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Original Article

Phenolic compounds and antioxidant activity of different parts of three mandarin varieties extracts: A comparative study

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Abstract

Background: Mandarin by-products, such as peels and seeds, are considered as one of the naturel sources of phenolic compounds. **Aim:** The objective of the present study was to compare the phenolic compounds contents and antioxidant activity of different parts (pulps, seeds, juices, and peels) of three mandarins varieties extracts. **Material and methods:** Total phenolic, flavonoid, and proanthocyanidin contents of the extracts were assessed while, the antioxidant activity was evaluated using three tests including ferric reducing power, free radical scavenging activity, and phosphomolybdate. **Results:** The Mediterranean Mandarin peels present a high total phenolics and flavonoids contents with values of 2445.62 mg GAE/100g FW and 609.78 mg QE/100g FW, respectively. Likewise, the clementine juice showed the highest proanthocyanins contents with a value of 46.67 mg CE/100g FW. Seeds and pulps of the three varieties present the lowest phenolic compound contents. Mediterranean Mandarin juice showed a strong ferric reducing power and phosphomolybdate with values of 1270.91 and 190.19 mg AAE/100g FW, respectively, however, the peels revealed a strong free radical scavenging activity with value of 259.89 mg AAE/100 g FW. Statistical analysis showed a positive correlation between the antioxidant activity and the phenolic compounds contents of the three studied mandarins varieties extracts. **Conclusion:** Among the analyzed cultivars, the Mediterranean mandarin variety may be considered as the most promising source of polyphenols and antioxidants, compared to the clementine and Satsuma varieties. Moreover, this study also highlights important properties of the fruit parts generally considered wastes.

Keywords: Mandarins, different parts, phenolics compounds, antioxidant activity.

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1 Introduction

The food and agricultural products processing industries produce significant amount of phenolic-rich by-products, which could be an important source of antioxidant compounds of natural origin[1]. Among fruits, citrus (*Punica granatum L.*) fruits are considered as one of the natural sources of antioxidants. In fact, they contain a considerable amount of ascorbic acid, flavonoids, and phenolics compounds [2,3]. The perception of these fruits lies mainly in their ascorbic acid contents and the antioxidant compounds. In addition, other components exhibit antioxidant activity such as polyphenols, flavonoids, and carotenoids etc. [4]. Mandarins present an important group of citrus fruits with pomelo and citron which are considered to be the original Citrus ancestors [5]. Mandarin fruits are considered as one of the natural resources of antioxidants, which contain an appreciable amount of ascorbic acid, flavonoids, and phenolic compounds. The peel is the richest source of bioactive phenolic compounds, especially flavonoids, with comparatively higher polyphenol content compared with the edible parts [6]. Flavonoids are represented in citrus fruits by two very peculiar classes of compounds: the polymethoxylated flavones and the glycosylated flavanones [7]. Orange juice is recognized as functional not only for its high vitamin C concentration, but also for its content of carotenoids and phenolic compounds [8].

Numerous epidemiological studies suggest that the consumption of fruits and vegetables decreases the incidence of several diseases such as cancer and cardiovascular disease. These beneficial effects are attributed to micronutrients such as antioxidants [9]. These secondary metabolites are involved in many facets of plant biological systems: pigmentation, growth and reproduction mechanisms, protection against predators, etc. More than 8,000 phenolic compounds have been identified, including 5,000 for the flavonoids subclass [10].

For a revaluation of citrus by-products, it would be appropriate to focus the attention on varieties with very different provenance and traits, in order to have an indication about the potential associated with the wastes of diverse cultivars [11]. Hence, estimation of the antioxidant power of the citrus fruit wasted part is required, to explore the potentials of their use in food manufacturing. Therefore, the aim of this study was to compare the phenolics, flavonoids, and proanthocyanidin contents as well as the antioxidant activity in four parts of three mandarin varieties

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(clementine (*Citrus clementina*), Satsuma mandarin (*Citrus unshiu* Marcovitch), and Mediterranean mandarin (*Citrus deliciosa* Tenore)). This study will be useful for a valorization of Algerian mandarin waste products, encouraging their use and thus favoring the recycling practices and the bioeconomy strategies.

2 Material and Methods

2.1 Samples preparation

Three different mandarins varieties (clementine (*Citrus clementina*), Satsuma mandarin (*Citrus unshiu* Marcovitch), and Mediterranean mandarin (*Citrus deliciosa* Tenore)) have been selected for this study. Those Algerian varieties were purchased from the shopping center of Ali Mendjeli, Constantine, Algeria in the seasons 2017–2018. Fruits were carefully selected (a desirable maturity and in appropriate sanitary conditions), washed, and peeled. The seeds were separated from the pulp. Three different edible parts (seeds, pulp, and peels) of each variety were separately lyophilized (Christ, Alpha 1-4 LD plus, Germany), ground with a crusher (IKA A 11B, Germany), and passed through a 500 µm sieve. However, the juices were centrifuged and filtered through filter paper. The powders and juices were conserved at 4°C until further analysis.

2.2 Phenolic compounds extraction

A mixture of 1g of each part (peel, seed, and pulp) powders and 25 mL of acetone (70%) was shaken in a water bath shaker for 60 min at 37 °C followed by centrifugation at 3000 g for 20 min and paper filtered. The obtained filtrates (extracts) were conserved at 4°C until further analysis.

2.3 Phenolic, flavonoid, and proanthocyanidins contents

Total phenolic contents (TPC) were estimated according to the method of Singleton & Rossi [12] as reported in our previous study [13]. One hundred microliters of extracts were mixed with 750 µL of Folin–Ciocalteu reagent (1/10) and 600 µL of sodium carbonate (7.5%). After 30 min of incubation, the absorbance was measured at 750 nm.

The flavonoid contents (FC) were estimated according to the method of Quettier-Deleu *et al.* [14]. Equal volumes of extract and aluminum chloride solution (2%) were mixed. The absorbance was measured at 430 nm after 15 min of incubation.

The proanthocyanidin contents (PrC) were estimated by butanol-HCl assay [15]. A volume of 500 µl of different extracts was mixed with 2 ml of butanol-HCl (95:5; v/v) and 0.1 ml of iron sulfate (2%). After incubated at 90°C for 1 h, the absorbance was measured at 530 nm.

The results of TPC and FC were expressed as milligram gallic acid (GA) and quercetin (Q) equivalents (E) per 100 gram of fresh weight (FW), respectively. While, the results of PrC were expressed as milligram cyaniding equivalent per 100 grams of fresh weight (mg CE/100g FW) using a molar extinction coefficient of cyanidin: ε = 34,700 L* mol^{-1} *cm^{-1}.

2.4 Antioxidant activities

The ferric reducing power (FRP) was evaluated according to the method of Yildirim *et al.* [16]. A volume of 1 mL of different extracts was mixed with 1 mL of phosphate buffer (200 mM, pH 6.6) and 1 mL of potassium ferricyanide (1%). After 20 min of incubation at 50°C, 1 mL of trichloroacetic acid (10%) was added followed by centrifugation at 1700 g for 10 min. One milliliter of supernatant was mixed with 1 mL of distilled water and 200 µL of ferric chloride (0.2%). The absorbance was measured at 700 nm.

The FRSA (DPPH: 1-diphenyl-2-picrylhydrazyl) was evaluated according to the method of Brand-Williams *et al.* [17]. A volume of 100 µL of the extract was added to 1 mL of DPPH (60 µM). After 30 min of incubation, the absorbance was measured at 517 nm.

The total antioxidant capacities of different extracts were evaluated also by the phosphomolybdenum method as described by Prieto *et al.* [18]. A volume of 1 mL of phosphomolybdenum solution (sulfuric acid 0.6 M, sodium phosphate 0.028 M and ammonium molybdate 0.004 M) was added to 100 mL of each extract. After 60 min of incubation at 90°C, the absorbance was measured at 695 nm.

The results of the three activities tested were expressed as milligram ascorbic acid equivalents per 100 grams of fresh weight (mg AAE/ 100g FW).

2.5 Statistical analysis

All analyses were carried out in triplicate and the results were reported as means ± standard deviation. The software STATISTICA® 5.5 was used to perform the analysis of ANOVA followed by the post hoc Turkey LSD test to analyze the significant differences between the means at 5% ($P<0.05$).

3 Results

3.1 Phenolic compound

The TPC of different parts (seeds, pulps, peels, and juices) of the three mandarins varieties studied are shown on Figure 1. The results showed that the highest TPC was recorded for the peels of three varieties followed by juices, pulps, and seeds. Indeed, the Mediterranean mandarin (*Citrus deliciosa*) peels present the highest TPC contents with a value of 2445.62 mg GAE/100g FW, followed by the clementine and Satsuma varieties.

In addition, the TPC of juice and pulp of the three varieties were varied between 504.38 and 1174.8 mg GAE/100 g FW and between 517.71 and 701.57mg GAE/100g FW, respectively. Likewise, the seeds were recorded the lowest contents with values oscillated between 298.95 and 374.66 mg GAE/100g FW. Statistical analysis revealed a significant difference (P<0.05) between the four fractions (seeds, pulps, peels, and juices) of each variety. Likewise, no significant difference (P>0.05) was observed between the pulps and seeds of the three varieties.

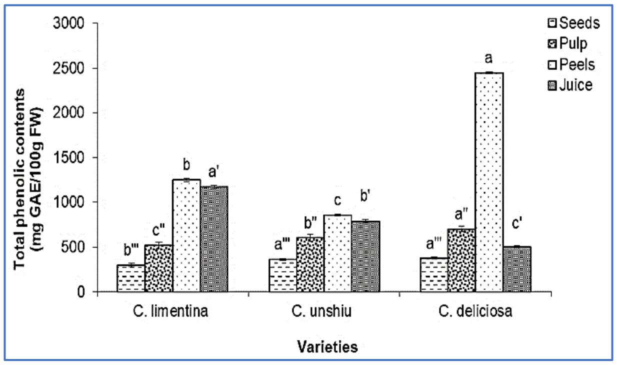

Figure 1: Total phenolic contents of different parts of the three mandarins varieties

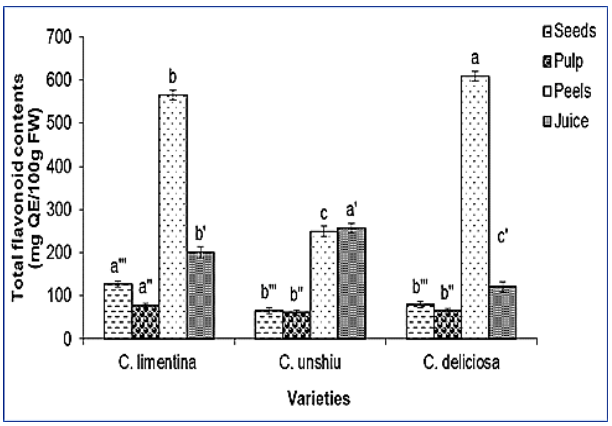

Figure 2: Total flavonoid contents of different parts of the three mandarins varieties

The flavonoid contents of different parts (seeds, pulps, peels, and juices) of the three mandarins varieties are shown in Figure 2. The results showed that the highest FCs were recorded for the peels of the three varieties with values of 609.78, 565.23, and 256.77 mg QE/100g FW for the Mediterranean mandarin, clementine, and Satsuma, respectively, followed by the juices, seeds, and pulps extracts. However, the lowest contents were recorded for pulps and seeds with values oscillating between 60.84 to 77.82 mg QE/100g FW and between 65.55 to 126.47 mg QE/100g FW, respectively. Statistical analysis revealed a significant difference (P<0.05) between the four parts of the Clementine and Mediterranean Mandarin varieties. While, no significant difference (P>0.05) was observed between the juices and the peels and between the seed and pulps of the Satsuma variety.

According to the results illustrated in Figure 3, the highest PrCs were recorded for clementine and Mediterranean Mandarin juices with a value of 46.67 and 27.55 mg CE/100g FW respectively, followed Mediterranean Mandarin (16.24 mg CE/100g FW) and Satsuma peels (14.95 mg CE/100g FW). Likewise, the pulps and seeds exhibited the lowest levels. Statistical analysis showed a significant difference (P<0.05) between the four parts of each variety and between each fraction of three varieties.

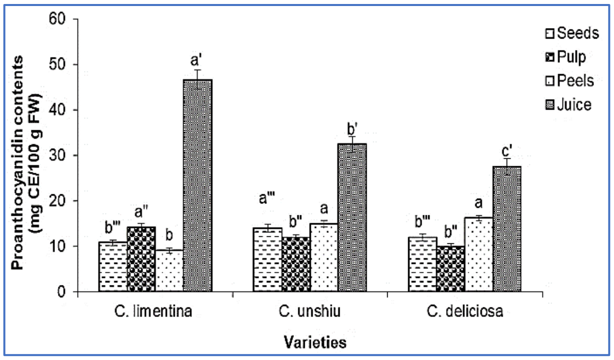

Figure 3: Proanthocyanidins contents of different parts of the three mandarins varieties

3.2 Antioxidant activity

The results of FRP, FRSA, and PhM of the different parts (seeds, pulps, peels, and juices) of the three mandarins varieties are summarized in Table 1.

The results showed that the juices of the three varieties presented a strong FRP with values of 1270.91, 1109.40, and 1091.39 mg AAE/100g FW for Mediterranean Mandarin, Satsuma, and Clementine, respectively. In addition, the activity of their peels varied between 253.049 to 495.41 mg AAE/100g FW. Likewise, the lowest activity was recorded for the pulps (varied between 58.76 and 72.15 mg AAE/100g) and seeds (varied between 72.81 and 78.50 mg AAE/100g FW). Statistical analysis showed a significant difference (P<0.05) between the ferric reducing power of peels and the juices of the three varieties of extracts. However, no significant difference (P>0.05) was observed between the pulps and the seeds' extracts. Likewise, a significant difference (P<0.05) was revealed between the peels and the juices of the three varieties.

Concerning the FRSA (DPPH), a strong activity was recorded for Mediterranean Mandarin peels (259.89 mg AAE/100g FW) followed by Clementine (258.37 mg AAE/100g FW), and Satsuma peels (211.18 mg AAE/100g FW). In addition, a strong activity was also recorded for the pulps and seeds of Mediterranean Mandarin with values of 197.06 and 189.57 mg AAE/100g FW, respectively. While the lowest activity was recorded for Satsuma (133.88 mg AAE/100g FW) and Mediterranean Mandarin (122.98 mg AAE/100g FW) juices. Statistical analysis revealed a significant difference (P<0.05) between the activity of the four parts of the three varieties and between the parts of each variety.

Regarding the phosphomolybdate activity, the results showed that the strong activity was recorded for peels of the three varieties with values that are varying between 172.95 and 190.19 mg GAE/100g FW. However, seeds and pulps presented the lowest activity with values varying between 66.05 and 90.88 mg

GAE/100g FW and between 87.97 and 144.49 mg GAE/100g FW, respectively. Statistical analysis revealed a significant difference ($P<0.05$) between the four parts of each variety as well as between each part of the three varieties.

A correlation between the quantified compounds and the values of the evaluated antioxidant capacity was carried out in order to determine the contribution of these compounds to the antioxidant activity tested (Table 2). A high correlation was observed between TPC and PrC of juices and FRSA and PhM with a correlation coefficient which was varied between r=0.95 and 0.99. In addition, a positive correlation was observed between TPC and TFC of the peels extracts and three antioxidant activity tested with a correlation coefficient "r" which was varied between r = 0.72 and r = 0.99. Furthermore, a positive correlation was observed between PrC and FRP and PhM of the seed extracts (r = 0.63 and r = 0.73, respectively) and between TPC of the pulps extracts and FRP and FRSA with coefficient value of r = 0.88 and r = 0.83 respectively. Likewise, a negative correlation was observed between phenolic compounds quantified of the juices and ferric reducing power (coefficient correlation varied between r = -0.83 and r = - 0.71).

In addition, a negative correlation was observed between PrC and FRP and PhM of the peels extracts with coefficient values of r=-0.31 and r=-0.40, respectively. A negative correlation was also observed between TFC and FRP, and between TFC and FRSA and between TPC and PhM of seeds extracts.

Table 1: Antioxidant activities of different parts of the three mandarins varieties: ferric reducing power (FRP), free radical scavenging activity (FRSA), and phosphomolybdate methods (PhM)

Parts	Varieties	FRP (mg AAE/100g FW)	FRSA (mg AAE/100g FW)	PhM (mg GAE/100g FW)
Seeds	*C. clementina*	78.503±0.86[b]	189.219±3.09[b]	90.880±1.87[a]
	C. unshiu	86.085±0.51[a]	194.639±1.81[a]	85.990±4.80[a]
	C. deliciosa	72.816±0.18[b]	189.575±1.33[b]	66.050±1.62[b]
Pulps	*C. clementina*	58.764±2.65[c]	190.859±1.82[b]	104.901±1.92[b]
	C. unshiu	67.971±0.77[b]	191.858±0.53[b]	87.970±1.87[c]
	C. deliciosa	72.154±0.90[a]	197.064±0.42[a]	144.490±1.92[a]
Peels	*C. clementina*	485.187±9.60[a]	258.375±0.75[a]	172.950±3.21[b]
	C. unshiu	253.049±8.57[b]	211.186±0.21[b]	135.970±5.33[c]
	C. deliciosa	495.417±6.99[a]	259.899±1.51[a]	190.190±2.39[a]
Juices	*C. clementina*	1091.390±7.92[c]	202.280±2.07[a]	108.201±3.08[c]
	C. unshiu	1109.407±10.60[b]	133.880± 2.71[b]	132.960±2.92[a]
	C. deliciosa	1270.910±11.97[a]	122.980±2.48[c]	117.210±3.23[b]

4 Discussion

The results of TPC of different parts of the three mandarins varieties obtained in the present study was in agreement with those obtained by Moulehi *et al.* [19] and Safdar *et al.* [20] for clementine seeds (210 mg GAE/100g FW) and Mediterranean Mandarin peels (2840 mg GAE/100g FW) cultivated in Tunisia and Pakistan, respectively. However, our results were higher than those obtained by Ramful *et al.* [21] for mandarin pulps grown in France (75 to 95 mg GAE/100g FW). While, Xu *et al.* [22] and Al-Juhaimi & Ghafoor [2] reported that the phenolic contents of mandarins juices cultivated in China and Saudi Arabia were 55.46% and 90 mg GAE/100 ml, respectively. In addition, Leontowicz *et al.* [23], Li *et al.* [24], and Garau *et al.* [25] reported that the total phenolic contents of peels were higher than the pulps extract of apple, pomegranate, and orange, respectively. Prasad *et al.* [6] showed that the high TPC was recorded for the peels (680 mg GAE/100g FW) followed by the pulps (140 mg GAE/100g FW) and seeds (10 mg GAE/100g FW) for *Canarium odontophyllum* Miq fruit.

Table 2: Correlation between phenolic compounds (TPC, TFC, and PrC) and antioxidant capacity (FRP, FRSA, and PhM)

	FRP	DPPH	PhM
		Seeds	
TPC	0.40	0.41	-0.79
TFC	-0.29	-0.56	0.55
PrC	0.68	0.73	-0.12
		Pulps	
TPC	0.88	0.83	0.20
TFC	-0.81	-0.49	0.49
PrC	-0.95	-0.88	-0.13
		Peels	
TPC	0.72	0.99	0.87
TFC	0.99	0.84	0.96
PrC	-0.31	0.33	-0.40
		Juices	
TPC	- 0.83	0.95	0.88
TFC	-0.79	0.23	0.60
PrC	-0.71	0.99	0.96

Concerning the TFC, the obtained results were in agreement with those reported by Moulehi *et al.* [19] and Ramful *et al.* [21] for seeds and pulps of Pakistan and France mandarins, respectively. Moreover, our results were lower than those obtained by Wang *et al.* [26] for mandarin peels grown in Japan (4920 mg QE/100g DM). In addition, Vanamala *et al.* [27] reported that the flavonoid content of orange juice was 55 mg QE/100 ml. Li *et al.* [24] reported that the high TFC were recorded for pomegranate peels (590mg QE/100g) followed by pulp (172 mg QE/100g). When, Prasad *et al.* [6] showed that the highest TFC were recorded for the peels, followed by the pulps, and seeds for the *Canarium odontophyllum* Miq fruit.

On the other hand, Negro *et al.* [28] reported that proanthocyanidin contents of seeds and peels of red grapes were 1380 and 80 mg CE/100g DM, respectively. Furthermore, Li *et al.* [24] showed that the highest proanthocyanidin contents of pomegranates was recorded for the peels (1000 mg CE/100g DM) followed by the pulp (500.3 mg CE/100g DM).

Under normal physiological conditions, phenolic compounds are compartmentalized in fruit cells. During juice pressing operations, cellular integrity is impaired. The phenolic compounds that generally accumulate within the vacuoles are solubilized. Overall, the phenolic potential of fruits depends on many external (climatic conditions, technical routes, origins) and internal factors (physiological state of the fruit, the position of the latter on the tree, genotype). The antioxidant compounds content of most fruits and vegetables may change pending on environmental conditions (temperature, radiation, etc.), driving methods (fertilization, irrigation, etc.) or, more broadly, production methods (conventional or organic) [5]. Additionally, the extraction techniques used as microwave, ultrasonic, etc. can significantly affect the phenolic compounds' extraction and release the soluble conjugate form of bioactive molecules [29].

For the antioxidant activity evaluated in the present study, the results showed that different parts of the three varieties exhibit a strong activity. The study carried out by Guo *et al.* [30] on orange, lemon, and dates, showed that the peels have a strong ferric reducing power than the pulps and seeds. Xu *et al.* [22] and Chaalal *et al.* [13] revealed different levels of FRP for mandarin juices and prickly pear seeds with values of 7.26 and 179.84 mg AAE/100g, respectively. In addition, Benchikh *et al.* [31] reported that the FRP of carob pulp was 1260 mg AAE/100g. Likewise, Safdar *et al.* [20] reported that the ferric reducing power of mandarin peels grown in Pakistan was 21.95%. Leontowicz *et al.* [23] reported that apple and pear peels showed a greater anti-free radical activity than the pulps. Moulehi *et al.* [19] and Safdar *et al.* [20] reported that the FRSA of seeds and peels of mandarin cultivated in Tunisia and Pakistan were 55.61% and 577.00 µg AAE/ml, respectively. In addition, Benchikh *et al.* [31] reported that the FRSA of carob pulp was1260 mg AAE/100g. Xu *et al.*[22] reported that the activity of citrus juices grown in China was 26.31% and 47.82% for Satsuma mandarin and lemons, respectively. Differences in cultivation region and practices, ripening stage, harvested conditions, and seasons could be the most factors leading to variation of the antioxidant activity [32]. Moreover, the antioxidant activity depends on the extraction solvent polarity, the extraction techniques as well as the process and the temperature of drying.

This positive correlation (higher or moderate) between the antioxidants assessed and the antioxidants activities evaluated of different parts of the three studied mandarins varieties could be explained by the contribution of the phenolic compounds quantified on the antioxidant activities tested. Besides, a negative correlation can be explained by the presence of other bioactive molecules in the extracts that contributed to those activities such as ascorbic acids, carotenoids, etc.

5 Conclusion

The aim of this work was to compare the phenolics amounts (TPC, TFC, and PrC) and the antioxidant activity (FRP, FRSA, and PhM) of different parts (seeds, pulps, peels, and juices) of three mandarin varieties. The obtained results showed a difference in the phenolic amount and antioxidant activity between fractions as well as between varieties. Indeed, the Mediterranean Mandarin peels presented higher phenolics compounds contents with values of 2445.62 mg GAE/100g FW for TPC, 609.78 mg QE/100g FW for TFC. While the clementine juice showed the highest PrC with a value of 46.67 mg C E/100g FW. For the antioxidant activity, the Mediterranean Mandarin peels exhibited strong activities compared to the other fractions, with the following values: 259.89 mg AAE/100g FW for FRSA and 190.19 mg GAE/100g FW for PhM, while, the juice extract presented a strong FRP with a value of 1270.91 AAE/100g FW. However, seeds and pulps showed the lowest phenolic amounts and antioxidant activity. A positive correlation was recorded between the antioxidant contents of different fractions and their antioxidant activities, with some exceptions. The present study confirms that the antioxidant compounds amounts are distributed differently in the different fruit fractions and the mandarin peels constitute an excellent source of bioactive compounds with significant antioxidant activity.

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