.5 mL of the reagent of Folin–Ciocalteu (10/100). After 6 minutes of incubation in the dark, 1.2 mL of sodium carbonate Na₂CO₃ (7.5 %) was added. The mixture was stirred and incubated in darkness for 2 hours, and the absorbance was taken at 760 nm. The total polyphenols content was estimated from linear regression equation 1 that is derived from calibration curve using Gallic acid as standard.

$$Y_1 = 0.009 X_1 + 0.057 ; R^2 = 0.999 \quad (1)$$

Where Y_1 is the absorbance and X_1 is the total polyphenols content expressed as mg of Gallic acid equivalent (GAE) per g of dry weight.

Total flavonoids content. The total flavonoids content in the fruit pulp samples was determined spectrophotometrically using the aluminium trichloride colorimetric method of Barbouchi et al., (2019). An aliquot volume of 1 mL of the sample (1 mg/mL) was added to 2.4 mL of distilled water and 0.3 mL of NaNO₂ solution (5 %). After 6 min, a volume of 0.3 mL of AlCl₃ (10 %) was added and the obtained solution was incubated for 5 min. After that 1 mL of NaOH (1M) was added. The obtained solution was left to stand for 10 min, and then the absorbance was recorded against a prepared reagent blank at 510 nm. The total flavonoids content was calculated from linear regression equation (2) that is derived from calibration curve using Catechin as standard.

$$Y_2 = 0.004 X_2 + 0.027 ; R^2 = 0.999 \quad (2)$$

Where Y_2 is the absorbance and X_2 is the total flavonoids content expressed in terms of Catechin equivalents (CE) in mg per g of dry weight. The data were presented on average ± standard deviation for the triplicates.

Total betacyanins content. The quantification of betacyanins was carried out by recording the absorbance at one single wavelength and utilizing the molar extinction coefficient of betacyanins. The method was reported previously (Chung et al., 2015). The betacyanins content was performed in triplicate and developed by the equation (3):

$$\text{Betacyanins content (mg / 100 g of fresh weight)} = \frac{A \times DF \times V \times MW \times 100}{\epsilon \times LW} \quad (3)$$

Where A is the measured absorbance at 538 nm (maximum absorbance of 538 nm was reported to quantify betacyanins), DF is the dilution factor, V is the solution volume of the sample (mL), L is the path length of 1 cm cuvette, W is the dried sample weight (g). MW and ϵ are the molecular weight and the molar extinction coefficient (MW = 550 g/mol; ϵ = 65 000 L/mol.cm in H₂O) were applied in order to quantify the betacyanins. The total betacyanins content was expressed as milligrams of betacyanins equivalents per gram of fresh weight. The data were presented for each assay on average ± standard deviation for the triplicates.

Betacyanins extraction and spectrophotometric analysis

One of the most distinctive characteristics of *O. dillenii* fruit is its color. Typically, 100 g of fruit pulp without seeds was macerated with 200 mL of solvent with occasional stirring. The chosen solvent was ethanol-water (50 % - 50 %). This choice was made since betacyanins are only soluble in water, so the extraction must be done with solvent containing water (Das et al., 2019). On the other hand, ethanol allows the pigments to be recovered from the pulp without co-extracting pectins, mucilaginous compounds, and other alcohol-insoluble solids. The sample was macerated for 24 h in the dark with occasional stirring. Afterwards, it was centrifuged at 5000 rpm at 15 °C for 10 min to remove the vegetal tissue residue. The supernatant was collected and filtered. The solvent was evaporated using a rotary evaporator. The yielded extract was resuspended in 20 mL of purified water and lyophilized for 48 h, in order to obtain a stable powder. The extracted powder was stocked at 4 °C in dark until their analysis.

The extracted betacyanins were characterized by a UV-Vis spectroscopy JP Selecta model UV-2005 to determine the wavelength of maximum adsorption. The whole visible spectrum between 250 and 800 nm was registered at constant intervals (λ = 2 nm) employing 1cm path length glass cells and distilled water as reference.

Stability study of betacyanins

In order to evaluate the stability, the influence of certain parameters such as pH, temperature and light on the stability of the extracted betacyanins, a UV-visible spectrophotometer JP Selecta model UV-2005, was commonly used.

The first set of experiments was carried out to evaluate the effects of pH as it is factor acting on the change in the color of betacyanins. Briefly, we prepared a series of buffer solutions covering the pH range from 0.5 to 13.5. In each tube we introduce the same quantity of betacyanins. After stirring, we measure the final pH of the solution. The pH measurements are conducted using a pH meter model HANNA Instruments pH211.

The second set of experiments was performed to evaluate the effects of different temperature. In this study, tubes filled with the tested sample and closed were placed in thermostatic water bath at different temperatures ranging from 20 to 100 °C for different incubation time periods ranging from 10 min to 72 hours. For the extract stored under refrigerated (4 °C) condition was also analyzed. The time 0 was considered as reference. Subsequently, the samples were analyzed, and the absorption spectra were recorded at 538 nm.

Then, we proceed to the evaluation of the samples which are stored in the light and in the dark, respectively. Samples that are stored in the dark are placed in test tubes wrapped in aluminum foil and kept in a dark place, while illuminated test tubes were exposed to natural daylight. Samples are analyzed at 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h in the first day and then once daily for a total of 3 days.

Finally, we evaluated the effect of adding sodium chloride salt (NaCl) as an additive on the extracted pigments. For this, different concentrations of sodium chloride between 0 and 10 g/L were added to the samples. Absorbance spectra are recorded, and the absorbance of the color change is evaluated. We noted that all experiments were performed in triplicate.

DPPH free radical scavenging assay

The power of the extracted betacyanins to quench DPPH free radicals was performed as described previously (Rahman et al., 2015; Wijewardhana et al., 2019). Briefly, a volume of 0.1 mL of the crude extract was mixed with 3.9 mL of DPPH solution freshly prepared by dissolving 24 mg of DPPH in 100 mL of ethanol. After incubation for 30 min of samples in darkness, the absorbance was then measured at 515 nm. Butylated hydroxytoluene (BHT) was utilized as a

positive control. All the tests were performed in triplicate. The inhibition percentages I (%) were calculated with the equation (4):

$$I (\%) = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100 \quad (4)$$

Where A_{control} is the absorbance of the negative control (containing no antioxidant) and A_{test} is the absorbance of the test corresponds to the sample. The antioxidant capacity has been expressed from the IC_{50} value, which is the concentration of the antioxidant that is necessary to trap 50% of DPPH in the test solution.

TAC total antioxidant capacity assay

The assessment of total antioxidant capacity for the extracted betacyanins was based on the phosphomolybdenum assay according to the protocol previously mentioned (Wijewardhana et al., 2019). Shortly, 0.3 mL of each sample was mixed with 3 mL of reagent solution (28 mM Na_3PO_4 ; 0.6 M H_2SO_4 and 4 mM $(NH_4)_6Mo_7O_{24}$). The samples were prepared in tubes and incubated for 60min at 95 °C. After cooling, we have registered the absorbance at 695 nm against the blank. The calibration curve was carried out in parallel on equal conditions, using ascorbic acid as a standard. Butylated hydroxytoluene (BHT) was employed as reference control. The total antioxidant capacity was calculated from equation (5) that are deduced from the calibration curve of ascorbic acid.

$$Y_3 = 0.0045 X_3 - 0.074 ; R^2 = 0.996 \quad (5)$$

Where Y_3 is the absorbance and X_3 is the total antioxidant capacity presented in milligram equivalents of ascorbic acid per gram of dry weight (mg AA / g of dry weight).

RESULTS AND DISCUSSION

Morphological characteristics

In order to define and identify the studied fruit, the determination of its morphological characteristics is a very important criterion that is necessary to start with. Therefore, Table 1 illustrates the results (mean ± standard deviation) of the main morphological characteristics of *O. dillenii* fruit.

Table 1 Morphological Characteristics of *O. dillenii* fruit*

Parameters	Values
Fruit mass (g)	32.90 ± 1.70
Fruit length (cm)	5.90 ± 0.40
Fruit width (cm)	3.20 ± 0.20
Pulp mass (g)	23.60 ± 0.90
Pulp length (cm)	3.90 ± 0.30
Pulp width (cm)	3.10 ± 0.20
Peel mass (g)	9.30 ± 0.60
Seed mass (g)	0.04 ± 0.01
Number of seeds / fruits	126.00 ± 6.00
Number of aerols / fruit	8.00 ± 1.00

*Mean values ± standard deviations of triplicate determinations are reported

The immature *O. dillenii* fruits are green but become reddish-purple as they ripen. They are fleshy, almost rounded pear-shaped berries. The average weight of the whole fruit was 32.90 ± 2.60 g that of the pulp and the peel were respectively 23.58 ± 0.90 g and 9.30 ± 1.19 g. The whole fruit was 5.94 ± 0.50 cm in length and 3.20 ± 0.20 cm in width. The pulp was 3.90 ± 0.30 cm in length and 3.10 ± 0.20 cm in width. In a similar way, these values are different from that of the *Opuntia ficus-indica* species, whose weight varies between 64.50 and 106.30 g, length between 6.68 and 7.58 cm and width between 3.92 and 5.29 cm (Chougui et al., 2013). The fruit peel is filled with a few areoles of about (8 ± 1). These areoles are made up of small spines, grouped in tufts and easy to detach. On the other hand, the juicy and mucilaginous pulp is characterized by an acidic taste. It is purple in color throughout and contains many small, rounded seeds about an average of 126 ± 6 seeds in a ripe fruit. The seeds are usually yellow or pale brown, and a single seed weighted about 0.04 ± 0.01 g. Admittedly these results are well within the range of literature data reported for *O. dillenii* fruit (Embaby et al., 2016).

Physicochemical properties

The analyzed physicochemical parameters of *O. dillenii* fruits are listed in Table 2 and compared favorably with literature.

The *O. dillenii* fruit has a relatively high moisture content with a percentage up to 93.95 ± 1.70 % and a low value of dry mass (6.05 ± 0.20 %). The value of moisture was higher than those observed in literature (Medina et al., 2007; Touil et al., 2010). The variation in moisture content could be attributed to the varietal factor, the degree of ripening, and the pedoclimatic factors (Inglese et al., 2017). However, the moisture amount in the fruit is an important parameter, as it relates to the juice content of the fruit.

The ash content was 0.47 ± 0.03 % and it represents the total amount of mineral salts fraction in the fruit pulp. Similar content of ash has been reported in literature (Medina et al., 2007; Touil et al., 2010). The degree Brix designates the rate of total soluble solids. Into this study, the rate of Brix found for the *O. dillenii* was 8.32 ± 0.08 %.

Table 2 Physicochemical parameters of *O. dillenii* pulp*

Parameters	Values
Moisture (%)	93.95 ± 1.70
Dry mass (%)	6.05 ± 0.20
Ash (%)	0.47 ± 0.03
° Brix (%)	8.32 ± 0.08
pH (20 °C)	3.55 ± 0.10
Acidity (g of citric acid /100g of fresh weight)	1.40 ± 0.10

*Mean values ± standard deviations of triplicate determinations are reported

The fully ripe *O. dillenii* fruits are characterized by a high acidity (1.40 ± 0.10 g of citric acid /100 g of fresh weight). The average pH of the pulp was approximately 3.55 ± 0.10. These values characterize *O. dillenii* fruit as an acidic food. Those results were practically identical to reported data (Medina et al., 2007; Embaby et al., 2016; Loukili et al., 2021). On the other hand, the pH values for *O. dillenii* were lower than values reported for the species of *Opuntia ficus-indica* where pH ranges between 5.3 and 7. Moreover, the titratable acidity for *Opuntia ficus-indica* were considerably lower than that of *O. dillenii*; it ranges between 0.04 - 0.08 g / 100 g (El-Gharras et al., 2006; Medina et al., 2007; Chougui et al., 2013). Besides, the concentrations of total polyphenols, flavonoids and betacyanins are presented in table 3.

Table 3 Total polyphenols, flavonoids and betacyanins content of *O. dillenii* pulp*

Parameters	Values
Total polyphenols content (mg of GAE /g of dry weight)	161.20 ± 2.90
Total flavonoids content (mg of CE / g of dry weight)	30.30 ± 0.70
Total betacyanins content (mg / 100g of fresh weight)	59.97 ± 0.30

*Mean values ± standard deviations of triplicate determinations are reported

The total polyphenols content was found in high amounts in the pulp of *O. dillenii* (161.20 ± 2.90 mg of GAE / g of dry weight). This concentration is an asset for health, since the consumption of foods rich in polyphenols could help prevent oxidative stress disorders, also it may help keep blood sugar levels within a healthy range. Moreover, it can significantly decrease the level of bad cholesterol (Chang et al., 2008). This total polyphenol amount agrees with that found in *O. dillenii* from Tenerife Island (Li et al., 2007), however it's higher than that found in *O. dillenii* from Taiwan (Chang et al., 2008), and lower than that registered for the *O. dillenii* cultivated in Egypt (Embaby et al., 2016).

The means of total flavonoids was lower than that of polyphenols. It was 30.32 ± 0.70 mg of CE per g of dry weight, this value was higher than that reported previously (Chang et al., 2008; Katanić et al., 2019). It must be recognized that flavonoids provide several advantages to the fruit such as sensory attributes and have good nutritional and health values. By virtue of their function, flavonoids can protect blood vessels from damage caused by cholesterol (Panche et al., 2016). In addition, various researchers have revealed the correlation between flavonoids and preventing some diseases such as neurodegenerative, cardiovascular and cancer diseases (Atrahimovich et al., 2021).

The average total betacyanins level obtained in the current study was about 59.97 ± 0.30 mg / 100 g of fresh weight. This level of betacyanins found was slightly higher to those shown by several other studies (Embaby et al., 2016; Tamba et al., 2019). This characteristic made *O. dillenii* fruit a quite promising source of betacyanins pigments, which would be suitable for industrial applications (Rehioui et al., 2021).

Besides, the amounts of all these indicated components are depended on the environmental and experimental considerations such as geographic site, growing conditions (climate and soil composition), degree of fruit maturity and storage conditions (Inglese et al., 2017).

Spectrophotometric analysis of the extracted Betacyanins

The betacyanins identification was investigated by UV-visible spectroscopy. This technique is relied on the property of molecules consisting in absorbing light radiations of a determined wavelength. Figure 3 displays the UV-visible absorption spectrum (250 - 800 nm) recorded for the betacyanins extracted from *O. dillenii* fruits at 298 K, and compared with the spectrum of a commercial beetroot red colorant (E162) as reference.

The spectrum shows that the betacyanins extracted from *O. dillenii* fruit absorbed light strongly with an intense main absorbance peak at a maximum of 538 nm. This band corresponds to the group of betacyanins, and mainly to betanin. This is in accord with the commercial colorant E126. In addition, there is also a second absorbance peak at the range 270 - 280 nm, which is attributed to the structure of cyclo Dopa since the vast majority of natural betacyanins are derived from the combination of cyclo-DOPA and betalamic acid (Sanchez-Gonzalez et al., 2013). The conjugated double bond system of the Betalamic acid moiety constitutes the

chromophore responsible for this absorption and consequently, the vivid color (Rodriguez-Amaya, 2018).

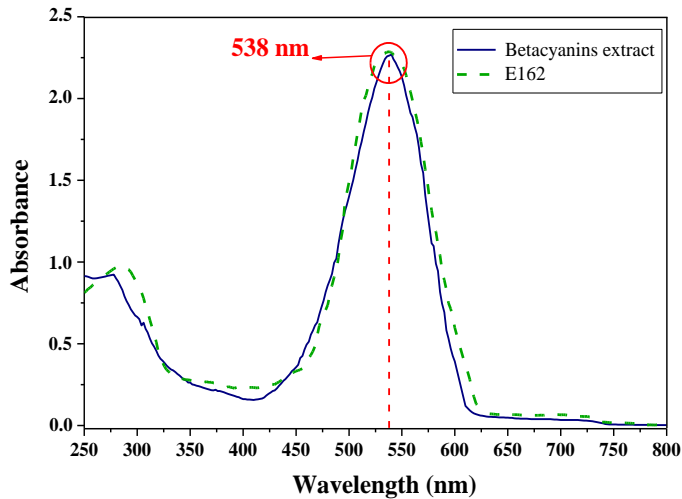


Figure 3 Visible light absorption spectra of the extracted betacyanins at 298 K.

pH effect on betacyanins stability

Figure 4 shows the spectra of the absorbance variation as a function of the wavelength λ for betacyanins in buffer solutions presenting different pH levels ranging from 0.5 to 13.5.

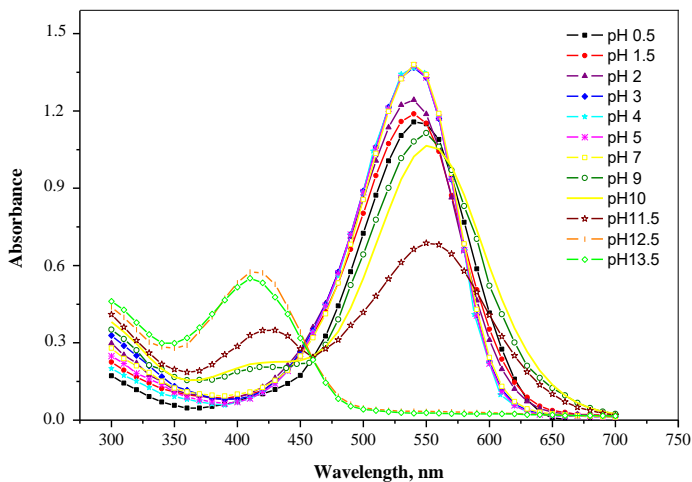


Figure 4 Absorbance spectra at different pH.

As it is conventionally observed from Figure 4, the absorbance is influenced by the pH of the solution. At $\text{pH} < 9$, the pigment has a main peak between 538 and 540 nm. For $\text{pH} < 2$, the wavelength remained in the vicinity of 540 nm, on the other hand, a slight hypsochromic shift of the absorbance maximum is noted. For pH of 9, 10 and 11.5, there is a bathochromic shift of the maximum absorbance, the main peak is in the vicinity of λ_{max} (550 nm). Beyond $\text{pH} = 12$, the main peak of the pigment is displaced near λ_{max} (410 nm). In parallel, the absorbance intensity of the visible spectra has decreased.

Thereby, the color of betacyanins is thus dependent on the pH. Indeed, betacyanins present various colors at different pH values. This change in color is due to the modification of the chemical structure of the molecule of betacyanins during the passage from the acid form to the basic form. In fact, at $\text{pH} < 9$, the solution is pink in color. From $\text{pH} = 9$ the solution turns purple, and beyond $\text{pH} = 12$ the betacyanins hydrolyze and the solution turns yellow.

Temperature effect on betacyanins stability

Figure 5 depicts the evolution of the absorbance as a function of time and temperature. As can be seen from Figure 5, at 4 °C and 20 °C, the absorbance of betacyanins is stable for up to 4 hours of incubation, then a slight decrease in the absorbance of betacyanins. When the temperature is increased to 40 °C, the results show that the absorbance of betacyanins decreases incessantly with the heating time. From 60 °C, betacyanins tend to degrade more quickly, since there is a clear and marked decrease in the absorbance of betacyanins.

We have found that over time betacyanins tend to degrade gradually regardless of temperature, even when stored in the refrigerator (4 °C). However, the increase in

temperature causes a gradual reduction in the pink color and eventually the appearance of a light yellow-brown color. The influence of temperature on the rate of absorption is probably due to the decomposition of the chemical structure of the molecule from different reactions, such as isomerization, deglycosylation, dehydrogenation or hydrolysis, which consequently reflected in loss of pink color (Bassama et al., 2020). All these data confirm that betacyanins are very sensitive to temperature; their degradation rate accelerates with the increase of temperature and heating time. The same results have been observed in several research studies concerning the influence of temperature on betacyanins (Caldas-Cueva et al., 2016 ; Wong et al., 2014).

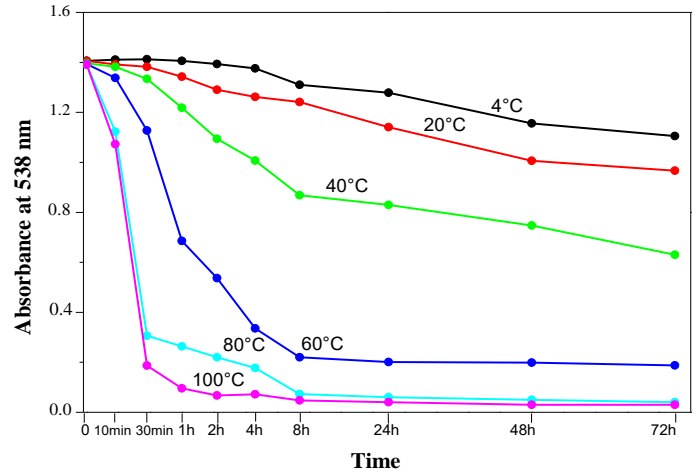


Figure 5 Evolution of the influence of temperature on betacyanins as a function of time

Light effect on betacyanins stability

Light is another crucial factor that affects the stability of betacyanins. Figure 6 presented the variation of absorbance as a function of time for samples stored in the dark and in the presence of light.

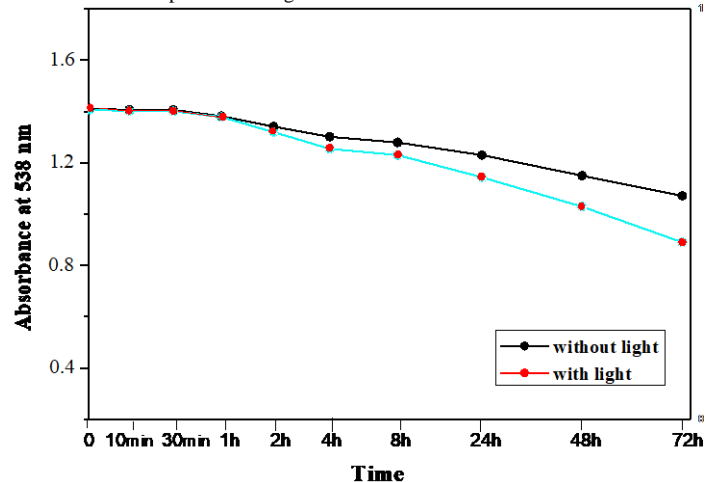


Figure 6 Variation of absorbance with and without light vs time.

According to Figure 6, we noticed that the shape of the curves with or without light begins to separate after one hour of exposure but keeps the same trend. In so far as the degradation of betacyanins under the effect of light is explained in the literature by the absorption of light which leads to the excitation of the electrons of the chromophore of betacyanins towards more excited states, causing a higher reactivity or lower activation energy for the molecule (Azeredo et al., 2009; Quina et al., 2018).

Effect of different salt concentration on the stability of betacyanins

In general, chloride salts are used in food industry as stabilizers agents, and they can have a similar effect on red colorants. For this, the effect of sodium chloride is evaluated as an additive to stabilize the extracted betacyanins. In order to optimize the adequate salt concentration, different amounts of NaCl (2, 4, 8, 10 g/L) are added to the samples and the absorbance spectra are recorded at 538 nm (Figure 7).

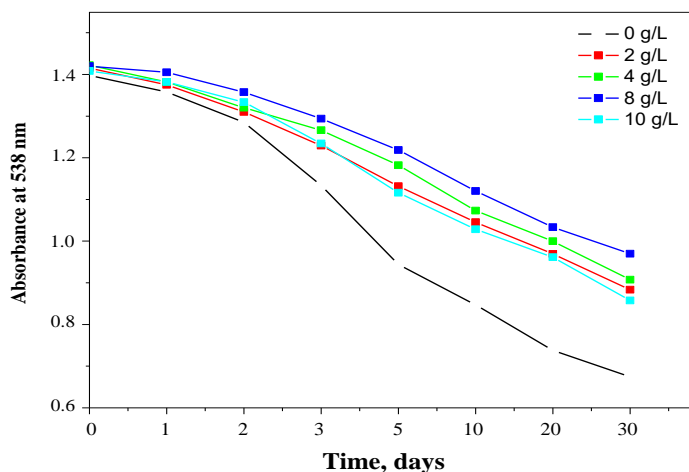


Figure 7 Absorbance spectra as a function of NaCl concentration and time.

The results illustrated in Figure 7 showed that adding different concentrations of sodium chloride could influence the stability of betacyanins. In fact, the presence of Na^+ cations and Cl^- anions contribute to delaying color loss, thereby improving color stability compared to the standard (without added salts). Moreover, it is interesting to note that, the addition of salt increased the intensity of the color with time. The concentration of 8 g/L of NaCl promoted a higher stability of the pigment than the other concentrations. However, if the salt concentration is higher, the color stability decreases, this may be because if the concentration of Na^+ cations is high, these can accelerate betacyanins to be regenerated into two products, betalamic acid and cyclodopa-5-Oglycoside, in unstable form.

Antioxidant properties

Recently, the use of natural antioxidants has generated ongoing interest, due to their therapeutic properties. They could be used in epidemiological studies to assess their application as functional products in food and pharmaceutical industries, and to replace synthetic antioxidants, which have been signaled to be toxic. It is obvious that the antioxidant activities of plant extracts cannot be characterized by a single method, but it is necessary to use at least two systems to establish the authenticity of the results. Within this context, we carried out the antioxidant property of the extracted betacyanins using two different tests: free radical scavenging activity (DPPH) and total antioxidant capacity (TAC). Butylated hydroxytoluene (BHT) is used as a standard for the positive control to make the comparison in the two tests. Indeed, BHT is a synthetic antioxidant that is widely known and used as an additive in the food, cosmetics, and pharmaceutical industries (Ousji et al., 2020).

DPPH free radical scavenging assay

The DPPH free radical reduction method is widely used to assess free radicals scavenging activity due to the ease of the reaction. The achieved results are reported in Table 4. In this test, the antioxidant capacity was measured colorimetrically in terms of IC_{50} . This parameter was determined graphically from the plot of the inhibition percentage as a function of different concentrations of the betacyanins. Generally, IC_{50} is inversely proportional to the antioxidant capacity of the compound because it reflects the amount of antioxidant necessary to reduce 50 % of the free radical. From the results presented in Table 4, the IC_{50} value for betacyanins extract was 2.41 ± 0.04 mg / mL. By comparison with the standard (BHT), we found that our value was significantly lower than the IC_{50} recorded for BHT (7.31 ± 0.05 mg/mL). Assuming that a lower value of IC_{50} value indicates a greater antioxidant activity of the compound (Soro et al., 2018), then betacyanins have a powerful antioxidant power. This power is due to the structural conformation of betacyanins which makes it possible to reduce the group susceptible to the DPPH radical due to its ability to release a hydrogen atom.

TAC total antioxidant capacity assay

The TAC assay is a quantitative method that gives a direct estimate of the overall antioxidant activity of extracted betacyanins and their ability to neutralize free radicals in a system.

From Table 4, TAC method expressed in ascorbic acid showed a significant antioxidant capacity of betacyanins with a value of 273.30 ± 1.40 mg AA / g of extract. This value is much higher than that of BHT standard (168.30 ± 1.05 mg AA / g of extract). This confirmed the strong antioxidant activity of the betacyanins extract since a higher value of TAC indicates a higher antioxidant power.

In line with these results, the antioxidant activity by both DPPH and TAC methods revealed that betacyanins extracted from *O. dillenii* fruit are a potent antioxidant, and this is owing to their chemical structure. Specifically, the betacyanins molecules are very good donors of electrons and protons. In fact, the basic

structural units of almost all betacyanins are made up of glycosides of aglycones (betanidine / isobetanidine) (Miguel, 2018), which contain proton donor groups as hydroxyl groups and imino groups which lead to higher antioxidant activity. Moreover, betacyanins molecules contain a monoglucosylated O-diphenol group and a cyclic amine group, whose partial structure resembles that of ethoxyquin, that has been reported as a highly potent antioxidant (Kanner et al., 2001). Ultimately, the used analytical methods (DPPH and TAC) are complementary and allowed us to confirm the obtained results and to clearly show that betacyanins extracted from *O. dillenii* fruit exhibited very strong antioxidant activity, suggesting that the betacyanins could become a useful source of both natural antioxidants and natural colorant agents.

Table 4 Antioxidant activities of the betacyanins extracted from *O. dillenii**

Bioactive compounds	Antioxidant activities	
	DPPH assay (IC_{50} (mg/mL))	TAC (mg AA/g)
Betacyanins	2.41 ± 0.04	273.30 ± 1.40
BHT	7.31 ± 0.01	168.30 ± 1.05

*Mean values \pm standard deviations of triplicate determinations are reported

CONCLUSION

In this work, we have focused on the study of the physicochemical characteristics of *O. dillenii* fruits grown in Morocco (Essaouira region). The obtained results showed that the fruit pulp is acidic and mostly made up of water with a ratio of 93.95 ± 1.70 %. The fruit pulp exhibited high contents of ash (0.47 ± 0.03 %) and degree Brix (8.32 ± 0.08 %). Moreover, the fruit pulp is rich in polyphenols with an amount of 161.21 ± 2.90 mg of GAE /g of dry weight, flavonoids with an amount of 30.30 ± 0.70 mg of CE /g of dry weight and a large amount of betacyanins pigment (59.97 ± 0.30 mg of BC / 100 g of fresh weight). The study of the stability of betacyanins revealed that several factors can affect its stability, the most important of which is high temperature. Regarding the in vitro antioxidant activity, the extracted betacyanins revealed significant antioxidant activity in the DPPH, and the TAC tests.

According to these results, cactus pear '*O. dillenii* fruits' are considered as a potential source of important bioactive compounds and pigments with intrinsic antioxidant activity. The outcomes would provide new avenues to further enhance the use of this cactus fruit species in the food, medicine, pharmaceutical and cosmetic industries.

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