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THE ANTIOXIDANT POTENTIAL OF FLESH, ALBEDO AND FLAVEDO EXTRACTS FROM DIFFERENT VARIETIES OF GRAPEFRUITS

Anna Muzykiewicz✉, Joanna Zielonka-Brzezicka, Adam Klimowicz

Chair and Department of Cosmetic and Pharmaceutical Chemistry, Pomeranian Medical University in Szczecin
Powstańców Wielkopolskich 72, 70-111 Szczecin, **Poland**

ABSTRACT

Background. The supplementation of antioxidants, in particular those of plant origin, may help to prevent the development of diseases caused by oxidative stress. Therefore, it is important to study plants for their antioxidant contents. Up to now, only a few reports on the antioxidant activity of different varieties and parts of grapefruit have been published. Therefore, the aim of this study was to evaluate the antioxidant potential of different parts and varieties of grapefruit. Moreover, the impact of different extraction parameters on the activity of the obtained extracts was estimated.

Materials and methods. Extracts of albedo, flavedo and flesh from three varieties of grapefruit – red, white and sweetie – were obtained using ultrasound-assisted extraction (time – 5, 10, 15 and 30 minutes; solvents – distilled water as well as 20, 40, 70 and 96% (v/v) ethanol). The samples were evaluated using the DPPH, ABTS, FRAP and Folin-Ciocalteu methods.

Results. The extracts of peel (in particular, those of albedo) showed higher antioxidant potential than the samples of flesh. In the majority of cases, the highest potential in the group of flesh and flavedo extracts was observed in the sweetie samples. The highest activity in the group of albedo samples was found in the white grapefruit extracts. Parameters such as the type of solvent and the extraction time had an impact on the antioxidant activity of the obtained extracts.

Conclusion. Grapefruit, in particular their peels, could be valuable sources of natural antioxidants. However, more detailed studies on the antioxidant properties of the studied plants are required.

Keywords: red grapefruit, white grapefruit, sweetie, flavedo, albedo, ultrasound-assisted extraction

INTRODUCTION

Fruit and vegetables are examples of the most important components of an adequately balanced and healthy diet. The results of many studies suggest that daily intake of the proper amount of fruit and vegetables could reduce the risk of death due to cardiovascular diseases and/or cancer. Current WHO recommendations include an intake of at least 400 g/day of such food ingredients. The results of several studies suggest that consumption of a minimum of 600 g of fruit and vegetables per day markedly reduces the risk of death due to neoplastic disorders, while 800 g the risk of death due to cardiovascular ones. Such studies and recommendations are important because these diseases are some of the most common causes of premature death (Aune et al., 2017).

Oxidative stress induced by an excess of free radicals has been proven to have an impact on the development of many diseases, such as cardiovascular and neurodegenerative disorders, and neoplastic, psychiatric and metabolic diseases. This phenomenon leads

✉anna.muzykiewicz@pum.edu.pl, https://orcid.org/0000-0003-1880-1140, phone 48 91 466 1630

to damage in very important body structures, such as lipids, proteins and nucleic acids. Antioxidant supplementation, based on the consumption of fruit and/or vegetables, is one of the methods which can protect organisms against harmful oxidative stress effects. Polyphenols (e.g. flavonoids and phenolic acids), vitamins and plant pigments (e.g. anthocyanins, carotenoids and betacyanins) are considered to be the most active plant antioxidants (Leong et al., 2018; Muzykiewicz et al., 2018a; 2018b).

Citrus fruit is a valuable source of biologically active compounds which are an important factor in reducing the risk of ischemic stroke (Gorinstein et al., 2004). One of the most commonly consumed citrus fruit is grapefruit. Several commercially available varieties of this fruit differ in areas such as the colour of the peel and flesh, as well as in the taste. The most popular is a white variety (*Citrus paradisi* Macfadyen) and a kind with red flesh (Ladaniya, 2008). Jaffa sweetie fruit (Oroblanco, *Citrus grandis* × *C. paradisi*) is a relatively new fruit growing in Israel and it is a hybrid of the giant orange fruit (pomelo, pummelo) and white grapefruit (Gorinstein et al., 2004). Recently, several reports on the antioxidant activity of grapefruit flesh and peel have been published and, moreover, there are also a few papers on the antioxidant potential of different parts of the peel. Grapefruit peel consists of the internal, white and spongy albedo and the external, narrow and coloured part – the flavedo (Danyluk et al., 2019). Therefore, the aim of this study was to evaluate the antioxidant potential of flesh extracts, and albedo and flavedo samples of oroblanco, white and red grapefruit. In addition, the impact of applied solvents (distilled water and 20, 40, 70 and 96% (v/v) ethanol) on the antioxidant activity of the extracts was investigated, as was the time of ultrasound-assisted extraction (5, 10, 15 or 30 minutes). To evaluate the antioxidant potential of the extracts, the DPPH, ABTS, FRAP and Folin-Ciocalteu techniques were applied.

MATERIALS AND METHODS

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 6-hydroxy-2,5,7,8--tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich, USA. Folin-Ciocalteu reagent, gallic acid and iron(III) chloride hexahydrate were from Merck, Darmstadt, Germany, whereas 36% hydrochloric acid, potassium persulfate, iron(II) sulfate heptahydrate, sodium carbonate anhydrous, sodium acetate anhydrous and 99.5% acetic acid were from Chempur, Poland. All the chemicals were of analytical grade.

Three types of grapefruit were purchased in the local market – red (Jaffa Star Ruby), white (Jaffa) and sweetie (Jaffa, oroblanco). All the fruits were from Israel. Parts of the fruit including the flesh, albedo and flavedo were separated from the fresh material and extracted immediately using ultrasound-assisted extraction at a frequency of 40 kHz, for 5, 10, 15 or 30 minutes. 96% (v/v), 70% (v/v), 40% (v/v) and 20% (v/v) ethanol, as well as distilled water, were used as extractants. The antioxidant potential of the extracts was assessed using four methods: DPPH, FRAP, ABTS and Folin-Ciocalteu (F-C), as described previously (Muzykiewicz et al., 2018a; 2018b). The activity was expressed as equivalents of the reference substances contained in the fresh raw material.

To evaluate the antioxidant potential of the extracts with DPPH, the following procedure was used: a 0.3 mM ethanolic DPPH solution (the test reagent) was diluted with 70% (v/v) ethanol to obtain an absorbance of 1.000 ±0.020 at 517 nm. 2850 µl of this solution was added to 150 µl of the studied extract, mixed and incubated at room temperature for 10 minutes. The absorbance was taken at 517 nm.

To evaluate the antioxidant activity of the grapefruit extracts, the ABTS method was also applied. A 7 mM solution of ABTS in 2.45 mM aqueous $K_2S_2O_8$ was prepared and, after 24 hours of incubation in the dark at room temperature, was diluted with 50% (v/v) methanol to obtain an absorbance of 1.000 ±0.020 at 734 nm. Then, 25 µl of the extract was added to 2500 µl of this solution. Measurements of absorbance were taken at 734 nm after 6 minutes of incubation. In both DPPH and ABTS methods, Trolox was used as the reference and activities were expressed as mg Trolox/g of fresh raw material.

To evaluate the ferric ion reducing power of the extracts, the FRAP method was applied. 1 volume of 10 mM TPTZ (in 40 mM HCl), 1 volume of 20 mM $FeCl_3$ and 10 volumes of 0.3 M acetate buffer (pH 3.6) were mixed to obtain the working solution. 80 µl of the extract was added to 2320 µl of this solution and

mixed vigorously. The absorbance was measured at 593 nm after 15 minutes incubation at room temperature. The results have been expressed as mg $FeSO_4$/g of fresh raw material, where iron(II) sulfate was used as the reference.

To determine the total polyphenol content, a 10% (v/v) aqueous solution of Folin-Ciocalteu reagent was prepared by dilution of the concentrated F-C solution and incubated in the dark at room temperature for 1 hour. 2700 µl of 5 mM Na_2CO_3 and 150 µl of extract were mixed with 150 µl of diluted F-C reagent. The absorbance of the samples was taken at 750 nm after 15 minutes incubation at room temperature. Gallic acid was used as the reference standard, so the total polyphenol content was expressed in mg gallic acid/g of fresh raw material.

All the extract measurements were done in triplicate. The data are presented as an arithmetical mean ±standard deviation (SD). The Pearson correlation coefficients (r) between the results obtained by the various methods were calculated. The statistical significance of the differences between the activity of the extracts from individual grapefruit varieties, as well as between the potential of the extracts from individual parts of each variety, was evaluated by the Wilcoxon's test. All statistical calculations were done using Statistica 13 PL Software (StatSoft, Polska). $p < 0.05$ was considered to be statistically significant.

RESULTS

The antioxidant activities of the studied grapefruit extracts evaluated using the DPPH, ABTS, FRAP methods, as well as the total polyphenol content determined using the F-C method, are presented in Tables 1–4.

The antioxidant activities evaluated using the DPPH method expressed as Trolox equivalents are summarized in Table 1. Some extracts of sweetie and white grapefruit showed no antioxidant properties. The highest potential was found for white grapefruit albedo extracts prepared using 20% (v/v) ethanol (15 min). No activity was found for most of its flesh samples. In the case of red grapefruit, the highest antioxidant potential was observed for flavedo extract in 20% (v/v) ethanol and flesh in 96% (v/v) ethanol, both extracted for 30 minutes. In the group of sweetie extracts, the highest activity was obtained for albedo extracts prepared using 70% (v/v) ethanol, with a 5-minute process.

Table 2 presents the antioxidant potential of red, white and sweetie grapefruit extracts determined using the ABTS method, also expressed as Trolox equivalents. Similar to the DPPH method, there are a few samples without antioxidant activity. The highest results were found for the albedo extracts in each group of samples prepared using the same fruit. It can be noted that, in the case of the red and white grapefruit, the most effective was the extraction using 70% (v/v) ethanol (30 min), whereas for sweetie, the process in 96% (v/v) ethanol (5 min). In the majority of cases, the activity of the flesh was markedly lower than that of the flavedo and albedo extracts.

In Table 3, the ferric ion reducing power as determined by the FRAP method is presented. Similarly, as in the other methods, the highest potentials were found in the albedo extracts. The highest result was observed in the albedo white grapefruit extract (96% (v/v) ethanol, 5 min). The lowest results of all the studied materials were found in the flesh extracts. In the case of the red grapefruit and sweetie, the most effective method was the extraction in 70% (v/v) alcohol, whereas for white grapefruit, the process using 96% (v/v) ethanol as an extractant was the most effective.

The total polyphenol contents determined by the F-C method and expressed as gallic acid equivalents are presented in Table 4. No total polyphenol content was observed in the majority of the flesh sweetie extracts. The highest content of these compounds was found in the albedo red grapefruit extract (70% (v/v), 15 min). In the case of the white variety, the highest concentration was found in the albedo extracts prepared in 20% (v/v) ethanol (5 min), whereas for sweetie extracts, it was found in 96% (v/v) ethanol (30 min).

The Pearson correlation coefficients between the obtained results with the applied methods and their statistical significance are presented in Table 5, whereas the differences between the obtained results are shown in Table 6. All the correlations were statistically significant ($p < 0.05$). The differences between the activity (evaluated by all methods) of each part of the individual grapefruit varieties were statistically significant ($p < 0.0001$). An assessment of the statistical significance of the differences between the antioxidant potential of extracts from the individual varieties

Table 1. Antioxidant potential of red, white and sweetie grapefruit part extracts, evaluated using DPPH method (mean ±SD)

Solvent	Extraction time min	Red grapefruit			White grapefruit			Sweetie		
		flesh	flavedo	albedo	flesh	flavedo	albedo	flesh	flavedo	albedo
		Trolox equivalents, mg Trolox/g raw material								
96% (v/v) EtOH	5	0.31 ±0.03	0.16 ±0.04	0.58 ±0.01	0.28 ±0.01	0.01 ±0.00	1.13 ±0.02	0.31 ±0.02	0.02 ±0.00	0.65 ±0.00
	10	0.27 ±0.03	0.35 ±0.04	0.52 ±0.05	0.38 ±0.01	NA	0.74 ±0.02	0.26 ±0.04	0.04 ±0.01	0.67 ±0.09
	15	0.37 ±0.04	0.21 ±0.02	0.56 ±0.02	0.27 ±0.01	NA	0.57 ±0.00	0.45 ±0.04	0.26 ±0.01	0.58 ±0.06
	30	0.70 ±0.02	0.21 ±0.01	0.61 ±0.03	0.56 ±0.02	NA	0.56 ±0.01	0.39 ±0.00	NA	0.78 ±0.06
70% (v/v) EtOH	5	0.10 ±0.02	0.09 ±0.01	0.62 ±0.01	NA	NA	1.14 ±0.02	NA	NA	1.04 ±0.03
	10	0.11 ±0.04	0.24 ±0.02	0.67 ±0.01	0.10 ±0.03	NA	0.73 ±0.01	0.16 ±0.04	NA	0.67 ±0.08
	15	0.12 ±0.02	0.40 ±0.05	0.65 ±0.02	NA	0.07 ±0.01	0.47 ±0.01	0.26 ±0.04	0.43 ±0.01	0.13 ±0.01
	30	0.40 ±0.00	0.50 ±0.01	0.62 ±0.01	NA	NA	0.77 ±0.03	0.54 ±0.03	0.27 ±0.01	0.43 ±0.06
40% (v/v) EtOH	5	0.09 ±0.01	0.01 ±0.00	0.44 ±0.00	NA	NA	0.94 ±0.02	0.28 ±0.01	0.11 ±0.03	0.16 ±0.03
	10	0.09 ±0.00	0.59 ±0.03	0.49 ±0.04	NA	NA	0.65 ±0.00	0.11 ±0.02	0.01 ±0.00	0.47 ±0.03
	15	0.12 ±0.03	0.26 ±0.05	0.46 ±0.01	NA	0.58 ±0.03	0.78 ±0.04	0.12 ±0.00	0.27 ±0.03	0.08 ±0.01
	30	0.16 ±0.02	0.49 ±0.02	0.56 ±0.01	NA	0.51 ±0.03	0.82 ±0.01	0.32 ±0.01	0.18 ±0.01	0.17 ±0.02
20% (v/v) EtOH	5	0.10 ±0.01	0.23 ±0.01	0.40 ±0.03	0.05 ±0.01	0.12 ±0.02	1.01 ±0.01	0.20 ±0.03	NA	NA
	10	0.03 ±0.00	0.43 ±0.02	0.43 ±0.04	NA	0.40 ±0.02	0.83 ±0.01	0.17 ±0.01	0.06 ±0.01	NA
	15	0.05 ±0.00	0.58 ±0.03	0.42 ±0.02	NA	0.49 ±0.02	1.18 ±0.01	0.06 ±0.00	0.08 ±0.02	0.05 ±0.01
	30	0.19 ±0.01	0.71 ±0.04	0.43 ±0.02	0.05 ±0.01	1.12 ±0.02	0.38 ±0.02	0.30 ±0.02	0.09 ±0.01	0.11 ±0.02
H_2O	5	0.04 ±0.01	0.26 ±0.02	0.35 ±0.01	NA	NA	0.37 ±0.03	0.07 ±0.00	NA	NA
	10	0.08 ±0.01	0.42 ±0.02	0.31 ±0.02	0.02 ±0.00	0.24 ±0.01	0.72 ±0.01	0.44 ±0.03	NA	NA
	15	0.13 ±0.03	0.21 ±0.03	0.44 ±0.00	NA	0.29 ±0.00	0.81 ±0.02	0.35 ±0.00	NA	NA
	30	0.09 ±0.01	0.30 ±0.03	0.45 ±0.02	NA	0.65 ±0.02	0.76 ±0.01	0.21 ±0.04	NA	0.09 ±0.00

NA – no activity.

Table 2. Antioxidant properties of red, white and sweetie grapefruit part extracts, evaluated using ABTS method (mean ±SD)

Solvent	Extraction time min	Red grapefruit			White grapefruit			Sweetie		
		flesh	flavedo	albedo	flesh	flavedo	albedo	flesh	flavedo	albedo
		Trolox equivalents – 28/5000, mg Trolox/g raw material								
96% (v/v) EtOH	5	0.98 ±0.07	0.30 ±0.05	1.84 ±0.01	0.14 ±0.03	2.32 ±0.11	7.37 ±0.07	0.83 ±0.09	2.82 ±0.37	13.21 ±0.39
	10	1.15 ±0.11	0.74 ±0.03	2.00 ±0.14	0.46 ±0.09	1.21 ±0.14	7.68 ±0.22	0.84 ±0.10	3.90 ±0.31	9.99 ±0.32
	15	1.43 ±0.09	0.57 ±0.08	2.10 ±0.06	0.26 ±0.07	1.33 ±0.10	11.45 ±0.29	0.47 ±0.08	4.30 ±0.33	7.21 ±0.21
	30	1.70 ±0.09	0.81 ±0.03	2.29 ±0.20	1.13 ±0.13	1.88 ±0.10	9.64 ±0.03	0.86 ±0.05	3.90 ±0.19	11.21 ±0.44
70% (v/v) EtOH	5	1.19 ±0.05	NA	1.92 ±0.11	NA	2.91 ±0.11	8.88 ±0.13	0.39 ±0.09	3.44 ±0.33	12.27 ±0.16
	10	1.40 ±0.02	0.76 ±0.03	2.70 ±0.07	0.13 ±0.02	2.26 ±0.12	11.59 ±0.24	0.08 ±0.01	3.71 ±0.11	13.10 ±0.51
	15	1.12 ±0.15	0.74 ±0.03	2.31 ±0.04	0.14 ±0.03	3.15 ±0.11	8.47 ±0.28	NA	6.94 ±0.11	10.84 ±0.33
	30	1.42 ±0.08	1.22 ±0.07	3.10 ±0.19	0.09 ±0.04	2.48 ±0.08	15.54 ±0.10	1.25 ±0.15	5.40 ±0.01	12.80 ±0.27
40% (v/v) EtOH	5	1.50 ±0.03	0.44 ±0.03	1.78 ±0.05	NA	2.55 ±0.05	6.13 ±0.22	NA	5.55 ±0.16	8.70 ±0.06
	10	1.52 ±0.17	1.49 ±0.13	2.36 ±0.16	0.26 ±0.02	2.38 ±0.03	5.56 ±0.15	0.10 ±0.03	3.15 ±0.22	8.82 ±0.38
	15	NA	0.42 ±0.03	0.99 ±0.09	NA	2.87 ±0.13	10.71 ±0.12	NA	5.06 ±0.33	9.21 ±0.30
	30	NA	0.62 ±0.06	1.66 ±0.09	NA	2.26 ±0.10	13.01 ±0.26	0.46 ±0.04	4.77 ±0.36	9.97 ±0.23
20% (v/v) EtOH	5	0.03 ±0.01	0.10 ±0.06	1.26 ±0.12	0.01 ±0.00	2.01 ±0.09	11.59 ±0.08	0.02 ±0.00	2.02 ±0.25	5.40 ±0.47
	10	NA	0.80 ±0.07	2.25 ±0.16	NA	3.00 ±0.07	8.78 ±0.35	0.40 ±0.03	2.70 ±0.31	8.29 ±0.28
	15	NA	1.14 ±0.13	1.62 ±0.08	NA	4.26 ±0.14	12.51 ±0.22	0.02 ±0.00	4.17 ±0.11	11.03 ±0.19
	30	0.24 ±0.01	1.20 ±0.09	1.69 ±0.17	NA	5.92 ±0.09	7.91 ±0.03	0.43 ±0.06	3.93 ±0.41	12.10 ±0.31
H_2O	5	0.04 ±0.01	0.36 ±0.07	0.83 ±0.07	NA	1.61 ±0.19	5.13 ±0.07	0.17 ±0.05	4.32 ±0.39	6.18 ±0.26
	10	0.12 ±0.03	0.60 ±0.03	0.56 ±0.14	NA	1.83 ±0.07	8.30 ±0.20	0.35 ±0.02	3.37 ±0.39	10.62 ±0.22
	15	0.06 ±0.01	0.21 ±0.01	1.04 ±0.05	NA	3.40 ±0.03	8.85 ±0.01	0.35 ±0.07	3.52 ±0.20	10.09 ±0.42
	30	0.01 ±0.00	0.36 ±0.03	1.21 ±0.03	NA	2.55 ±0.08	11.79 ±0.00	0.43 ±0.06	3.29 ±0.37	8.78 ±0.20

NA – no activity.

Table 3. Ferric ion reducing power of red, white and sweetie grapefruit part extracts, evaluated using FRAP method (mean ±SD)

Solvent	Extraction time min	Red grapefruit			White grapefruit			Sweetie		
		flesh	flavedo	albedo	flesh	flavedo	albedo	flesh	flavedo	albedo
		$FeSO_4$ equivalents, mg $FeSO_4$/g raw material								
96% (v/v) EtOH	5	0.49 ±0.00	0.93 ±0.02	2.00 ±0.05	0.68 ±0.02	1.44 ±0.08	4.08 ±0.18	0.58 ±0.01	0.98 ±0.02	2.45 ±0.03
	10	0.60 ±0.01	1.33 ±0.02	1.98 ±0.01	0.73 ±0.01	1.21 ±0.08	3.27 ±0.08	0.72 ±0.04	1.70 ±0.00	2.62 ±0.08
	15	0.82 ±0.00	1.10 ±0.02	2.17 ±0.03	0.62 ±0.04	1.45 ±0.04	3.33 ±0.01	0.79 ±0.01	1.57 ±0.09	2.02 ±0.06
	30	1.26 ±0.02	1.13 ±0.04	1.99 ±0.06	0.96 ±0.02	1.56 ±0.04	2.80 ±0.07	0.87 ±0.07	1.43 ±0.04	2.79 ±0.06
70% (v/v) EtOH	5	0.44 ±0.01	0.87 ±0.03	1.99 ±0.02	0.36 ±0.02	1.57 ±0.07	3.72 ±0.04	0.44 ±0.02	1.21 ±0.03	3.14 ±0.16
	10	0.36 ±0.02	1.17 ±0.01	2.12 ±0.03	0.60 ±0.02	1.85 ±0.06	3.10 ±0.09	0.48 ±0.03	1.52 ±0.04	3.60 ±0.16
	15	0.41 ±0.01	1.39 ±0.02	2.23 ±0.01	0.55 ±0.02	3.37 ±0.12	2.46 ±0.05	0.61 ±0.05	2.71 ±0.00	2.48 ±0.02
	30	0.76 ±0.01	1.54 ±0.08	2.10 ±0.09	0.54 ±0.02	2.67 ±0.03	3.73 ±0.16	1.08 ±0.03	2.20 ±0.01	3.25 ±0.12
40% (v/v) EtOH	5	0.40 ±0.04	0.80 ±0.00	1.50 ±0.02	0.43 ±0.00	1.27 ±0.04	2.59 ±0.04	0.60 ±0.05	1.87 ±0.09	1.82 ±0.04
	10	0.40 ±0.01	1.76 ±0.00	1.77 ±0.04	0.64 ±0.02	2.07 ±0.02	2.16 ±0.05	0.50 ±0.01	1.53 ±0.02	2.19 ±0.07
	15	0.44 ±0.04	1.27 ±0.00	1.59 ±0.05	0.59 ±0.03	2.24 ±0.18	2.85 ±0.03	0.49 ±0.01	1.98 ±0.00	2.44 ±0.06
	30	0.62 ±0.03	1.47 ±0.03	1.99 ±0.06	0.43 ±0.02	2.01 ±0.20	2.91 ±0.12	0.73 ±0.02	2.15 ±0.03	2.77 ±0.10
20% (v/v) EtOH	5	0.44 ±0.02	0.84 ±0.03	1.42 ±0.05	0.52 ±0.00	1.44 ±0.03	2.82 ±0.17	0.41 ±0.02	0.98 ±0.01	1.19 ±0.00
	10	0.46 ±0.05	1.39 ±0.05	1.73 ±0.03	0.38 ±0.02	1.69 ±0.11	2.49 ±0.13	0.48 ±0.01	1.24 ±0.03	1.75 ±0.04
	15	0.38 ±0.02	1.59 ±0.03	1.78 ±0.07	0.54 ±0.03	2.02 ±0.14	3.80 ±0.04	0.39 ±0.02	1.82 ±0.01	2.13 ±0.02
	30	0.66 ±0.04	1.89 ±0.05	1.69 ±0.03	0.57 ±0.06	3.20 ±0.08	1.72 ±0.03	0.67 ±0.08	1.58 ±0.06	2.83 ±0.16
H_2O	5	0.46 ±0.02	1.11 ±0.01	1.54 ±0.01	0.61 ±0.01	1.10 ±0.05	1.37 ±0.01	0.47 ±0.01	1.10 ±0.01	1.29 ±0.04
	10	0.62 ±0.01	1.41 ±0.05	1.61 ±0.04	0.51 ±0.02	1.56 ±0.07	2.04 ±0.01	0.98 ±0.06	1.10 ±0.02	1.68 ±0.04
	15	0.50 ±0.01	1.53 ±0.05	1.64 ±0.02	0.42 ±0.00	1.60 ±0.02	2.78 ±0.22	0.86 ±0.02	1.24 ±0.01	1.56 ±0.01
	30	0.56 ±0.00	1.23 ±0.00	1.90 ±0.01	0.43 ±0.00	2.29 ±0.04	2.37 ±0.10	0.58 ±0.02	1.54 ±0.05	1.85 ±0.07

Table 4. Total polyphenol contents of red, white and sweetie grapefruit part extracts, evaluated using Folin-Ciocalteu method (mean ±SD)

Solvent	Extraction time min	Red grapefruit			White grapefruit			Sweetie		
		flesh	flavedo	albedo	flesh	flavedo	albedo	flesh	flavedo	albedo
		Gallic acid equivalents, mg gallic acid/g raw material								
96% (v/v) EtOH	5	0.26 ±0.07	0.41 ±0.07	1.16 ±0.07	0.14 ±0.01	0.07 ±0.01	1.02 ±0.04	NA	0.30 ±0.00	1.06 ±0.04
	10	0.25 ±0.01	0.43 ±0.03	1.14 ±0.01	0.40 ±0.03	0.29 ±0.03	0.86 ±0.01	NA	0.62 ±0.03	1.30 ±0.03
	15	0.20 ±0.04	0.41 ±0.04	1.20 ±0.03	0.20 ±0.06	0.13 ±0.02	1.03 ±0.03	NA	0.43 ±0.03	1.40 ±0.03
	30	0.66 ±0.05	0.43 ±0.05	1.24 ±0.06	0.39 ±0.01	0.15 ±0.02	0.98 ±0.07	0.07 ±0.03	0.46 ±0.00	1.69 ±0.01
70% (v/v) EtOH	5	0.05 ±0.03	0.33 ±0.03	1.29 ±0.03	0.02 ±0.00	0.54 ±0.02	0.78 ±0.00	NA	0.27 ±0.01	1.28 ±0.04
	10	0.14 ±0.01	0.53 ±0.03	1.47 ±0.08	0.29 ±0.03	0.12 ±0.03	0.75 ±0.01	NA	0.28 ±0.04	1.53 ±0.03
	15	0.14 ±0.02	0.59 ±0.03	1.85 ±0.07	0.33 ±0.05	0.50 ±0.02	1.07 ±0.05	NA	0.85 ±0.02	0.50 ±0.00
	30	0.38 ±0.07	0.49 ±0.01	1.16 ±0.08	0.26 ±0.07	0.08 ±0.01	1.01 ±0.03	0.22 ±0.00	0.53 ±0.01	0.72 ±0.07
40% (v/v) EtOH	5	0.05 ±0.03	0.25 ±0.03	1.22 ±0.03	0.32 ±0.03	0.11 ±0.01	1.16 ±0.06	0.20 ±0.03	0.58 ±0.01	0.75 ±0.01
	10	0.34 ±0.09	0.96 ±0.09	1.22 ±0.01	0.07 ±0.01	0.11 ±0.00	0.62 ±0.03	NA	0.95 ±0.03	1.07 ±0.05
	15	0.10 ±0.01	0.64 ±0.05	1.09 ±0.01	0.14 ±0.03	0.66 ±0.03	0.88 ±0.05	NA	0.40 ±0.03	0.47 ±0.00
	30	0.14 ±0.01	0.77 ±0.01	1.37 ±0.05	0.14 ±0.03	0.39 ±0.03	0.84 ±0.05	NA	0.31 ±0.03	0.90 ±0.03
20% (v/v) EtOH	5	0.20 ±0.01	0.38 ±0.01	1.17 ±0.10	0.14 ±0.03	0.03 ±0.01	1.27 ±0.05	0.25 ±0.03	0.62 ±0.01	0.81 ±0.05
	10	0.09 ±0.03	0.59 ±0.02	1.58 ±0.11	0.12 ±0.01	0.12 ±0.01	0.47 ±0.01	NA	0.56 ±0.03	1.09 ±0.02
	15	0.03 ±0.01	0.56 ±0.00	1.19 ±0.07	0.16 ±0.03	0.17 ±0.04	1.09 ±0.02	NA	0.48 ±0.00	1.09 ±0.00
	30	0.47 ±0.01	0.73 ±0.03	1.24 ±0.04	0.19 ±0.03	0.42 ±0.05	0.49 ±0.01	NA	0.28 ±0.00	1.09 ±0.03
H_2O	5	0.05 ±0.02	0.58 ±0.05	1.19 ±0.07	0.07 ±0.00	0.16 ±0.03	0.43 ±0.00	NA	0.59 ±0.02	0.46 ±0.00
	10	0.49 ±0.01	0.57 ±0.03	1.02 ±0.08	0.05 ±0.01	0.16 ±0.03	0.75 ±0.01	NA	0.59 ±0.02	0.73 ±0.03
	15	0.14 ±0.04	0.43 ±0.03	1.16 ±0.08	0.05 ±0.02	0.56 ±0.02	1.52 ±0.19	NA	0.97 ±0.01	0.64 ±0.01
	30	0.12 ±0.03	0.74 ±0.02	0.85 ±0.05	0.03 ±0.01	0.59 ±0.03	1.24 ±0.08	NA	1.19 ±0.05	0.75 ±0.05

NA – no activity.

Table 5. The correlation coefficients (r) and statistical significance (p) between the obtained results

	ABTS	FRAP	F-C
DPPH	0.466*	0.675*	0.583*
ABTS		0.805*	0.542*
FRAP			0.677*

*$p < 0.00001$.

Table 6. The differences between obtained results evaluated using the Wilcoxon's test

Red grapefruit	
Flesh – albedo	$z = 7.651$, $p < 0.0001$
Flesh – flavedo	$z = 4.768$, $p < 0.0001$
Albedo – flavedo	$z = 7.453$, $p < 0.0001$
White grapefruit	
Flesh – albedo	$z = 7.700$, $p < 0.0001$
Flesh – flavedo	$z = 6.437$, $p < 0.0001$
Albedo – flavedo	$z = 7.170$, $p < 0.0001$
Sweetie	
Flesh – albedo	$z = 7.276$, $p < 0.0001$
Flesh – flavedo	$z = 6.651$, $p < 0.0001$
Albedo – flavedo	$z = 6.767$, $p < 0.0001$
Red – white	$z = 3.919$, $p < 0.0001$
Red – sweetie	$z = 3.074$, $p < 0.001$
White – sweetie	NS

NS – not statistically significant ($p > 0.05$).

(taking into account all the analyzed parts, as well as the results obtained with all the methods) showed that only in the case of the activity of the white grapefruit and sweetie extracts are these differences not statistically significant ($p > 0.05$).

DISCUSSION

Analysis of the antioxidant potential of the extracts prepared from different parts of white and red grapefruit, as well as those from the sweetie (oroblanco) hybrid showed that the peel extracts have a higher activity than the flesh extracts. This observation is in accordance with the study by Gorinstein et al. (2004), who evaluated the activity of the flesh and peel of white grapefruit and sweetie extracts. They found that the grapefruit peels were characterized by a significantly higher content of phenolic acids than the flesh. Zielonka-Brzezicka et al. (2018a; 2018b) observed a higher antioxidant activity in peel extracts when compared to flesh samples for some other tropical fruits. An analysis of the antioxidant potential of each peel part – albedo and flavedo – showed that, regardless of the measurement technique, a higher activity was found in the albedo extracts. Yerlikaya et al. (2016) evaluated the antioxidant activity of albedo and flavedo extracts from different citrus fruits – sour orange (*C. aurantium*), grapefruit (*C. paradisi*) and bergamot (*C. bergamia*). In their study, the albedo of all the fruit samples showed a higher potential, including the total phenolic compound content. Moreover, the extracts of grapefruit albedo contained almost two times more phenolic compounds and were characterized by twenty times higher activity determined by the ABTS method when compared to the flavedo extracts. In our study, the comparison of the antioxidant activity of extracts from different varieties of the analyzed fruit showed the highest potential for the extracts of sweetie for the majority of the flesh and flavedo samples (taking into account the results obtained by all the applied methods). In the group of albedo extracts, the white grapefruit extracts showed the highest antioxidant activity.

Taking into account the antioxidant activity of each analyzed fruit part extract evaluated with all the applied techniques, the highest results were obtained in the sweetie extracts, whereas the lowest were found in the red grapefruit samples. It should be noted that the antioxidant activity of the extracts from each grapefruit variety was different and depended on the applied method of evaluation. For the DPPH method, the highest results were obtained most frequently for the sweetie and white grapefruit extracts. The highest potential evaluated by the ABTS technique was found for the sweetie extracts, whereas the white grapefruit extracts showed the highest ferric ion reducing power. The highest content of polyphenols was found in most cases in the red grapefruit extracts. Gorinstein et al. (2005) compared the antioxidant activity of the peel and flesh of white and red grapefruit. In both varieties, the highest potential was determined by the DPPH

method. In addition, the highest total polyphenol content evaluated by the F-C technique was observed in the red grapefruit extracts (for both the peel and the flesh). Moreover, in the case of both grapefruit, the peel extracts were characterized by a higher potential than the flesh samples. A similar observation was noted in our study. The comparison of the antioxidant activity obtained for the red and white grapefruit extracts showed that the red grapefruit extracts demonstrated a higher activity, determined by the DPPH, ABTS and F-C techniques, when compared to the samples prepared from the white variety. Gorinstein et al. (2004) compared the activity of the peel and flesh extracts of Israeli white grapefruit and sweetie fruit (harvested from 2003–2004). Contrary to our results, they observed a higher content of antioxidants in the sweetie fruit extracts. In our study, the white grapefruit extracts showed a higher potential evaluated by the DPPH, FRAP and F-C technique. Higher antioxidant activity was only observed in the sweetie extracts evaluated using the ABTS method. This may indicate that the antioxidant activity of the grapefruit extracts may depend on the year the raw material is harvested.

In evaluating the effects of extracting the solvent on the antioxidant activity of the obtained extracts, it was found that the highest antioxidant activity occurred in the majority of the ethanolic extracts. In contrast, the antioxidant potential of the aqueous extracts determined by the F-C method was not high, except in the total polyphenol content in the white grapefruit albedo extracts. Ye et al. (2015) also confirmed the effects of the solvent and its concentration on the antioxidant potential of plant extracts. Based on studies of the antioxidant activity of sunflower florets extract, they postulated that solvent polarity had an effect on the antioxidant potential of extracts, including the polyphenol content. They observed the highest potential for samples prepared in medium-polar extractants such as aqueous solutions of ethanol and methanol (50%), in contrast to extracts prepared in high (water) and low (ethyl acetate) polarity solvents. The observation that the aqueous solutions of ethanol seem to be more efficient solvents than water for extracting compounds with antioxidant potential was confirmed in our study. The effects of solvent concentration on the antioxidant activity of extracts was also noticed by Sun et al. (2015) in their studies on the antioxidant potential of propolis extracts. The choice of solvent and the extraction time seem to be important factors in the extraction efficiency of antioxidants. Azmir et al. (2013) suggested that time should be optimized depending on the type of plant material. In our study, it was rather difficult to establish the most favourable time for ultrasound-assisted extraction to obtain extracts of the highest activity. However, a higher potential of the extracts was predominantly observed in 30-minute extractions. Other studies confirm the beneficial effects of prolonged ultrasound-assisted extraction on the antioxidant activity of the extracts (Muzykiewicz et al., 2018a; 2018b). In contrast, Xu et al. (2017) tried to optimize the time of the ultrasound-assisted extraction of *Limonium sinuatum* to obtain extracts with the highest antioxidant potential, and found that 10-minute extractions seem to be more efficient than 30-minute ones. This observation confirmed that the extraction time should be optimized depending on the plant material to be used and the extraction solvent.

To sum up, the white part of the peel (albedo) of white and red grapefruit, and of sweetie fruit in particular, seems to be a valuable source of natural antioxidants. However, it should be taken into account that, due to its content of more than 100 biologically active compounds such as flavonoids and furanocoumarins, grapefruit juice could affect the pharmacokinetics of certain drugs (Hu et al., 2016; Mouly et al., 2017; Theile et al., 2017). Therefore, despite the beneficial effects of grapefruit antioxidants, patients should consult a physician or pharmacist on the possible interaction between the applied drug and citrus juice or other citrus products.

CONCLUSIONS

In our study, the highest antioxidant activity evaluated using the DPPH, ABTS and FRAP methods was found in the albedo extracts, particularly those of white grapefruit, whereas the lowest was found in the flesh samples. However, in most cases, the highest antioxidant potential of flesh and flavedo samples was observed in the sweetie extracts. Taking into account the extractant, in most cases, the highest results were obtained in the ethanolic extracts. Water seems not to be a good solvent for grapefruit extraction. The impact of extraction time on antioxidant activity is ambiguous,

so this activity may depend on other factors. However, prolonging the extraction time can enhance the antioxidant activity of the obtained extracts.

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