

Physicochemical Characterization of Pomegranate (*Punica Granatum L.*) Native to Jordan During Different Maturity Stages: Color Evaluation Using the Cielab and Cielch Systems

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ABSTRACT

Punica granatum L. study is interesting for the functional properties associated to its Phenolic compounds and high volumes of production of this fruit in Jordan. The pomegranate type "pale arils" from Abu Ziyad valley were evaluated during different stages between 20 to 140 days after full bloom, with techniques of chelation, colorimetry and spectrophotometry. The results show that the increasing of sphericity (0.79 to 0.91), size diameter (65.58 to 90.41 mm), juice yield (46.03 to 55.67%), arils mass (31.70 to 61.23%), TSS total soluble solids (11.01 to 16.80), pH (2.7 to 3.6) and anthocyanins (7.43 to 12.41 Cyannidine 3 glucoside/100 g) were associated with the decreasing of thickness of the rind (4.9 to 2.77 mm), TTA Titrable total acidity (3.5 to 1.1%), TPC Total phenolics (389.61 to 251.52 mg Gallic acid/100 g), flavonoids (0.73 to 0.40 mg Catechine/g), tannins (8940.11 to 3324.79 ppm), AA Antioxidant Activity (63.41 to 48.84%) and Ascorbic acid content (28.91 to 9.48 mg/100 g); presenting significant differences $p \geq 0,5$. The paleness of rind and arils is positively related to the anthocyanin content expressed by "a" and "C" color coordinates. The actual investigation demonstrates that high values TSS-pH, low content of tannins and TTA could be successfully indicators of maturity stages rather than the concentration of anthocyanins.

Keywords: Anthocyanins, antioxidant capacity, aril juice color, flavonoids, phenols.

INTRODUCTION

The Abu Ziyad valley forms part of rift valley depression where it has an elevation range between 0 to 100 m below sea level, presenting an annual average temperature of around 12°C and rainfall ranging from 350–300 millimeter (Fig. 1).

The growing scientific interest of pomegranate is due to the functional properties produced for its *Phenolic* compounds. The term "functional" is understood as the potentially positive effect of a food on health beyond basic nutrition (Pérez, 2014). Pomegranate would represent a potential market due to the rise production in the last production years: 2016, 2017 and 2018 from 12000, 16000 to 21000 tons respectively, with projection to a probable rise (<http://moa.gov.jo/ar>).

Nowadays the arils, rind, seeds, or mixture of these fractions of the pomegranate (Fig. 2), are used in the treatment of arteriosclerosis and constitute subjects of continuous studies for their possible anti-carcinogenic, anti-microbial and antiviral properties (Shams *et al.*2011).

Ascorbic acid is one of the compounds of the pomegranate, which acts as antioxidant, and cannot be synthesized by the human body, having to be taken from vegetables and fruits.

TPC are organic compounds with variable molecular structures, which contain at least one phenol group and an aromatic ring linked to a hydroxyl group.

Anthocyanins are water-soluble pigments responsible of the coloration in leaves, flowers and fruits. They are glycosides linked to a sugar by means of a glycosidic bond.

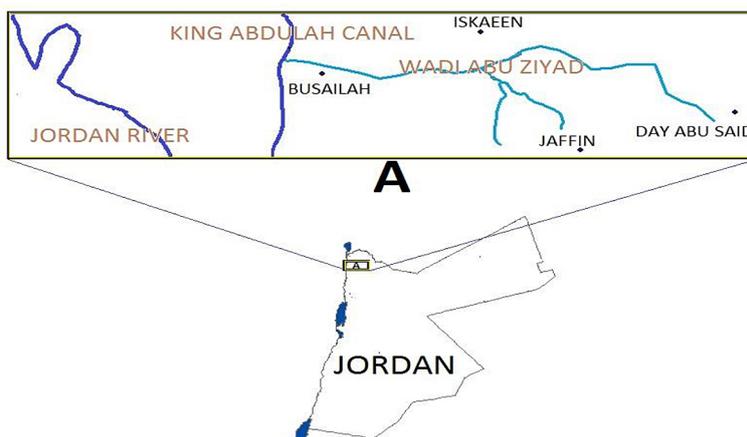


Fig. 1. Location of Abu Ziyad Valley (A) on Jordan map

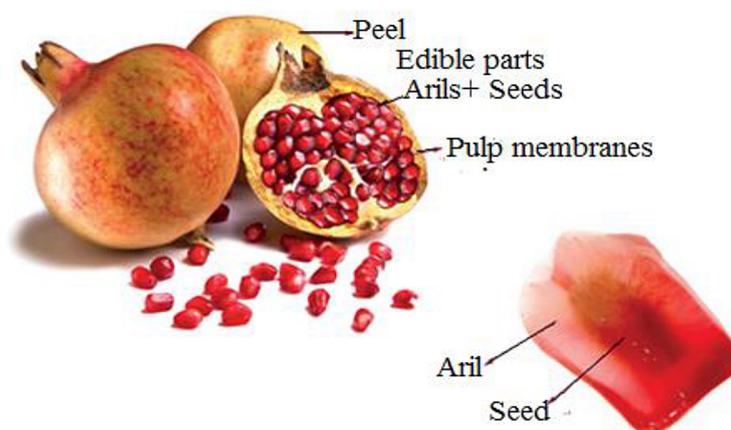


Fig. 2. Parts of *Punica Granatum L.*

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Condensed Tannins or *Proanthocyanidins*, are polymeric molecules of flavonoids soluble in water and insoluble in organic solvents, which contains non-nitrogenous structures. They produce a slight degree of astringency, bitterness and characteristic odor in plants acting as defense against herbivores. *Flavonoids* act as chemical indicators, producing characteristic as flavor, sweetness and aroma to the fruits.

As far as is known, there is no literature available about the characteristics of the pomegranates of the Abu Ziyad valley. Hence, the conducted study is focused on the identification and comparison of the phenolic-chemical and physical parameters across different maturity stages. Furthermore, the color characteristics have not been extensively analyzed in this type of studies, for this reason it is taken into account within the physical parameters.

MATERIALS AND METHODS

The experimental studies were carried out during the periods of September 2019 to August 2020, in the Departments of Chemical Engineering of the Universities Technical of Tafilah-Jordan and Valladolid-Spain.

Physical Characterization

The selection of the samples was made at random. They were weighed by an electronic 0,001g scale (Metler model AE100). The equatorial diameter, latitudinal length and rind thick were determined by Vernier digital precision 0,001 caliper. The *moisture* content was determined by applying the AOAC method (1980. №20.013). *Real density* was calculated as the quotient weight and volume.

Sphericity (ϵ) was quantified by using the method followed by Pérez (2004). The variables a , b , c are diameters being c the longest diameter and ϵ is calculated using the equation:

$$\epsilon = (abc)^{1/2} / c \quad (1)$$

Determination of the color

The *color of the rind* samples was measured by means of a Minolta model CR-410 colorimeter (Pro-Cereal Laboratories, Valladolid-Spain). The color was assigned using the Cartesian coordinates L , a , b of the CIELab system. The parameter a moves between green and red of the space color, where $+a$ and $-a$ represent redness and greenness degree respectively. The parameter b moves between blue and yellow where the $-b$ and $+b$ indicate the blueness and yellowness degree, respectively. Luminosity L represents the clarity degree of the sample, which moves between $L = 0$ (black, maximum darkness) and $L = 100$ (white, maximum clarity). Chroma "C" describes the percentage of purity ($C=100$) and the faintness of the color ($C=0$). Chroma is calculated using the equation:

$$C = (a^2 + b^2)^{1/2} \quad (2)$$

The CIELCh polar system includes the hue angle h , which describes the predominant color that an observer perceives, where 0° =red-purple, 90° =yellow, 180° =bluish-green and 270° blue (McGuire, 1992). Hue angle is calculated by the following equation:

$$h = \arctan(b/a) \quad (3)$$

The *color of the arils juice* was determined by spectrophotometry, using the method provided by Eaton *et al.* 1995 and Alvarez (2018) for liquid samples.

Statistical analysis

Three replicates were taken from each sample and the results were used for statistical analysis and reported as mean \pm SD. The regression analysis and Pearson's correlation coefficients were calculated by the SPSS program and values of differences $p < 0.5$ were considered significant.

Chemical characterization

The samples of arils juice were characterized in the following parameters:

TTA expressed as % citric acid was measured with the AOAC method 1980 №942.15. It was calculated using the equation (4), where V_{NaOH} is the base volume (cc) and m the sample mass (g).

$$TTA = V_{NaOH} \cdot N_{NaOH} \cdot 192.100 / (3m \cdot 1000) \quad (4)$$

TSS was measured using a ABBE Carl Zeiss refractometer 89553 model and reported as Brix degrees at 25°C . The *maturity index* (*MI*) was calculated by using the equation:

$$MI = \text{Brix} / (TTA) \quad (5)$$

Ascorbic acid (vitamin C) content was determined following the AOAC(1980) № 967.21 official method, and reported as mg. Ascorbic acid/100 grams.

TPC was determined by applying the Folin-Ciocalteu method (Makkar *et al.* 2000), reporting the results as mg. Gallic acid (GAE)/100g.

The *total flavonoids* content of the arils extract was quantified using the method proposed by Shams *et al.* 2011; which is based on the chelation technique with AlCl_3 . The results are expressed as mg. Catechin /g dry extract.

The *concentration of anthocyanins* was evaluated quantifying the predominant anthocyanin present, cyanidine 3-glucoside. The official method AOAC (2003), No 2005.02 was followed, expressing the result as mg cyanidine 3-glucoside/100 g.

Condensed tannins were quantified using the Bate-Smith assay (Cáceres *et al.* 2013). The results were reported as mg.catechin/gram of sample.

Antioxidant capacity was evaluated by means of the method described by Moon and Terao (1988). The absorption at 517 nm was used to calculate the antioxidant capacity as follows:

$$\text{Antioxidant Activity} = (1 - [A_{\text{sample } 517\text{nm}} / A_{\text{standard } 517\text{nm}}]) \cdot 100 \quad (6)$$

RESULTS AND DISCUSSION

Physical characterization

The fruit samples were taken between 20 to 140 days after full bloom. The parameters evaluated are shown in Fig. 3, which presented significant difference $P < 0.05$. At full ripening stage (140 days), the physical analysis recorded maximum values of diameter $D = 90.41\text{mm}$; latitudinal length 82.03mm and juice yield 55.67% , which revealed an increasing of 39% , 41% and 20% compared with the measurements at the first maturity stage (Fig. 3A). The results at ripening stage of diameter, latitude length; arils mass and juice yield were 8% ; 14% ; 6.6% and 12.14% higher than the results presented by Zarei *et al.* 2011 for the “Rabba e Fars” pomegranate at same maturity stage.

The increasing of diameter size, moisture, mass of arils and yield juice along maturity stages, were associated with the reduction of thickness rind across all maturity stages consequence of the ripening of the fruit (Fawole *et al.* 2011). The values of sphericity reported were closer to one at stages near to full ripening ($\epsilon=0.91$) (Fig. 3A).

The values recorded across all the maturity stages for the thickness of rind and % seeds, revealed a clear inverse relationship between them (Fig. 3A); corroborated for Pearson’s coefficients close to one (Fig. 3B), and were in accordance with Gozlekei *et al.* (2011) for “Turkish pomegranate”.

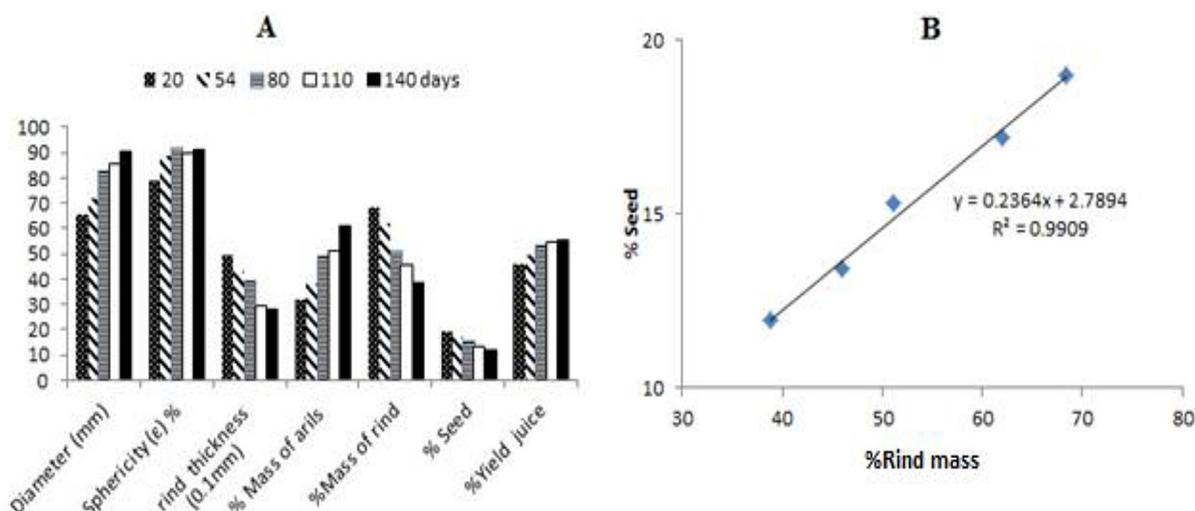


Fig. 3. Evolution of the physical properties (A) and correlation of percentage seed and rind mass (B)

Chemical evaluation

Some of the main chemical and phenolic parameters evaluated are presented in Figure 4. The increasing TSS values recorded from 11.01 to 16.81° Brix; denoted a clear increase of sugars level from the unripe to full ripe stage together with MI (from 3.51 to 15.27) associated with the maturity process. The maximum TSS and MI reached for the ripe fruit were not associated with the peculiar pale color of arils; MI being exclusively affected by acidity, sweetness and genetic of the fruit.

The pH and TTA are relevant properties for the conservation of the fruits contributing greatly to their stability (Pérez, 2017). The values of TTA and pH recorded, ranged from 3.5% to 1.1% and from 2.7 to 3.6 respectively; revealing continuous decrease during the maturity stages due to change in the content of malic and citric acids (Fig. 4A). The results demonstrate a clear inverse correlation between TTA and pH in accordance with the work presented by Labbe *et al.* (2016) for Chilean pomegranates variety “Wonderful”, “Chaca” and “Copa”.

The TPC results, ranged from 389.61 to $251.54\text{mg Gallic ac./100g}$. The ascorbic acid content decreased from 28.9 to 9.48mg/100g and the Antioxidant activity (AA) ranged from 63.41% to 48.845 . The recorded values of ascorbic acid content, TPC and AA showed a tendency to continuously decrease across all maturity stages, leading to deduction of a direct relationship between them similar to the results presented for

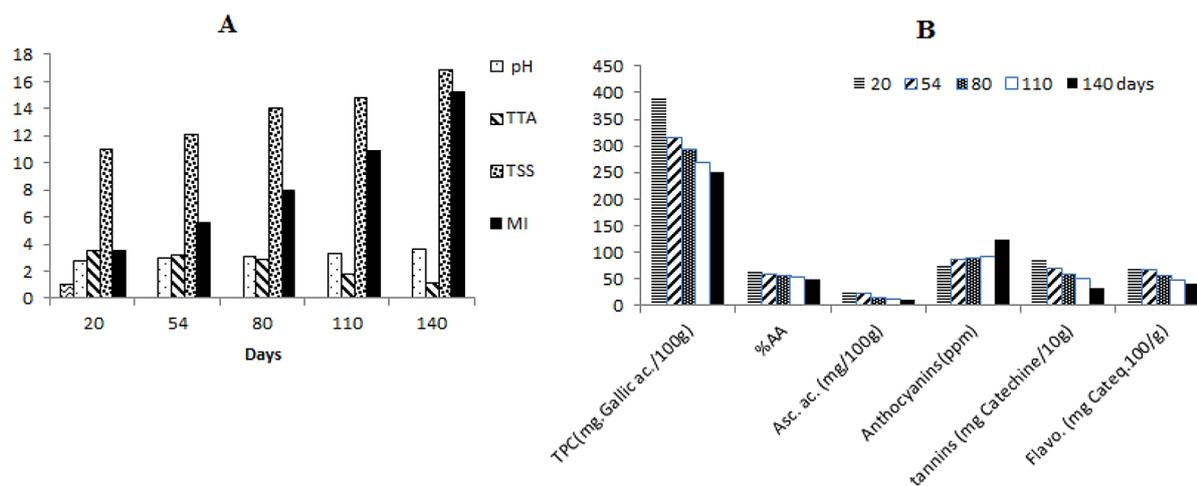


Fig. 4. Change of chemical properties

Gil *et al.* (2000) and Fisher (2011). Probably, this correlation occurs because of the existence of functional similarities between the Ascorbic acid content, TPC and AA. Ascorbic acid acts as an active antioxidant in oxidation and reduction reactions, associating the content of ascorbic acid to the antioxidant capacity of fruits, stimulating each other, and then creating metabolites that increase the TPC levels (Kulkarni *et al.* 2005). The decreasing of TPC content with the increasing of MI during maturity stages (Fig. 4 A-B), probably occurs due to the participation of TPC in the biosynthesis mechanism of flavylum anthocyanin ring at maturation stage, with the consequent reduction of TPC levels in concordance with Kulkarni *et al.* (2005).

The *Anthocyanins* content recorded the values from 7.43 to 12.41 mg cyanidine-3-glucoside/100g, increasing along maturity stages, which contributes directly to the appearance of color across maturity stages, similar to the studies presented by Radunic *et al.* (2014).

The paleness of the arils and rind of Abu Ziyad’s pomegranate was justified by the low anthocyanins content reached across all maturity stages. The anthocyanins levels at full ripening stage obtained in this study (12.4111 mg. Cyanidin-3-glucoside/100 g), were at range of the values reported by Garcia (2016) for pale pomegranates from “Mollar Elche” – Spain (11 mg. Cyanidin-3-glucoside/100g), and falling within the range specified for Zaouayet *et al.* (2012) for Tunisian pomegranate; from 5;10 to 49 mg/100 ml with an average of 25 mg/100ml (Fig. 5B).

Other varieties of pomegranate showed high values of anthocyanins content ranging from 24 to 100 mg Cyanidine-3-glucoside/100g, such as the case of pomegranates from “Mexico-Queretaro” (Diaz, 2014), “Wonderful” and “Chaca” (Labbeet *et al.* 2016) and “Rabb e Fars” (Zarei *et al.* 2001), depending mainly of the pomegranate variety (Fig. 5A).

The results of *Condensed Tannins* and *Flavonoids* showed an inverse correlation with *MI*.

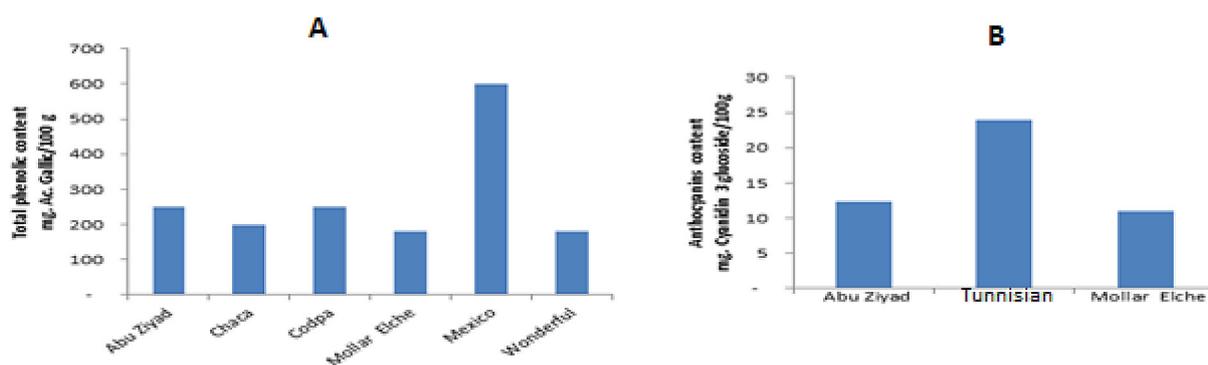


Fig. 5. Anthocyanins and TPC contents for different cultivars of pomegranate at full ripening stage

Table 1. Results of CIELAB and CIELCH coordinates

Fraction	ARILS					RIND				
	20	54	80	110	140	20	54	80	110	140
C	22,04 ±0,31	22,50 ±0,13	22,81 ±0,42	23,61± 0,31	25,01 ±0,11	24,61 ±0,21	24,02 ±0,31	25,03 ±0,20	26,60 ±0,19	27,30 ±0,61
h	87,39 ±1,1	69,13 ±0,81	61,18 ±0,54	51,53 ±0,67	53,94 ±0,43	83,01 ±0,35	73,34 0,53±	63,94 ±1,02	55,72 ±1,51	48,52 ±1,41
L	72,03 ±0,32	70,50 ±1,67	70,11 ±1,45	69,91 ±0,88	69,32 ±0,76	79,52 ±0,67	71,24 ±0,56	71,04 ±1,76	70,12 ±1,65	72,11 ±1,87
a	1,10 ±0,13	8,01 ±0,09	11,03 ±0,54	14,70 ±0,67	17,05 ±0,87	3,04 ±0,64	7,18 ±0,46	11,02 ±0,17	15,01 ±0,28	18,12 ±0,34
b	22,10 ±0,71	21,04 ±0,98	20,11 ±1,09	18,52 ±0,76	19,07 ±0,57	24,52 ±0,47	23,11 ±0,53	22,51 ±0,65	22,05 ±0,58	20,51 ±0,71

* Three replicates for each sample were represented as mean ± standard deviation.

The Condensed Tannins and Flavonoids content decreased from 8940.1 to 3324.79 ppm and from 0.73 to 0.4 mg Catechine/g respectively. The decreasing of the flavonoids content during all maturity stages may be because the flavonoids structure as” (o) condensed tannin” is synthesized as anthocyanins, through an increase in enzymatic activity as anthocyanins synthase (AS) and 3-glycosyl transferase (3GT) until the ripening stage is achieved (Labbe *et al.* 2016; Critosto *et al.*2000).

Analysis of the physical parameter of color

The results of color coordinates are presented in Table 1, Figure 6 and 7. Analysis of the results of the CIELab Cartesian coordinates for “*aril juice*» and “*rind*” during maturity stages, shows

that the +a coordinate gradually increased (red-ness) while the +b and L coordinates decreased and the highest values of +a were achieved at full ripening stage.

The increasing of +a means that the green hue was gradually replaced by the red one. The decreasing of +b indicate that the blue pigments were replaced for the yellow color and the slight fall-off in L values indicated that the brightness has decreased only a little bit during fruit maturation of (Manera *et al.* 2012). Similar results were observed for the pomegranates variety” cv. Rubi”(Olaniyiet *al.* 2013) and “Mollar” ascensions (Gil *et al.* 1995).

The values C and h for arils juice and rind, revealed an inverse relation between them, indicating the quadrant of color diagram and the angle

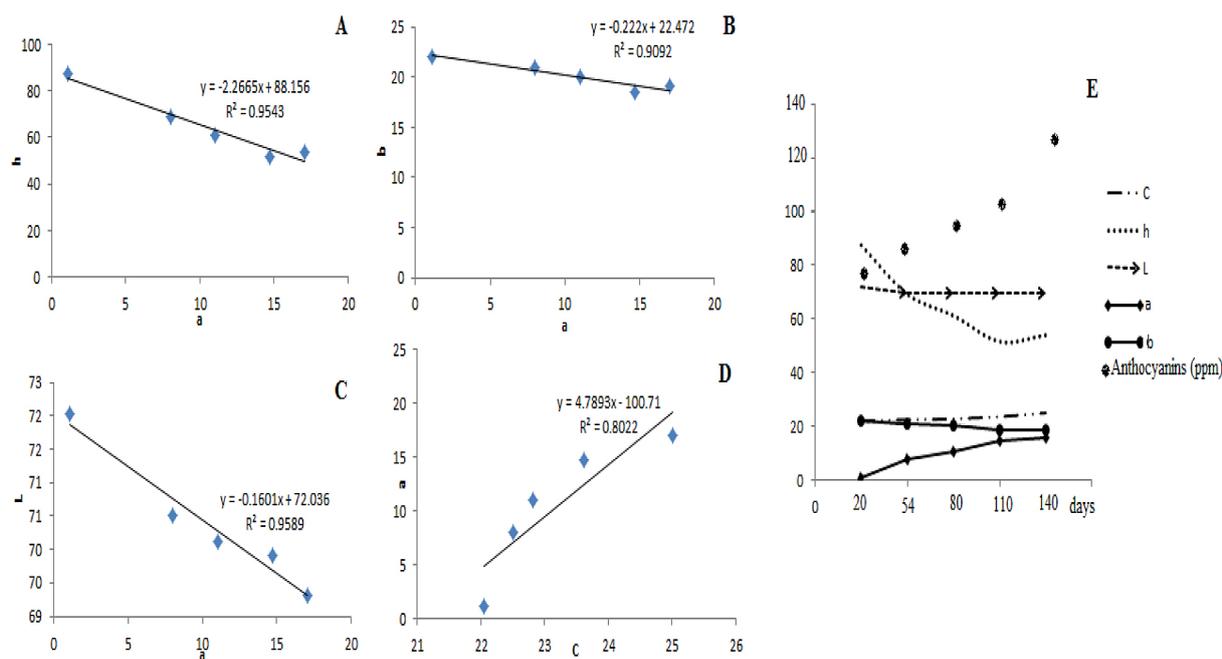


Fig. 6. Correlation between CIELab and CIELCh coordinates and evolution respect to the Anthocyanins content

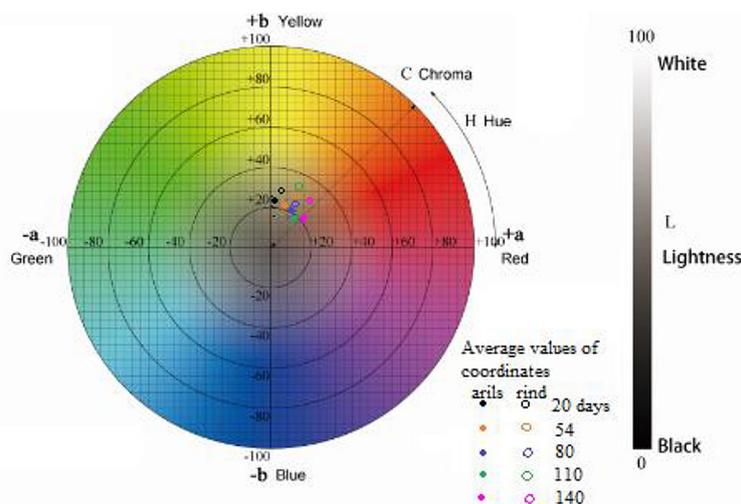


Fig. 7. CIELab and CIELCh coordinates in the color space

with respect to the axis of coordinate $+a$. These results are similar to the discussed for Manera *et al.* (2012) for pomegranate accession “Mollar de Elche 15”. The correlations of the coordinates a , L , b and C , h are demonstrated by the Pearson’s coefficients close to one, represented in Figure 6A, B, C, D. The evolution of the coordinates of redness $+a$ and saturation C for aril and rind fraction, would indicate that they are associated with the gradual increasing of the anthocyanins during maturity stages (Fig. 6E), associated for Pearson’s correlation factors close to one, shown in the Figure 6D.

CONCLUSIONS

The high MI, sweetness, low levels of tannin and acidity achieved for Abu Ziyad’s pomegranates, make this variety of fruit, suitable for fresh consumption. The “aril paleness”, is justified by the low anthocyanin content reached during the maturity stages. The study of physical properties revealed that the thickness of rind across maturity stages showed an inverse correlation with arils mass, percentage of seeds and arils moisture. The inherent color between arils and rinds is due to an interaction between the phenolic- chemical components, exchanged through the porous structure of the arils and rind. Which can be detected by the closeness of “ $+a$ ”, “ C ” and “ b ” coordinates of these fractions in the color diagram. Finally this investigation demonstrated that high values TSS-pH, low content of TTA, and total tannins could be successfully use as indicators of maturity stages rather than the concentration of anthocyanins.

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