

# Relationships between Fruit Acceptability and Health-Case of Seven Pomegranate (*Punica granatum* L.) Juices

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## ABSTRACT

The objective of this study was to evaluate acceptability and instrumental measurements (titratable acidity, total soluble sugar, total phenolic, flavonoids, anthocyanins, and condensed tannin contents as well as antioxidant capacity) of seven Tunisian pomegranate cultivars to relate fruit acceptability and beneficial health. Seventy one participants were asked to complete a questionnaire and to indicate their level of acceptance, on a nine point-hedonic scale, for fruit size, sweetness, bitterness, juice color and juice overall acceptability. Taste and aril color were the main criteria to select Pomegranate. Significant differences occurred for all the acceptability tests. Titratable acidity determines the taste and important inter-specific variability in the phenolic contents as well as for the antioxidant capacities was noticed. Anthocyanin and condensed tannin were contributing to the organoleptic properties while bitterness acceptability was negatively correlated to the antioxidant capacity. Also, a relationship was found between the fruit size acceptability and the antioxidant capacity.

**Keywords:** Pomegranate Juice; Acceptability Tests; Instrumental Measurements; Phenolic Contents; Antioxidant Capacity

## 1. Introduction

“The eyes are the gatekeeper to the mouth”. Color was suggested to be the most influential quality factor, as consumers have expectations of overall quality based on color [1]. Most of the yellow, red and blue colors in fruit are related to the flavonoids [2]. Flavonoids are a group of polyphenolic compounds naturally present in a wide variety of fruit and vegetables. Fruit and vegetable consumption decreases the risks of coronary heart disease and lung cancer probably to their phenolic compounds [3].

Phenolics are secondary metabolites synthesized by plants, both during normal development and in response to stress conditions such as infection, wounding and UV radiation, among others [4]. In plants, phenolics may act as phytoalexins, antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective agents against UV light, among others. In food, phenolics may contribute to their organoleptic properties,

such as, bitterness, astringency, color, flavor, odor and oxidative stability [5].

Among colored fruits, containing phenolics there is the Pomegranate [6,7]. The Pomegranate (*Punica granatum* L. Punicaceae) is a fruit becoming more popular because of its healthy properties [8]. These healthy properties come from the high antioxidant activity of the phenolic compounds content of the Pomegranate fruit [8,9]. However, it is surprising that the literature focused on the antioxidant properties of the phenolics but little is yet known about their contribution to the organoleptic properties of the Pomegranates.

Thus, the present study aims at relating the phenolic contents to the organoleptic properties of the Pomegranate juices as perceived by a panel of participants. To achieve this objective, a collection of seven Tunisian pomegranate cultivars differing in fruit internal color (from light pink to deep pink), taste (from sour-sweet to sweet) and fruit size (from small to large) was examined. The results were used to test for correlations between acceptability tests, physicochemical properties, phenolic con-

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tents and antioxidant capacity. This study allows identifying a possible link between the acceptability of Pomegranates and the protective effect of their phenolic compounds.

## 2. Materials and Methods

### 2.1. Participants

Seventy one adults, eligible and volunteers (40 men and 31 women) were recruited through ad wall in the Institute of Arid Regions of Medenin in Tunisia during the two last weeks of October 2008. Criteria for participation were: age from 18 to 55 years, regular consumer of pomegranate, no experience in sensory analysis, not being allergic to any food and availability for the test. Students and staff of the Institute of Arid Regions who work-ed on pomegranate were excluded in this study as it was thought they may affect the outcomes with their advanced skills on pomegranate taste. Participants reported their answers on individual ballots.

### 2.2. Samples

Seven Tunisian pomegranate cultivars differing in fruit size, juice color and juice taste were examined (Table 1). The fruits were classified as large when their diameter

was superior to 95 mm, they were medium if their diameter was from 75 to 95 mm and they were small when their diameter was inferior to 75 mm. The juice color was determined visually while the juice taste was defined according to their sugar to acidity ratio [10]. A sugar to acidity ratio between 8 and 24 indicated sour-sweet cultivars, while a ratio from 25 to 98 referred to sweet cultivars. The cultivars included in the present study were chosen according to customary consumer purchasing practices and the answers to the questionnaire on perceptions and consumption of pomegranate. The denominations of the cultivars refer, in Tunisian, to the population where they belong or to the taste of the fruit [11]. The cultivars were selected from mature fruits grown in the collection of the Tunisian National Germplasm of pomegranate located at Zerkin (33°45'N, 10°16'E) [11]. They were cultivated under homogenous conditions, without any special management (no fertilizers, no irrigation except natural rainfall). Approximately, fifteen kg of each cultivar were picked at harvesting maturity in the last week of September 2008 and in the last week of October 2008. According to Mirdehghan and Rahemi [12], the harvest maturity for pomegranate is achieved, when the arils' weight is greater than that of the peel. Fruits were transported by ventilated car to the laboratory, and trans-

**Table 1. Codes, cultivars, main characteristics, mean value and standard deviations of instrumental analyses of the seven Tunisian Pomegranate (*Punica granatum*) studied.**

Codes	Cultivars	Main characteristics			TSS (°Brix)	TA (% MA)	TSS/TA	CD	TPC (mgGAE/L)	TFC (mgQE/L)	TAC (mgCGE/L)	CTC (mgCE/L)	ABTS IC <sub>50</sub> (mg/L)
		Fruit size <sup>A</sup>	Juice color <sup>B</sup>	Juice taste <sup>C</sup>									
CH 4	Chelfi 4	L	LP	Sweet	14.8 ± 0.2	0.2 ± 0.01	59.2 ± 20	0.3 ± 0.01	1073 ± 44	329 ± 15	93 ± 3	2070 ± 0.1	755 ± 21
KL 11	Kalaa 11	L	P	Sweet	14.0 ± 0.1	0.5 ± 0.02	27.8 ± 5	0.5 ± 0.01	1075 ± 30	120 ± 5	188 ± 7	2826 ± 0.1	498 ± 18
GR 2	Garoussi 2	L	DP	Sour-sweet	16.5 ± 0.2	1.8 ± 0.05	9.2 ± 4	1.7 ± 0.04	413 ± 21	268 ± 8	20 ± 1	1436 ± 0.1	1073 ± 22
ZG 11	Zaghouani 11	M	P	Sweet	14.5 ± 0.1	0.4 ± 0.01	36.2 ± 10	1.5 ± 0.03	1316 ± 41	679 ± 21	40 ± 2	2507 ± 0.1	638 ± 21
JB 1	Jbeli 1	M	DP	Sweet	15.8 ± 0.2	0.4 ± 0.01	40.0 ± 20	0.8 ± 0.01	1382 ± 62	1100 ± 32	77 ± 3	2217 ± 0.1	492 ± 17
CH 2	Chelfi 2	S	LP	Sweet	15.0 ± 0.2	0.5 ± 0.02	30.0 ± 10	1.0 ± 0.02	1232 ± 31	264 ± 7	25 ± 1	1458 ± 0.1	1342 ± 33
NB 1	Nabli 1	S	P	Sweet	15.0 ± 0.2	0.4 ± 0.01	37.5 ± 20	0.6 ± 0.01	4570 ± 185	211 ± 8	433 ± 23	5299 ± 0.2	493 ± 15
	Mean				15.1 ± 0.2	0.6 ± 0.02	34.3 ± 13	0.9 ± 0.02	1580 ± 59	424 ± 14	125 ± 6	2545 ± 0.1	756 ± 21
	Relevant SD				0.8	0.5	15.0	0.5	1357	346	148	1317	333
	CV (%)				6	90	44	57	86	82	118	52	44

All values are reported as ± standard deviation; <sup>A</sup>Fruit sizes were based on diameter measurements. Diameter > 95 mm = Large fruit (L); 75 mm < diameter < 95 mm = Medium fruit (M); diameter < 75 mm = Small fruit (S); <sup>B</sup>Juice color was visually determined. LP = Light Pink; P = Pink; DP = Dark Pink; <sup>C</sup>Taste was determined according to Melgarejo, Salazar & Artés, (2000). 8 < TSS/TA < 24 = sour-sweet cultivars; 25 < TSS/TA < 98 = sweet cultivars; TSS = Total Soluble Sugars expressed as degrees Brix (°Brix); TA = Titratable Acidity expressed as percent of malic acid (% MA); TSS/TA = sugars to acidity ratio; CD = Color Density; TPC = Total Phenolic Content expressed as mg gallic acid equivalent per liter of juice (mg GAE/L); TFC = Total Flavonoids Content expressed as mg quercetin equivalent per liter of juice (mg QE/L); TAC = Total Anthocyanins Content expressed as mg cyaniding-3-glucoside equivalent per liter of juice (mg CGE/L); CTC = Condensed Tannins Content expressed as mg catechin equivalent per liter of juice (mg CE/L); ABTS IC<sub>50</sub> = radical scavenging capacity on ABTS radical expressed as concentration of the extract required to reduce 50% of the initial ABTS free radical; Relevant SD = Relevant standard deviation; CV = coefficient of Variation = Relevant SD to mean in percent.

ferred to a 4°C store room on the same day as they were harvested. To avoid possible contamination of the juices with the metabolites produced by microorganisms, fruits with cracks, cuts, sunburn and other defects in husk were disposed of and only healthy fruits of uniform size and appearance were arranged in one row in wooden boxes containing packing material during the experiments. Whole fruits were preserved for the fruit size acceptability while others were squeezed to juices for further analysis. For thus, fruits for each cultivar were manually peeled, ground in a commercial turmix blender (Moulinex) for 30 s, and filtered through muslin cloth (0.5 mm). The homogenate juices obtained were freshly utilized for physico-chemical analyses and acceptability tests.

### 2.3. Sensory Analyses

#### 2.3.1. Questionnaire

Participants were asked to complete a one-page questionnaire (Table 2) developed and administered by the author, on their consumption of Pomegranate. The questionnaire included three types of questions: 1) eat, 2) purchase and 3) medicinal effects. For each question, participants indicated their responses by a cross in front

of the answer(s) corresponding to their choice. Each participant indicated his answer(s) on an individual questionnaire.

#### 2.3.2. Acceptability Tests

Prior to the acceptability tests, participants were given a presentation outlining the methodology and the procedure of the sensory methods.

Participants evaluated the samples in one session (30 minutes). They were asked to assess, in a first time, the acceptability of the fruit size and the juice color, and, in a second time, to assess the acceptability of sweetness, bitterness and juice overall acceptability. The assessments were done by using a nine-point hedonic scale, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely [13]. The samples were presented with 3-digit codes in a balanced order to account for first order and carry-over effects [14]. The whole washed fruits were placed on white plates and the juices were presented in transparent glasses at room temperature. The assessments were done in a well ventilated and at

**Table 2. Questionnaire on perceptions and consumption of pomegranate (N = 71).**

Questions on perception and consumption of pomegranate	Frequency	% Responding
1. How often do you eat pomegranate?		
a. Once a day	14	20
b. Every two days	21	29
c. Once a week	11	15
d. Twice a week	26	36
2. What is the most important thing when purchasing pomegranate?		
a. Fruit size	15	21
b. Fruit color	23	33
c. Aril color	11	15
d. Hand-evaluated texture	16	22
e. Cultivar	6	9
3. How do you eat pomegranate?		
a. Salads	6	8
b. Desserts	17	24
c. Fresh fruit	30	42
d. Juice	18	26
4. What is the most important thing when eating pomegranate?		
a. Aril color	19	27
b. Taste	45	63
c. Texture	1	2
d. Nutrition	6	8
5. Would the pomegranate have medicinal effects?		
a. No	22	31
b. Yes	33	46
c. Maybe	16	23
6. If yes or maybe, pomegranate would be:		
a. Anti-carcinogenic	5	7
b. Anti-inflammatory	61	86
c. Anti-alzheimer	1	2
d. Anti-atherosclerotic	4	5

ambient temperature room of the Institute of Arid Regions. Participants were provided with mineral water, unsalted crackers, and expectorant cups to cleanse the palate between sample evaluations. They were instructed to pause for one min between samples.

## 2.4. Instrumental Analyses

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma-Aldrich-Fluka (Saint-Quentin France).

### 2.4.1. Physico-Chemical Properties

Titrateable Acidity (TA) was determined by potentiometry, using a pH meter, with a 0.1N NaOH solution to a pH of 8.1. The results were expressed as percentage of Malic Acid (%MA). A hand refractometer type OPTECH K7 1319 (Optical Technology, Munich, Germany) graduate of 0° - 32° Brix was used to determine the Total Soluble Solids (TSS). Results were expressed in degrees Brix (°Brix). The sugar to acidity ratio (TSS/TA) was calculated using the relation between Total Soluble Solids by Acidity. Color Density (CD) was determined by measuring the absorbance at 420, 520 and 620 nm in a cell of 1 mm using a spectrophotometer Shimadzu (Shimadzu Corporation, Kyoto, Japan) UV-1201.

### 2.4.2. Phenolics Content

The phenolics of each fruit extract were determined by the method of Geogé *et al.* [15]. The diluted aqueous solution of each extract (0.5 mL) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 mL). The mixture was allowed to stand at room temperature for 5 min before adding sodium carbonate solution (75 g/L in water, 2 mL). An hour later, the absorbances were measured at 765 nm against a water blank. A standard calibration curve was plotted using gallic acid (0 - 300 mg/L). The results were expressed as mg of gallic acid equivalents (GAE)/L of juice.

#### 1) Flavonoids Content

The flavonoids were estimated according to the Dowd method as adapted by Arvouet-Grand, *et al.* [16]. A diluted methanolic solution (4 mL) of each plant extract was mixed with a solution (4 mL) of aluminium trichloride (AlCl<sub>3</sub>) in methanol (2%). The absorbance was read at 415 nm after 15 min against a blank sample consisting of methanol (4 mL) and plant extract (4 mL) without AlCl<sub>3</sub>. Quercetin was used as reference compound to produce the standard curve. The results were expressed as mg of quercetin equivalents (QE)/L of juice.

#### 2) Anthocyanins Content

Total anthocyanin contents were measured with the pH differential absorbance method, as described by Cheng and Breen [17]. Briefly, absorbance of the extract was

measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid-potassium chloride, 0.2 M) and 4.5 (acetic acid-sodium acetate, 1 M). The wavelength reading was performed after 15 min of incubation. Anthocyanin contents were calculated using a molar extinction coefficient ( $\epsilon$ ) of 29600 (cyanidin-3-glucoside) and absorbance of  $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$ . Results were expressed as mg cyanidin-3-glucoside equivalents/L of juice.

#### 3) Tannins Content

Proanthocyanidins reactive to vanillin were analyzed by the vanillin method [18]. One milliliter of extract solution was placed in a test tube together with 2 mL of vanillin (1% in 7 M H<sub>2</sub>SO<sub>4</sub>) in an ice bath and then incubated at 25°C. After 15 min, the absorbance of the solution was read at 500 nm. Concentrations were calculated as (+)-catechin (mg/L of juice) from a calibration curve.

### 2.4.3. Antioxidant Capacity

Antioxidant capacity was measured using the improved ABTS method. The radical scavenging capacity of antioxidants for the ABTS (2, 2'-azinobis-3-ethylbenzothiazoline-6-sulphonate) radical cation was determined as described by Re *et al.* [19]. ABTS radical was generated by mixing a 7 mM of ABTS at pH 7.4 (5 mM NaH<sub>2</sub>PO<sub>4</sub>, 5 mM Na<sub>2</sub>HPO<sub>4</sub> and 154 mM NaCl) with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted with ethanol to give an absorbance of  $0.70 \pm 0.02$  units at 734 nm using Helios Alpha spectrophotometer (Thermospectronic, USA). For each sample, the diluted methanol solution of the extract (100  $\mu$ L) was allowed to react with fresh ABTS solution (900  $\mu$ L), and then the absorbance was measured 6 minutes after initial mixing. Ascorbic acid was used as a standard and the capacity of free radical scavenging was expressed by IC<sub>50</sub> (mg/L). Values calculated denote the concentration required to scavenge 50% of ABTS radical.

## 2.5. Statistical Analysis

All measurements were expressed as mean  $\pm$  standard deviation of triplicate measurements. Standard deviations (SD) did not exceed 5% for the majority of the values obtained. Acceptability data was collected manually on a MS Excel spreadsheet and was recorded with "dislike extremely" equal to one, "like extremely" equal to nine, and intermediate points numbered appropriately. Analysis of Variance (ANOVA) was performed on the qualitative data obtained from the acceptability tests with  $\alpha$  set at 0.05. Friedman's test [20], employing a significance level of  $\alpha < 0.05$ , was applied to the results of the hedonic test, to separate the means of the acceptability tests. Standardized Principal Component Analysis (PCA) was per-

formed on the mean ratings among the participants for all acceptability tests. Hierarchical cluster analysis (HCA) with the Ward criteria was applied on the first two principal components of the PCA [21]. To compare the variability in quantitative physico-chemical composition of the samples having different averages and expressing in different units, the coefficient of variation (CV) was used. It expresses the standard deviation as a percentage of the mean. The data will be considered homogeneous if the CV is less than 15%, and conversely, the data will be considered heterogeneous if the CV is greater than 15%. A Pearson correlation analysis was carried out between the instrumental measurements with a 95% significance level. A Partial Least Square (PLS) regression analysis was performed to relate the acceptability data (Y-variables) to the instrumental data (X-variables). Regression coefficients for the relations between variables revealed by the PLS regression were estimated by jack-knifing, and significance levels were determined. All the data were collected in spread sheets and the statistical analyses were done using MS Excel Xlstat 2009 (Addinsoft, New York).

### 3. Results and Discussion

#### 3.1. Questionnaire

The participants were asked to complete a questionnaire (Table 2) about their perceptions and consumptions of pomegranate. The external sensory attributes, such as appearance, color, size and hand-evaluated texture, which are evaluated by the consumer prior to consumption, are intrinsic quality cues. Taste and oral texture evaluated at the time of consumption are experience-quality attributes.

Among intrinsic quality cues, appearance (fruit size and fruit color) was the major factor (one of two) in the purchase of pomegranate fruits for the participants. Of all appearance factors, fruit color (one of three) was suggested to be the most influential quality factor, as participants have expectations of overall quality based on color, while fruit size (one of five) was considered to have minor influence on the consumer evaluation of Pomegranate quality. To our knowledge, no study was conducted on the intrinsic quality cues of pomegranates. Nevertheless, according to Kays [1], color was suggested to be the most influential quality factor, as participants have expectations of overall quality based on color, such as color cues for banana ripeness. The same author notes that at times, color expectations of quality may not be valid because, for example, some orange (*Citrus* spp.) cultivars are at their optimum when they are green, not orange as most participants perceive.

When experience-quality attributes, participants may

be intending to consume pomegranate because of its beneficial health consequences (one of ten), but taste (two of three) and aril color (two of seven) were the fundamental quality that must be satisfied for continued consumption. According to Kays [1], taste is ranked more highly than texture and appearance as a contributor to overall liking for food products; however, color and texture of horticultural products are more frequently cited as consumer quality attributes. Also, pomegranates were preferred as fresh fruit (three of seven) and juice (one of four). These results confirm those of Marlette [22], who found that apple sauce was preferred to whole apples. Thus, fruits would be preferred if they required no preparation, were ready-to-eat or not too difficult to eat.

Results from this study show that the size of the fruit, the taste, the color of the arils and the consumption of the Pomegranate as fresh fruit were the main criteria for participants to select and consummate pomegranate. Thus, these criteria were considered to select the plant material. In order to homogeneity experimental conditions, pomegranate juices were used also for the acceptability tests and the instrumental analyses.

#### 3.2. Acceptability Tests

As seen in Table 3, significant differences ( $p < 0.05$ ) existed among pomegranate juices for all the acceptability tests. The participants liked most of the pomegranate juices (as indicated by mean scores  $> 5$ ) and the average scores for the pomegranate juices were particularly high. Also, participants preferred the juices from the cultivars CH 2 and NB 1 and the cultivar JB 1 received the highest score for juice color acceptability.

To determine the relationships between the juice over-

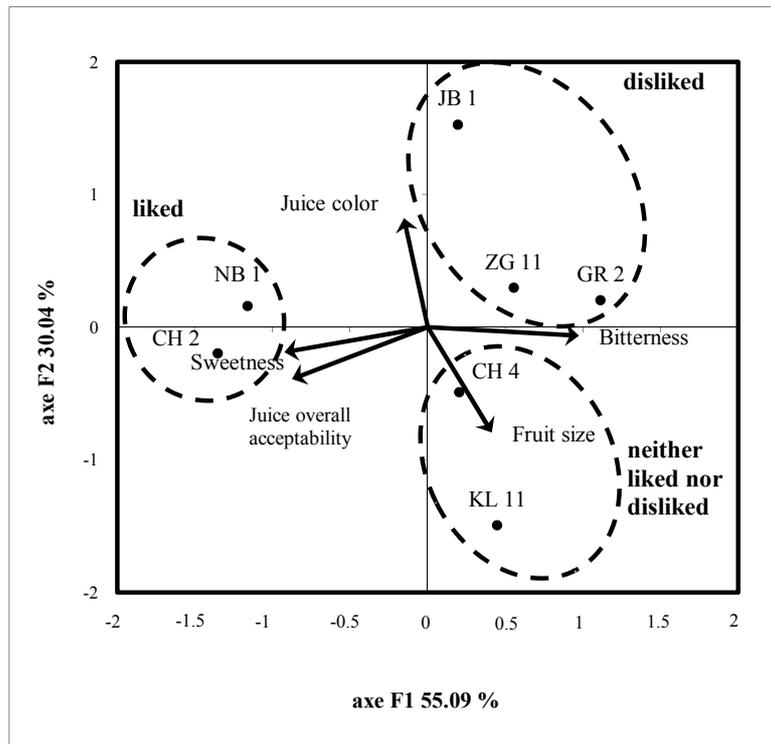
**Table 3. Mean scores (n = 71) for acceptability tests of the seven Tunisian Pomegranate (*Punica granatum*) cultivars studied<sup>A</sup>.**

Cultivars	Fruit size	Juice color	Sweetness	Bitterness	Juice overall acceptability
CH 2	6.42 <sup>b</sup>	5.98 <sup>a</sup>	7.94 <sup>ab</sup>	2.06 <sup>a</sup>	7.20 <sup>ab</sup>
CH 4	7.85 <sup>b</sup>	5.23 <sup>a</sup>	4.57 <sup>ab</sup>	4.01 <sup>a</sup>	6.20 <sup>ab</sup>
GR 2	7.02 <sup>b</sup>	5.14 <sup>a</sup>	3.10 <sup>ab</sup>	5.29 <sup>a</sup>	4.75 <sup>ab</sup>
JB1	5.04 <sup>b</sup>	7.82 <sup>a</sup>	4.00 <sup>ab</sup>	3.57 <sup>a</sup>	5.00 <sup>ab</sup>
KL 11	8.14 <sup>b</sup>	1.34 <sup>a</sup>	4.71 <sup>ab</sup>	4.17 <sup>a</sup>	5.83 <sup>ab</sup>
NB1	4.81 <sup>b</sup>	4.02 <sup>a</sup>	6.29 <sup>ab</sup>	1.43 <sup>a</sup>	6.71 <sup>ab</sup>
ZG 11	5.76 <sup>b</sup>	3.94 <sup>a</sup>	4.96 <sup>ab</sup>	4.63 <sup>a</sup>	4.56 <sup>ab</sup>

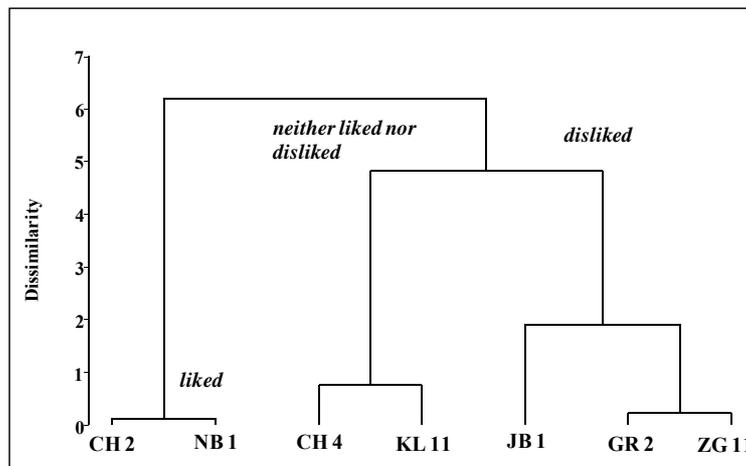
<sup>A</sup>Scores were based on a nine-point hedonic scale with 1 = dislike extremely; 5 = neither like nor dislike; and 9 = like extremely. <sup>ab</sup>Means with different letters within each line indicate significant differences at  $p < 0.05$  (Friedman test).

all acceptability of the seven cultivars and the acceptability of the sweetness, the bitterness, the juice color and the fruit size, a PCA was performed. Principal Components 1 (PC1) and 2 (PC2) accounted for 55% and 30% of the total variance, respectively. As shown in **Figure 1**, it seems that participants have clearly distinguished cultivars from each other, since the products were dispersed in the space of the representation. To improve the interpretation of the PCA map, a HCA on the PC1 and PC2 was performed. Three classes of cultivars were revealed (**Figure 2**). A

first class, named *liked*, includes the cultivars CH 2 and NB 1. A second class corresponds to the *neither liked nor disliked* cultivars, such as, CH 4 and KL 11 and a third class, namely, *disliked*, was composed of the cultivars GR 2, JB 1 and ZG 11. By representing the three classes of cultivars on the PCA biplot, the *liked* class was closest to the juice overall acceptability and the acceptability of the sweetness. The *neither liked nor disliked* and the *disliked* classes seem to be grouped with the bitterness and diametrically opposed to the juice overall acceptability.



**Figure 1.** PCA map of consumer acceptability of the seven Tunisian Pomegranate (*Punica granatum*) cultivars studied. Forms dashed represent the three classes of cultivars defined by the HCA.



**Figure 2.** Dendrogram obtained by HCA on the first two principal components of the PCA.

Although, the significant negative correlation ( $R = -0.82$ ,  $p < 0.05$ ) found between the sweetness and the bitterness acceptability explains the natural opposition between the two attributes. Also, the significant correlations found between the juice overall acceptability and the bitterness acceptability on one side ( $R = -0.83$ ,  $p < 0.05$ ), and the sweetness acceptability ( $R = 0.82$ ,  $p < 0.05$ ), on the other, confirmed that heightened perception of bitterness was the principal reason for food rejection [23]. Other significant differences, less expected, were found. In fact, Friedman's test indicated highly significant differences ( $p < 0.05$ ) between fruit size and juice color acceptability, in a hand, and fruit size and bitterness acceptability, in another hand. There is no report, to our knowledge, on the consumer acceptability of Pomegranate.

Thus, it can be concluded that the sweetness accounted for most of the variation in overall acceptability and the consumer rejection of the pomegranate juices was mainly related to the bitterness rather than to the color of juice or the fruit size.

### 3.3. Physico-Chemical Properties

**Table 1** shows the average value and the standard deviations for the physico-chemical properties of the seven pomegranate juices analyzed. As seen, the pomegranate juices differed little in TSS ( $CV = 6\%$ ) with values ranging from 14.0 °Brix for the cultivar CH 4 to 16.5 °Brix for the cultivar GR 2. However, great differences ( $CV = 90\%$ ) were observed in TA with values ranging from 0.2% MA for the cultivar CH 2 to 1.8% MA for the cultivar GR 2. The TSS/TA ratio gives an indication of the sweetness of fruits. The higher the TSS/TA ratio, the sweeter is the fruit. With the highest values for TA and TSS, GR 2 was the sourness cultivar (TSS/TA = 9.2) while with lowest values for TA and TSS, CH 2 was the sweetness cultivar (TSS/TA = 59.2). The TSS of the studied cultivars was close to 15.1 °Brix, both sweet and sour-sweet, thus, the TA was the factor which determines the taste of the Pomegranate. This result was confirmed by the significant negative correlation ( $R = -0.85$ ,  $p < 0.05$ ) found between the TSS/TA ratio and the TA. So, it can be concluded that the taste of the pomegranate juices is inversely related to their TA. Similar conclusion was advanced for orange [24]. **Table 1** shows also that there were great differences ( $CV = 57\%$ ) with respect to the instrumental measurement of color of the pomegranate juices. Juice from the cultivar CH 2 was differentiated as the lighter sample ( $CD = 0.3$ ) while juice from the cultivar GR 2 was the darkest ( $CD = 1.7$ ) one; NB 1 cultivar presented intermediate values of lightness ( $CD = 1.0$ ). The higher CD of the pomegranate juices could be explained by the heat units accumulated (difference be-

tween the daily average temperature and 25°C) prior to harvest. Indeed, the intensity of the red color of fresh Israeli pomegranate arils was found to be inversely related to the sum of heat units accumulated during fruit development and ripening [25].

### 3.4. Phenolic Contents

As shown in **Table 1**, the Total Polyphenols Content (TPC) varied greatly ( $CV = 86\%$ ) among the seven cultivars, and the juice of the cultivar ZG 11 was characterized by the highest TPC with 4570 mg GAE/L, while the lowest TPC was found in the juice of the cultivar GR 2 with 413 mg GAE/L. Özgen, *et al.* [6] reported a TPC ranging between 1245 and 2076 mg GAE/L for six pomegranate varieties grown in the Mediterranean region of Turkey and Sepúlveda *et al.* [7] found a TPC from 676 to 1236 mg GAE/L for ten different genotypes of Chilean pomegranate juices. Indeed, phenolic levels increase during exposure to biotic and abiotic stresses, such as wounding, drought, metal toxicity and nutrient deprivation and the highest values of phenolics corresponded to the varieties most sensitive to drought stress [4]. Hence, it can be deduced that the cultivar ZG 11 was the most sensitive to drought stress. This cultivar could be a good source of phenolics.

One of the important quality factors of pomegranate marketing is color. Dietary flavonoids are polyphenolic compounds responsible for the orange, red and blue colors in fruits and vegetables [2]. Also, anthocyanins are a largest group of flavonoids known to intervene in the color of the pomegranate juices [9]. So, Total Flavonoid Content (TFC) and Total Anthocyanin Content (TAC) were measured (**Table 1**). Besides, the cultivar KL 11 exhibited the highest TFC (1100 mg QE/L) while the highest TAC was found in the cultivar ZG 11 (433 mg CGE/L). When consumed regularly, these juices may contribute a significant amount of flavonoid to the diet. Ames [3] suggested that the consumption of flavonoid-rich foods protects against human diseases associated with oxidative stress, like cancer. Also, variability among cultivars was greatest for the TFC ( $CV = 82\%$ ) and TAC ( $CV = 118\%$ ). However, the TAC found in this study (20 - 433 mg CGE/L), was three to ten times greater than this reported by Özgen, *et al.* [6] (6 - 41 mg CGE/L) for six Turkey pomegranate juices. On the other hand, Sepúlveda *et al.* [7] founded a TAC around 676 to 1236 mg CGE/L for ten Chilean pomegranate juices. In fact, the variability in the TAC could be explained by the climatic conditions. The extreme temperatures may decrease anthocyanin content by accelerating degradation [26].

Although, Tibe *et al.* [27] described a relationship between condensed tannin content and color. Thus, the

Condensed Tannins Content (CTC) was determined. The pomegranate juices studied exhibited a CV of 52% and the cultivar ZG 11 was characterized by the highest CTC (5299 mg CE /L). Gil *et al.* [9] found a CTC of 417 mg/L for juice of “Wonderful” pomegranate variety of California while Mousavinejad, *et al.* [28] reported that the “Sweet Aalak” Iranian cultivar showed a tannin contents of 320 mg/L juice in comparison to the seven other cultivars studied. However, in other species, such as cowpea, Tibe, *et al.* [27] found that brown colored seeds of cowpeas contain more tannins than cream colored seeds. Therefore, it is not surprising that, the cultivar GR 2 with a red juice color had less tannin than other light colored cultivars in this study. It was unexpected; however, that the cultivar JB 1, with red juice color had a relatively high CTC which may be specific to this cultivar.

When a Pearson correlation was carried out with individual instrumental measurements, a high significant positive correlation ( $R = 0.91$ ,  $p < 0.05$ ) was found between the TPC and the TAC; this suggests that the pomegranates studied are anthocyanin-rich fruits. Another high significant positive correlation ( $R = 0.97$ ,  $p < 0.05$ ) was found between TAC and CTC. This result supports earlier studies [29] that found that anthocyanins and condensed tannins are related through a common biochemical pathway. So, it is important to consider the TAC and the CTC to balance pomegranate juice color.

By studying antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing, Gil *et al.* [9] concluded that the variations in the phenolic composition may be due to differences among varieties and also, to maturity, horticultural practices, geographic origin, growing season, postharvest storage conditions and processing procedures. To deepen the knowledge on the variation of the phenolic composition of pomegranate juice, the climatic and edaphic influences were limited, as well as, the horticultural practices by collecting the pomegranates from the same site where the culture conditions were homogenous, without fertilizers and irrigation. Also, the postharvest storage conditions of the pomegranate and the processing procedures for the determination of the phenolic composition were the same. So, it can be concluded that the observed differences in terms of phenolic contents of the juices depend on cultivar type or/and ripening season.

### 3.5. Antioxidant Capacity

The results from free radical scavenging revealed that pomegranate juices differed (CV = 44%) in their antioxidant capacities. Relatively high levels of TPC were observed in ZG 11 and KL 11 cultivars, so they decreased capacity toward ABTS radical scavenging. The hierarchy for antioxidant capacity was KL 11 > ZG 11 > CH 4 >

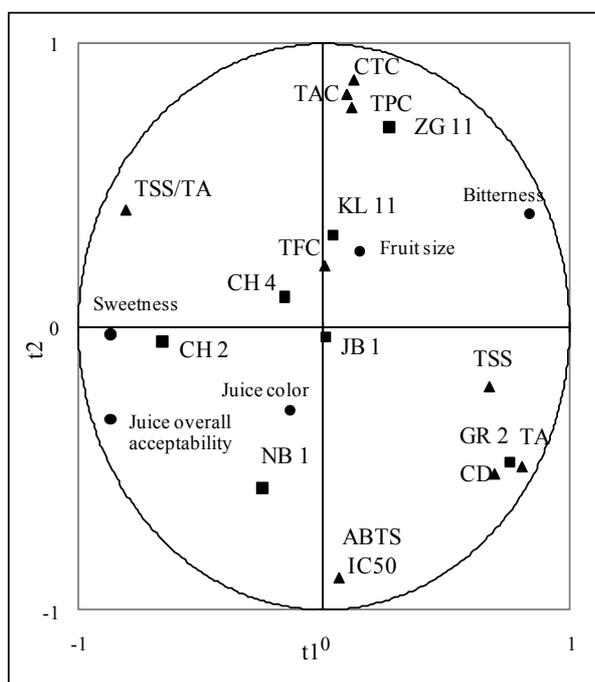
JB 1 > CH 2 > GR 2 > NB 1. Also, antioxidant capacity of the cultivar KL 11 was about three times higher than that of the cultivar NB 1. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may have, therefore, health-promoting effects in the prevention against diseases linked to oxidative stress. Thus, the juices of KL 11 and ZG 11 cultivars may be considered as a source of natural antioxidants for drug products because of their high antioxidant capacity. These results confirm previous works [6, 9,30] about the antioxidant activity of pomegranate juices.

In the present study, phenolic contents were not significantly correlated with antioxidant activity in the ABTS assay. However, the results of exhaustive work of two groups [9,30] on antioxidant activity of pomegranate juices suggested that anthocyanins play only a minor role in this activity. Thus, it can be concluded that the flavonoids, including anthocyanins, and condensed tannins play a minor role in the antioxidant activity of the pomegranate juices studied. Nevertheless, Seeram *et al.* [8] found that synergism between the antioxidants in a mixture makes the antioxidant activity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants.

### 3.6. Correlation between Consumer Acceptability Tests and Instrumental Analyses

The PLS regression analysis carried out to relate the five acceptability tests (variables Y) to the nine instrumental analyses (variable X), leads to the map shown in **Figure 3**. With a global  $R^2$  between Y and ( $t_1$ ,  $t_2$ ) equal to 0.45, the quality of the regression was low. Also, **Figure 3** shows that fruit size and juice color acceptability cannot be related to pomegranate characteristics since they are located in the centre of the graphical display. Therefore, it does not seem useful to take this acceptability into account. Similarly, it seems that TFC doesn't play an important role in explaining the consumer acceptability of the pomegranate. Therefore, these variables were removed from the analysis. In this approach, a new PLS regression was carried out. The quality of the new PLS regression was noticeable higher than the previous one ( $R^2 = 0.71$ ). By comparing the new map (figure not shown) obtained with the previous one, it can be noticed that the new map is an extract of the **Figure 3**. This confirms the stability and the robustness of the PLS regression analysis.

As shown in **Figure 4**, the sweetness acceptability appeared significantly positively correlated to the CD. Also, the acceptability of bitterness was significantly positively correlated to the TAC. In fact, Zampini, *et al.* [31], found



**Figure 3.** Partial Least Squares regression map showing the correlation between consumer acceptability data and instrumental data in the seven Pomegranates studied (■ = cultivars as indicated in Table 2; ● = acceptability attributes; ▲ = instrumental variables; TSS = Total Soluble Sugars expressed as degrees Brix (°Brix); TA = Titratable Acidity expressed as percent of malic acid (% MA); TSS/TA = sugars to acidity ratio; CD = Color Density; TPC = Total Phenolic Content expressed as mg gallic acid equivalent per liter of juice (mg GAE/L); TFC = Total Flavonoids Content expressed as mg quercetin equivalent per liter of juice (mg QE/L); TAC = Total Anthocyanins Content expressed as mg cyaniding-3-glucoside equivalent per liter of juice (mg CGE/L); CTC = Condensed Tannins Content expressed as mg catechin equivalent per liter of juice (mg CE/L); ABTS IC<sub>50</sub> = radical scavenging capacity on ABTS radical expressed as concentration of the extract required to reduce 50% of the initial ABTS free radical).

a significant main effect of flavor on color. Indeed, they revealed that orange-colored solutions were rated as sweeter when they had an orange flavor than when they were flavorless, thus color cues might exert a stronger influence on people's flavor identification responses. Acceptability of bitterness was also significantly negatively correlated to the antioxidant capacities. However, cancer researchers even proposed that heightened bitterness might be a positive feature, allowing consumers to select broccoli sprouts with the highest glucosinolate content [32]. As was also indicated in **Figure 4**, the juice overall acceptability was correlated to the CTC. Previous studies have shown that condensed tannins contribute to the bitterness taste of fruits [23]. The correlations between TAC, CTC and, respectively, bitterness and juice overall accep-

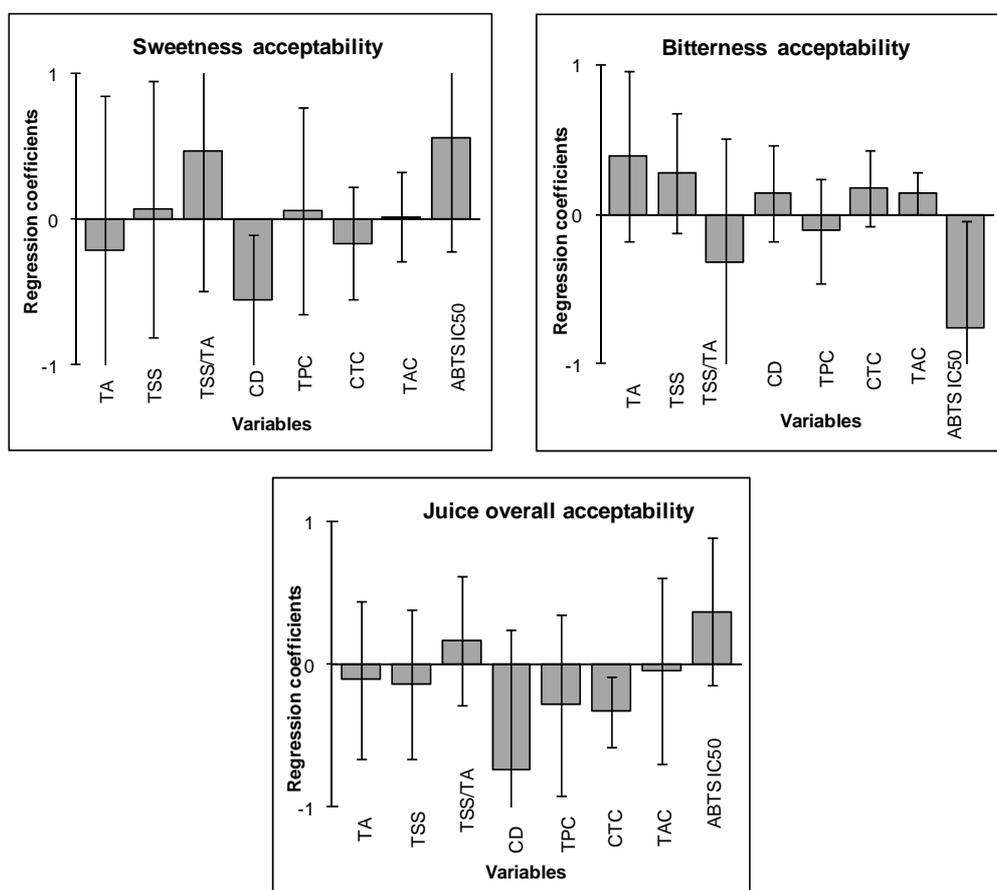
tability explain the contribution of phenolic compounds to organoleptic properties of the Pomegranates. Nevertheless, according to Gil *et al.* [9], phenolic compounds are connected to environmental factors, varieties, growing season, postharvest storage conditions and processing procedures. As the cultivars of this study have been grown in the same germplasm, it can be concluded that the organoleptic properties of the Pomegranates studied, like the juice color and the taste were obviously closely connected to the cultivar type or/and ripening season. Further research is needed to investigate which factors may be responsible for the organoleptic properties of the Pomegranates.

According to the current study, it can be concluded that the most appreciated juices were not the most beneficial to health while the fruit size could have a relationship with the beneficial effects of pomegranate on health. To our knowledge, no work relates the consumer acceptability to the antioxidant capacity of the pomegranate. Meanwhile, bitterness in plant foods has been described as a sensory defect with a major economic effect. Responding to taste-driven consumer demand, the food industry generally debittering plant food what improves the taste, but reduces the beneficial effects on health [23]. To avoid these problems, it can be suggested the consumption of cultivars with high concentration of polyphenols, and high antioxidant properties and reduction of the bitterness by addition of sugar, such as in the case of wines. The addition of sugar is a relatively inexpensive option to mask the bitterness while preserving the bioactive potential of the polyphenols. However, further studies should be undertaken to elucidate the duality product acceptability and health benefits.

#### 4. Conclusions

The frequency of respondents to the questionnaire showed that three of seven of the participants eat Pomegranate as fresh fruit and that the size of the fruit, the taste and the color of the aril were the main criteria for participants to select Pomegranate. This study also found that the taste of the pomegranate juices was inversely related to their TA. In addition, the juices studied appeared to have a great variability in terms of TPC, CTC, TFC and TAC, as well as, for the antioxidant capacities. PLS regression revealed that the juices most appreciated by participants were not the most beneficial to health. However, a significant correlation was found between the fruit size acceptability and the antioxidant capacity of the Pomegranate, so, a relationship could be established between the size of the Pomegranate and its health benefits.

By limiting the climatic and edaphic influences, as well as, the horticultural practices by collecting the pomegranates



**Figure 4.** 95% Jack-knife confidence intervals of the Partial Least Squares regression coefficients in the Partial Least Squares regression of sweetness, bitterness and juice overall acceptability on instrumental analyses (Variables with confidence interval includes the value 0 are significant ( $p < 0.05$ ); TSS = Total Soluble Sugars expressed as degrees Brix ( $^{\circ}$ Brix); TA = Titratable Acidity expressed as percent of malic acid (% MA); TSS/TA = sugars to acidity ratio; CD = Color Density; TPC = Total Phenolic Content expressed as mg gallic acid equivalent per liter of juice (mg GAE/L); TFC = Total Flavonoids Content expressed as mg quercetin equivalent per liter of juice (mg QE/L); TAC = Total Anthocyanins Content expressed as mg cyaniding-3-glucoside equivalent per liter of juice (mg CGE/L); CTC = Condensed Tannins Content expressed as mg catechin equivalent per liter of juice (mg CE/L); ABTS IC<sub>50</sub> = radical scavenging capacity on ABTS radical expressed as concentration of the extract required to reduce 50% of the initial ABTS free radical).

from the same site, it can be concluded that the observed differences in terms of phenolic contents and organoleptic properties of the pomegranate juices studied depend on cultivar type or/and ripening season. Although the primary function of food is to provide nutrients, its secondary function concerns organoleptic properties such as taste and its tertiary function is to prevent disease. However, results of this study appeared that the secondary and tertiary functions are linked.

The results of this study could be used to understand the complex nature between the acceptability of a product, as perceived by untrained subjects, and its benefits on health.

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