

Effects Of Storage Temperature and Juice Pulp on The Content of Bioactive Compounds and Antioxidant Activity of Pomelo

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Abstract

Antioxidants are known for their health benefits, capable of neutralizing free radicals in the body, preventing several chronic diseases and reducing oxidative stress. Through this research work we were interested in comparing the contents of bioactive substances and evaluating the antioxidant activity of the juice according to its conservation method at two different temperatures (4°C and -20°C) and the influence of the presence or absence of the juice pulp. We have selected three varieties of pomelo cultivated at INRA-Kenitra: Pomelo thompson, Pomelo star ruby and Pomelo marsh. The extraction and quantification of flavonoids and total phenolic compounds was carried out according to the aluminum trichloride (AlCl₃) and Folin-Ciocalteu methods respectively. The method applied to measure antioxidant activity was free radical scavenging using DPPH (2,2- diphenyl-1-picrylhydrazyl). Our results indicated that the juice containing pulp has a high antioxidant property compared to the juice without pulp. The effect of freezing the juice decreases its titratable acidity and °Brix, but freezing the juice preserves a large amount of flavonoids and phenolic compounds with minimal difference in the three accessions studied.

Keywords: Pomelo, pulp, antioxidant activity, total phenolics, DPPH.

INTRODUCTION

Rutaceae is a large family, many of them have economic importance as fruit, medicinal and ornamental species. Citrus is undoubtedly the most important genus of this family; this genus includes citrus fruits that present a great diversity of morphological characters and a worldwide distribution [1]. The most produced citrus fruits in the world are oranges (73 million tons), mandarins (33 million tons), yellow and green lemons (17 million tons) and pomelos (9 million tons) [2]. Pomelo under the scientific name *Citrus. paradisi Macf.* is native to tropical and subtropical regions of Asia and has been cultivated in China for 2000 years [3]. It has a unique shape, flavor, and taste, all quality attributes that attract consumers, this is consumed in the form of fresh fruit juice and candied fruit peel [4]. The albedo part of the pomelo skin (spongy white) contains natural chemicals such as flavonoids, cellulose, pectin and essential oils [5]. Although pomelo has been widely used in the food and pharmaceutical industries, its use as well as products made from its peel as a biosorbent to remove toxic organic compounds and heavy metals from wastewater are still insignificant [6]. It has been reported that the phytoconstituents in pomelo juice are useful for the prevention of chronic diseases [7]. In recent years, *Citrus paradisi Macf* has gained importance, mainly due to the presence of bioactive substances (carotenoids, lycopene, polyphenols, flavonoids, limonoids, fiber, and vitamin C) that have protective effects against diseases such as oxidative stress, hyperglycemia, and hypercholesterolemia [8] [9] [10].

Due to its important health-promoting property, the presence and absence of the pulp and the storage temperature were the main factors limiting the shelf life of this species [11]. Fewer studies and data are available on the changes in phytochemistry and total antioxidant activity of fresh juice and after

its storage period at different temperatures [12]. The objective of this study is to evaluate the effect of storage conditions of pomelo juice in the presence and absence of pulp on total phenolic content, flavonoid content, antioxidant activity and on some physicochemical parameter.

MATERIAL AND METHODS

Plant material

The samples of three varieties of Pomelo: pomelo thompson, pomelo star ruby and pomelo marsh grafted on citrange troyer, planted in June 1995 in the experimental field El Menzeh of INRA-Kenitra. They were harvested during the two companies 2018/2019 and 2019/2020 to have better results.



Figure 1: Accessions of the pomelo

Preparation of the pomelo juice

Once brought to the laboratory, the extraction of the juice is done manually with a citrus press. We chose to work on three different storage conditions, the analysis of the juice was done directly from the freshly squeezed juice, after its conservation at 4°C for 10 days and at -20°C for 90 days in the presence and absence of the pulp.

Total soluble solids (TSS)

Total soluble solids were determined using a manual refractometer (Model: HSR-500 Atago, Tokyo, Japan). The refractive indices were recorded and converted to Brix (°Brix). All measurements were performed in triplicate, after performing each analysis, the refractometer prism was cleaned thoroughly with distilled water.

Titrateable acidity (TA)

The titration of the acidity requires the use of a sodium hydroxide solution 0.1563N of which 10 cm³ of this solution correspond to 1% of acidity, expressed as anhydrous citric acid. The verification of the soda solution is carried out with the oxalic acid solution 0.1563N. The neutrality of soda is adjusted when 10 cm³ of this solution neutralizes 10 cm³ of the oxalic acid solution. The color reagent to detect the turning point is phenolphthalein 1%. The titration is performed using a Hirschmann™ (Titration Unit Opus™) [13].

Antioxidant activity content (AAC)

To determine the antiradical role (scavenging activity) of our samples by the DPPH test. A volume of 2 ml of DPPH solution was added to 50 µL of pomelo juice extract. At the same time, a negative control of DPPH was prepared. The absorbance reading is taken at 515 nm, after 30 minutes of incubation in

the dark and at room temperature [14]. The percentage of inhibition is expressed by the following formula:

$$PI (\%) = ((A0 - A1) / A0) \times 100$$

With:

A0: absorbance of the control at 515 nm.

A1: absorbance of the sample at 515 nm.

Total flavonoid content (TFC)

The total flavonoid content was determined by the aluminum chloride colorimetric method [15]. A volume of 1ml of the aluminum trichloride (AlCl₃) at 2% m/v, this volume was mixed with 1ml of diluted extracts. The mixture was then shaken vigorously, followed by a 30-minute incubation in the dark and spectrophotometric measurement at 510 nm. Referring to a calibration curve performed under the same conditions with the reference flavonoids of quercitrin.

Total polyphenol content (TPC)

To determine the total polyphenol content, we opted for the colorimetric method of Folin-Ciocalteu [16]. A quantity of 1ml of each sample is pipetted into dark test tubes, followed by the addition of 500µl of Folin - Ciocalteu reagent (diluted 10 times with distilled water). After incubation for 2 min. 2.5 ml of 20% sodium carbonate Na₂CO₃ is added, then kept in the dark for 30 min at room temperature. The absorbance was measured at 760 nm. A calibration curve performed under the same conditions using gallic acid as standard.

Statistical analysis

For each accession, three replicates of juice with and without pulp were analyzed, all determinations were also performed in triplicate. The analysis of variance was performed using the ANOVA method and Duncan's test for post-hoc analysis at $p < 0.05$; only the variables with a confidence level higher than 95% were considered significant. At the same time, Pearson's correlation test was performed to determine the correlations between antioxidant content and antioxidant activity of pomelo juice in the presence and absence of the pulp. These analyses were performed using IBM(R) SPSS(R) Statistics Version 25 software.

RESULTS

Physicochemical characterization:

The analysis of variance of the variable "juice percentage" shows a highly significant effect of fresh juice, pomelo star ruby with and without pulp has the highest juice percentage (45.30% and 44.33%) while pomelo marsh with and without pulp has the lowest juice percentages (33.67% and 34.67%). At 4°C pomelo ruby with pulp has the highest juice content (42.67%) while pomelo thompson with pulp has the lowest juice content (31%). At -20°C pomelo ruby without pulp has the highest juice content (47.67%) as opposed to pomelo marsh without pulp has the lowest percentage of juice (37%).

The analysis of variance of the soluble solids content variable is significant ($p < 0.05$), the fresh pomelo marsh juice without pulp has the highest °Brix ($12,78 \pm 0,122$) and the pomelo ruby accession without pulp has the lowest ($10,167 \pm 0,034$). At 4°C pomelo marsh without pulp has the highest value of the

accessions (12,33 ± 0,242) while pomelo ruby with pulp has the lowest one (9,751 ± 0,148). At -20°C the pomelo marsh accession without pulp has the highest °Brix (11,746 ± 0,161) and pomelo ruby with pulp has the lowest value (7,8787 ± 0,123).

However, analysis of variance showed that there is a highly significant effect for the parameter "titratable acidity", fresh juice of pomelo marsh without pulp has the highest acidity value (1,94 ± 0,1) and pomelo ruby with pulp has the lowest acidity content (1,4133 ± 0,0577). At 4°C pomelo thompson without pulp has the highest titratable acidity content (1,803 ± 0,0145) while pomelo ruby with pulp has the lowest value (1,3733 ± 0,056). Also, at -20°C the pomelo thompson accession without pulp has the highest value (1,67923 ± 0,051) while pomelo ruby without pulp has the lowest titratable acidity (0,736 ± 0,265).

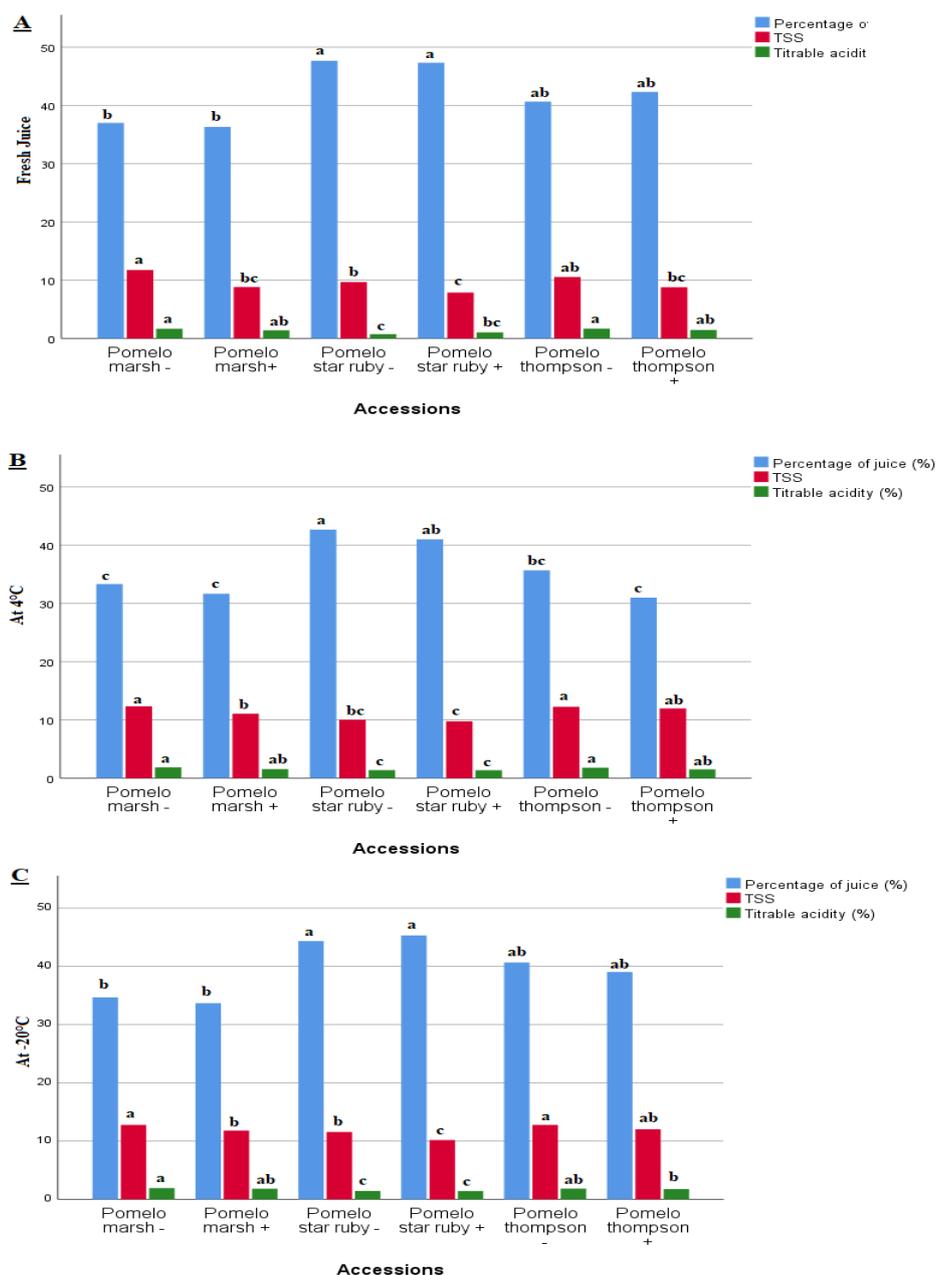


Figure 2: Variations in juice percent, TSS and titratable acidity of fresh pomelo juice (A), stored at 4°C (B) and at -20°C (C).

Bioactive properties of pomelo extracts

Total flavonoid content (TFC)

The analysis of variance showed that there is a highly significant effect for the flavonoid content, the fresh juice of pomelo thompson with pulp represents the highest value (519.04 mg QE 100 ml⁻¹) while pomelo ruby without pulp has the lowest value (372.46 mg QE 100 ml⁻¹). At 4°C pomelo marsh juice without pulp has the highest flavonoid content (388.44 mg QE 100 ml⁻¹) and pomelo ruby without pulp has the lowest value (120.41 mg QE 100 ml⁻¹). At -20°C pomelo thompson juice without pulp has the highest flavonoid content (453.28 mg QE 100 ml⁻¹) and pomelo ruby without pulp has the lowest flavonoid content (269.27 28 mg QE 100 ml⁻¹).

Table 1: Total flavonoid content of fresh pomelo juice and stored pomelo juice at 4°C and -20°C with and without of pulp.

Genotypes	Pulp	Total flavonoid content (mg QE 100 ml ⁻¹)		
		Fresh juice	At 4 °C	At -20 °C
Pomelo thompson	+	519,04 a ± 0,79	215,38 c ± 0,45	438,21 ab ± 0,19
	-	488,44 ab ± 0,79	203,05 c ± 0,64	453,28 a ± 0,40
Pomelo marsh	+	416,3 bc ± 0,79	292,55 b ± 0,34	307,62 c ± 0,66
	-	443,24 b ± 0,45	388,44 a ± 0,32	341,87 c ± 0,69
Pomelo ruby	+	482,05 ab ± 0,79	354,2 ab ± 0,91	412,64 ab ± 0,34
	-	372,46 d ± 0,13	120,41 d ± 0,51	269,27 d ± 0,19

Different letters show a significant difference and the same letters show a non-significant difference between genotypes. (+: with pulp, -: without pulp).

Total polyphenol content (TPC)

The statistical analysis of variance for the variable "total polyphenol content" showed that there is a significant effect for the polyphenol content, the fresh juice of Pomelo thompson with pulp represents the highest content of total phenolic compounds (260.97 mg GAE 100 ml⁻¹) while Pomelo marsh without pulp has the lowest content (117.68 mg GAE 100 ml⁻¹) At 4°C Pomelo thompson juice with pulp has the highest content of total phenolic compounds (97.76 mg GAE 100 ml⁻¹) and the juice of the same accession without pulp has the lowest content of total phenolic compounds (48.57 mg GAE 100 ml⁻¹). At 20°C the juice of Pomelo ruby with pulp represents the highest content of total phenolic compounds (51.61 mg GAE 100 ml⁻¹) while the lowest content was obtained in Pomelo marsh without pulp (85.75 mg GAE 100 ml⁻¹).

Table 2: Total polyphenol content of fresh pomelo juice and stored pomelo juice at 4°C and -20°C with and without pulp.

Genotypes	Pulp	Total polyphenol content (mg GAE 100 ml ⁻¹)		
		Fresh juice	At 4 °C	At -20 °C
Pomelo thompson	+	260,97 a ± 0,63	97,76 a ± 0,04	148,66 a ± 0,37
	-	158,01 cd ± 0,28	48,57 d ± 0,01	92,68 bc ± 0,7
Pomelo marsh	+	249,77 ab ± 0,34	72,50 c ± 0,13	137,18 b ± 0,20
	-	117,68 d ± 0,97	62,06 cd ± 0,3	85,75 c ± 0,71
Pomelo ruby	+	257,38 a ± 0,14	81,89 ab ± 0,02	151,61 a ± 0,09

- 166,37 c ± 0,01 73,18 c ± 0,02 94,23 bc ± 0,47

Different letters show a significant difference and the same letters show a non-significant difference between genotypes. (+: with pulp, -: without pulp).

Antioxidant activity content (AAC)

The statistical analysis of variance shows a significant effect for the variable "antioxidant activity", the fresh juice of Pomelo thompson with pulp has the highest content of antioxidant activity 68.23%, while Pomelo thompson without pulp has the lowest content of antioxidant activity 25%. At 4°C Pomelo thompson with pulp has the highest content 44.07% while Pomelo marsh without pulp has the lowest content of antioxidant activity 3.9%. After storage of the juice at -20°C the Pomelo thompson accession with pulp has the highest antioxidant activity content 58% while the Pomelo thompson accession without pulp has the lowest antioxidant activity content 11.37%.

Table 3: Total antioxidant activity content of fresh pomelo juice and stored pomelo juice at 4°C and -20°C with and without pulp.

Genotypes	Pulp	Antioxidant activity content		
		Fresh juice	At 4 °C	At -20 °C
Pomelo thompson	+	68,23% a ± 0,09	44,07% a ± 0,12	58% a ± 0,43
	-	25% c ± 0,73	5% cd ± 0,73	11,37% d ± 0,83
Pomelo marsh	+	63,5% a ± 0,91	11,57% b ± 0,67	34,93% b ± 0,63
	-	58,83% ab ± 0,63	33% ab ± 0,15	44,3% ab ± 0,77
Pomelo ruby	+	44,63% b ± 0,30	3,9% d ± 0,15	19,17% cd ± 0,81
	-	42,86% b ± 0,67	7,33% c ± 0,67	23,93% c ± 0,28

Different letters show a significant difference and the same letters show a non-significant difference between genotypes. (+: with pulp, -: without pulp).

Correlation analysis of bioactive characteristics

The correlations between total polyphenols (TPC) and flavonoids (TFC) and antioxidant activity (AAC) analyzed as a function of the presence and the absence of pulp at 4°C and -20°C using Pearson correlation analysis are presented in the following tables. There are significant correlations between antioxidant activity and antioxidants at 4°C in the presence of pulp such as TFC and TPC. A strong and positive correlation was found between TPC and AAC values (r = 0.785). This indicates that total polyphenols in pomelo juice samples are among the major bioactive compounds with potential reducing capacity. At the same time, a high and negative correlation was observed between TFC and TPC values as well as between TFC and AAC values with correlation coefficients of r = -0.991 and r = -0.860 respectively.

Table 4: Pearson correlation analysis of pomelo juice with pulp stored at 4°C.

	TPC	AAC	TFC
Total polyphenol content (TPC)	1	,785*	-,991**
Antioxidant activity content (AAC)	,785*	1	-,860**
Total flavonoid content (TFC)	-,991**	-,860**	1

*. Correlation is significant at the 0.05 level (two-tailed).

** . Correlation is significant at the 0.01 level (two-tailed).

However, the study of the correlation between total polyphenol content, antioxidant activity and flavonoid content at 4°C without pulp showed only one strong and negative correlation between total polyphenol content and antioxidant activity content ($r = -0.811$), while no significant correlation was observed between TPC values and TFC as well as between TFC values and AAC.

Table 5: Pearson correlation analysis of pomelo juice without pulp stored at 4°C.

	TPC	AAC	TFC
Total polyphenol content (TPC)	1	-,811**	-0,093
Antioxidant activity content (AAC)	-,811**	1	0,624
Total flavonoid content (TFC)	-0,093	0,624	1

** . The correlation is significant at the 0.01 level (two-tailed).

The correlation between total polyphenol content, antioxidant activity content and flavonoid content at -20°C without pulp showed the absence of correlation between the last three contents.

Table 6: Pearson correlation analysis of pomelo juice without pulp stored at -20°C.

	TPC	AAC	TFC
Total polyphenol content (TPC)	1	-0,348	0,255
Antioxidant activity content (AAC)	-0,348	1	0,428
Total flavonoid content (TFC)	0,255	0,428	1

The antioxidant activity content of samples stored at -20°C without pulp was negatively and strongly correlated with total polyphenol content ($r = -0.715$), while no significant correlation was observed either between total polyphenol content and flavonoid content or between total polyphenol content and antioxidant activity content.

Table 7: Pearson correlation analysis of pomelo juice without pulp stored at -20°C.

	TPC	AAC	TFC
Total polyphenol content (TPC)	1	-,715*	-0,097
Antioxidant activity content (AAC)	-,715*	1	-0,499
Total flavonoid content (TFC)	-0,097	-0,499	1

*. The correlation is significant at the 0.05 level (two-tailed).

DISCUSSION

The health properties of pummelos are of great interest, This is due to their content of natural antioxidant substances that seem to be associated with the prevention of degenerative processes and the reduction of the risk of some chronic diseases such as cancer [17]. Among these natural nutritional antioxidants present in these fruits, phenolic compounds stand out as they possess a wide range of therapeutic properties for medical and clinical applications such as anti-inflammatory,

antihypertensive, diuretic, analgesic and hypolipidemic activities [18]. Flavanones constitute 98% of the total flavonoids present in grapefruit [19] [20]. In addition, citrus phenolics are relevant in terms of quality, as they influence visual appearance (pigmentation and browning) and taste (astringency and bitterness) [21].

According to the present study our accessions are juicy, their total soluble solids content of fresh juice varied from 10 to 12% which is in accordance with *Nishad et al* (2018) [22] *Nhi et al* (2020), [23] and *Yang et al* (2020) [24]. We noticed that the percentage content of juice, °Brix and titratable acidity content are highly influenced by the method of preservation as well as with and without pulp in the juice. The results indicated that the total soluble solids and titratable acidity content of fresh grapefruit juice showed a slight decrease after storage at 4°C, while storage of the juice at -20°C showed a remarkable decrease in TSS, This decrease can be attributed to the consumption of sugar due to the beginning of fermentation, therefore the storage of juice at 4°C is not very recommended, especially if it is going to be for a long time [25],, while it was found that the titratable acidity content of the pomelo juice stored at -20°C has decreased slightly. Our results are in line with those of *Alaz et al* (2007) [26].

Most flavonoids (flavanones and flavanone glycosides) found in citrus plants are rarely found in other plants. The major flavanone glycosides in pomelo, grapefruit, and mandarin are naringin, hesperidin, and narirutin [27] [28]. Our accessions contain an appreciable amount of flavonoids especially in fresh juice, the presence or absence of pulp affects this factor according to the genotype, and by comparison the flavonoid content of juice with pulp was higher than without pulp for all accessions except for Pomelo marsh where the content of juice without pulp is slightly higher than the juice with pulp which is in line with the results of *J. Wang et al* (2020) [29]. The change of storage mode of pomelo juice also influences the flavonoid content, we noticed that at -20°C the content decreased compared to storage of juice at 4°C. The relatively higher yield of flavonoids in pomelo could be due to differences in variety and intense sunlight conditions (a characteristic of tropical fruits), both of which can induce flavonoid accumulation in the fruit [30]. Therefore, the flavonoid composition of citrus fruits varies considerably depending on the genetic origin of the fruit, the time of harvest of the fruit and the parts used [31] [32].

The results of the present research indicate that the total phenolic compound content of fresh pomelo juice with pulp is higher compared to the juice without pulp. Our results are in agreement with those of *Ani et al* (2018) and *Mäkynen et al* (2013) who also reported total phenolic compound contents were in the same range as ours [33] [34]. The values obtained for the variable total phenolic compounds content allowed us to deduce that a preservation of the juice at -20°C is better than a preservation at 4°C, since it keeps a relatively high content compared to the content obtained from the fresh juice.

The DPPH stable radical scavenging model is a widely used method to assess free radicals, *Pichaiyongvongdee et al* (2014) provided similar results to ours for the distribution of antioxidant activity content of fresh pomelo juice [35]. After analyzing the obtained results, we found that the contents of the accessions with pulp during the conservation at -20°C presents a slight decrease of the antioxidant activity very remarkable in pomelo thompson, in front of the conservation of the juice at 4°C where we noticed a strong decrease to the content of the antioxidant activity very observed in Pomelo marsh with pulp. Both, without pulp, the conservation of juice at 4°C has recognized a huge

decrease in the content of antioxidant activity, compared to a considerable decrease during the conservation of juice at -20°C.

The correlation of pomelo juice with pulp stored at 4°C between total polyphenol content, flavonoids and antioxidant activity were very high, as shown in Table 5. The data showed that a high content of total polyphenols increases the antioxidant properties hence their considerations as the main antioxidant of citrus fruits according to Sun *et al* (2002) [36]. The increase in total polyphenol content also increased the antioxidant efficiency of fruits as reported by *Proteggente et al* (2003) [37]. In addition, the correlation of pomelo juice without pulp stored at 4°C and -20°C is high, *Pichaiyongvongdee and Haruenkit* (2009) reported that the antioxidant properties measured by DPPH assays gave good correlations with total polyphenol content in pomelo juice [38].

CONCLUSION

The data obtained from this study allowed to roughly estimate the physicochemical properties, phenolic composition and antioxidant property of pomelo juice, these vary according to the juice storage temperature and the presence or absence of juice pulp. Pomelo Star Ruby remains the juiciest accession despite the change in storage temperature while Pomelo thompson and Pomelo marsh without pulp show the highest °Brix and titratable acidity contents of fresh juice, juice stored at 4°C and -20°C. Statistically significant differences in flavonoid content, total phenolic content and DPPH antioxidant activity were observed in the studied accessions. Therefore, Pomelo thompson with pulp is the accession with the highest total polyphenol content and antioxidant activity despite the change in storage conditions. These results aim to promote pomelo cultivation in Morocco and worldwide, to provide information on the health-promoting compounds present in Thai pomelo fruits and to present citrus fruits as an important source of antioxidants in the food.

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