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DETERMINATION OF ANTIOXIDANT CAPACITY OF ALCOHOLIC EXTRACTES FROM PEEL OF CITRUS FRUITS

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Abstract

Citrus fruits have a high antioxidant capacity due to the high content of volatile oils, polyphenolic compounds, flavonoids, carotenoids, bitter substances existing in the pericarp of the fruit. More and more studies show that these fruits can be used successfully to treat oxidative stress and chronic, degenerative diseases.

In this paper, three alcoholic extracts were made from the peel of mandarin, orange and grapefruit fruits and it was found following the Folin-Ciocâlteu and colorimetric analysis that the peel of grapefruit fruits contains the highest amount of flavonoids and polyphenolic compounds with antioxidant effect demonstrated by the DPPH, FRAP and ABTS methods.

Keywords: peel of citrus fruits, antioxidant, flavonoids, polyphenolic compounds

INDTRODUCTION

Although citrus fruits are originating in Asia, they can be found in all tropical and subtropical countries. World production in 2006 was over 124 million tonnes (Duthie et al., 2003, Fierascu et al., 2014).

Citrus fruits and peels are rich in natural antioxidants (flavonoids, phenolic acids) and the alcoholic extract of mandarins, oranges and grapefruits is beneficial in treating chronic diseases: diabetes, cardiovascular disease, cancer, respiratory diseases, etc. (Rafiq et al., 2016, Zou et al., 2016, Fraga et al., 2019) and in the fight against free radicals. In the shell are polymethoxylated flavonoids and glycosylated flavonoids which are found exclusively in citrus and are specific to each species where they can act as a marker of counterfeiting/denaturation in commercial juices (Gattuso et al., 2007, Mouly et al., 1994, Ooghe and Detavernier, 1997, Arias, Ramon-Laca, 2005).

There are many studies that have shown the antioxidant action of citrus by various chemical methods of analysis, but all in this paper are used the Folin-Ciocâlteu method, the DPPH method (uses 2,2-diphenyl-1-picryhydrazine reagents), the colorimetric method, the ABTS (2,2´-azino-bis-3-ethylbenzthiozoline-6-sulphonic acid) and FRAP method (ferric reducing / antioxidant power) (Zou et al., 2016, Lee et al., 2015, Rivero-Pérez et al., 2007, Benzi et al., 1999, Ou et al., 2001) because they have many advantages

and do not require the use of very expensive equipment (Zou et al., 2016, Nihal et al., 2006).

MATERIAL AND METHOD

CHEMICALS

UV-VIS PG Instruments T70 + spectrophotometer, reagents FRAP, ABTS, DPPH were purchased from Sigma Aldrich USA, Folin - Ciocâlteu reagent from Merck Darmstadt Germany, gallic acid and quercetin were supplied by Silver Chemicals Romania. The ultrapure water was prepared using a Millipore system (Millipore, Bedford, USA). All other analytical grade reagents were purchased by Silver Chemicals Romania.

PLANT MATERIALS

For the preparation of citrus extracts, fruits purchased from the local market in Romania, Bihor Oradea were used in the winter of 2018.

The peels of *Citrus sinensis L., Citrus reticulata* and *Citrus aurantium* were shredded, dried and stored at room temperature until the extract was made. The orange, grapefruit and mandarin extracts were prepared using the Soxhlet device in order to determine the total content of polyphenolic compounds.

METHODS

The peels of orange, mandarin and grapefruit were subjected to direct microscopic analysis using a digital microscope and an optical microscope.

Determination of content of polyphenols in citrus peels can be done by several methods: Folin-Ciocâlteu method and colorimetric method. The Folin-Ciocâlteu method determine the phenolic hydroxyl groups in citrus extracts, in a basic medium with sodium carbonate. The colorimetric method determine the concentration of flavonoids in the extracts (Istudor, 1998, Chen et al., 2015, Everette et al., 2010, Jurca et al., 2016, Dae-Ok et al., 2003, Zhishen et al., 1999).

Antioxidant capacity of citrus peel extracts was determined using DPPH, FRAP, ABTS methods. DPPH analysis was performed with 1,1-diphenyl-2-picrylhydrazyl reagent. It has an intense purple color in the solution, a color that changes to pale yellow or colorless when neutralized by flavonoids. Its color change offers the possibility to observe and visually the neutralization reaction of DPPH with compounds that have antioxidant capacity. FRAP is a simple, spectrophotometric method based on the reduction of the ferric tripyridyltriazine complex to the ferrous tripyridyltriazine complex by a reducting, the reaction taking place at acidic pH. or TEAC is based on the ability of antioxidants to reduce cation radicals

(ABTS +) a green-blue chromophore that absorbs at 734 nm, the method being done compared to a standard Trolox solution (Jurca et al., 2016).

RESULTS AND DISCUSSION

By microscopic analysis of the external surface of both citrus species *(Citrus aurantium, Citrus paradisi*), as can be seen in figures 1 and 2, the secretory structures have a round or oval shape, of different sizes, arranged in a certain order.

The secretory pockets in the orange fruit have an oval shape and in the case of the grapefruit fruit the secretory pockets are in greater numbers, even in the albedo area of the fruit and have a round shape.

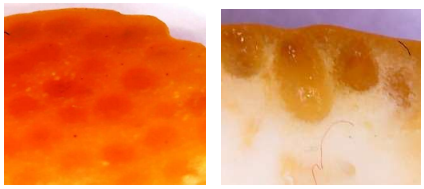

Fig. 1. Secretory pockets of the orange fruit

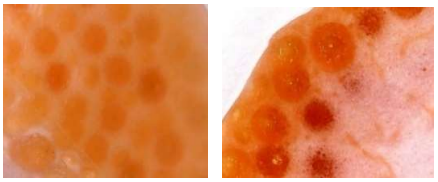

Fig. 2. Secretory pocket of the grapefruit fruit

A microscopic analysis performed using an optical microscope on successive cross sections through the epicarp of the orange and grapefruit fruit identified internal secretory pockets, larger or smaller depending on the stage of development. Microscopic preparations were studied at 10x and 40x objective. The internal space of the secretory pocket consists of flattened, parenchymal cells, closely joined together in 2-3 rows, the last row lining the secretory pocket forms the layer of secretory cells of the pocket. The secreted drops of volatile oil can only be seen in the fresh sections. The internal secretory pockets identified in the two species are differentiated by shape and number. In orange fruit, as shown in figures 3 and 4, the secretory pockets are oval, elongated, and are found only in the flavedo area of the fruit.

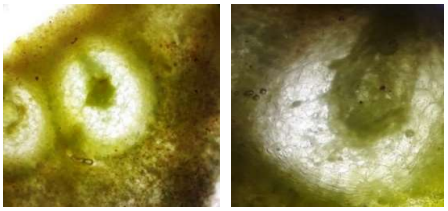

Fig.. 3. Oval internal secreting pockets, observed in the orange fruit

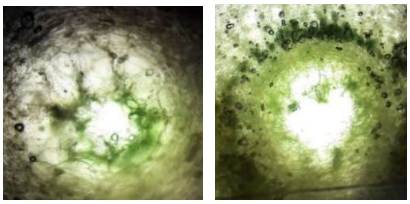

Fig. 4. Round internal secretory pockets, observed in the grapefruit fruit

The total polyphenol content determined by the Folin-Ciocalteu method is shown in table 1.

Table 1

Calculation of the average total polyphenol concentration of citrus peel extracts expressed in mg GAE/100 g dry product

Sample	Sample absorption read at 765 nm	Sample concentration (mg GAE/100 g dry matter)	Average sample concentration (mg GAE/100 g dry matter)
Orange	0.7065	584.9629	585.0123 ±0.4692
	0.7060	584.5926	
	0.7072	585.4815	
Grapefruit	0.7488	616.2963	615.8765±1.8024
	0.7458	614.0741	
	0.7501	617.2592	
Mandarine	0.6151	517.2592	519.6296±2.3704
	0.6211	521.7037	
	0.6187	519.9259	

From analysis of data provided in table 1 it is observed that the highest concentration of total polyphenols is found in the ethanolic grapefruit peel extract (615.8765 ± 1.8024), followed by the orange peel extract (585.0123 ± 0.4692) and then the extract from mandarine peel (519.6296 ± 2.3704).

Reactive oxygen species are involved in many human pathological diseases, so flavonoids are compounds that have antioxidant capacity and can fight free radicals. The amount of flavonoids determined is given in table 2.

Table 2.

Calculation of the average flavonoid concentration of citrus peel extracts expressed in mg EQ/100 g dry product

Sample	Sample absorption read at 510 nm	Sample concentration (mg EQ/100 g dry matter)	Average sample concentration (mg EQ/100 g dry matter)
Orange	1.5913	1930.1368	1933.2042 ±6.3769
	1.5991	1939.5811	
	1.5911	1929.8946	
Grapefruit	1.6025	1943.6978	1947.8548±4.1570
	1.6078	1950.1150	
	1.6075	1949.7518	
Mandarine	0.9940	1206.9258	1205.9168±3.9553
	0.9899	1201.9615	
	0.9956	1208.8631	

Analysis of data provided by table 2 leads to conclusion that the highest concentration of flavonoids, so the highest antioxidant capacity has the alcoholic extract of grapefruit peel (1947.8548 ± 4.1570), followed by the orange peel extract (1933.2042 ± 6.3769) and the mandarine peel extract (1205.9168 ± 3.9553).

Antioxidant capacity determined by the DPPH method on the three extracts is presented in table 3.

Table 3.

DPPH test results on citrus peel extracts (orange/grapefruit/mandarin)

Sample	Blanc absorbance	Sample absorbance	Percentage of inhibition, %	Average percentage of inhibition, %
Orange	0.6959	0.0381	94.5394	94.4915±0.2395
		0.0372	94.6831	
		0.0400	94.2520	
Grapefruit	0.6959	0.0322	95.4016	95.5890±0.2437
		0.0293	95.8327	
		0.0293	95.8327	
Mandarin	0.6959	0.0895	87.1389	87.2490 ±0.1532
		0.0869	87.5125	
		0.0898	87.0958	

Analysis of data provided by table 3 leads to conclusion that the highest percentage of inhibition, so the best ability to neutralize free radicals has the alcoholic extract of grapefruit peel (95.5890 ± 0.2437), followed by the orange peel extract (94.4915 ± 0.2395) and the mandarin peel extract (87.2490 ± 0.1532).

The antioxidant capacity determined by the FRAP method on the three alcoholic extracts from the citrus peels is shown in table 4.

Table 4.

Results of the FRAP method on citrus peel extracts (orange/grapefruit/mandarin)

Sample	Sample absorbance read at 595 nm	Sample concentration (µmoli TE/100 g dry sample)	Average sample concentration (µmoli TE/100 g dry matter)
Orange	1.263	69.1647	69.6835±0.5188
	1.274	69.8118	
	1.278	70.0470	
Grapefruit	1.327	72.9294	72.6745±0.2549
	1.319	72.4588	
	1.322	72.6353	
Mandarin	0.801	41.9882	42.4196±0.4314
	0.810	42.5176	
	0.814	42.7529	

Analysis of data provided by table 4 leads to the conclusion that the highest antioxidant capacity, by the FRAP method, has the alcoholic grapefruit extract (72.6745 ± 0.2549), followed by the orange peel extract (69.6835 ± 0.5188) and the mandarin peel extract (42.4196 ± 0.4314).

The results obtained after performing the ABTS method for determining the antioxidant capacity are shown in table 5.

Table 5.

Results of the ABTS method on citrus peel extracts (orange/grapefruit/mandarin)

Sample	Sample absorbance read at 595 nm	Sample concentration (µmoli TE/100 dry matter)	Average sample concentration (µmoli TE/100 g dry matter)
Orange	0.047	160.8471	159.7059±2.2823
	0.046	157.4236	
	0.047	160.8471	
Grapefruit	0.086	239.0921	246.5077±9.2695
	0.088	244.6538	
	0.092	255.7772	
Mandarin	0.072	151.0994	147.6003±3.4991
	0.069	144.8011	
	0.070	146.9005	

Analysis of data provided by table 5 leads to conclusion that the highest antioxidant capacity, by the ABTS method has the alcoholic extract of grapefruit peel (246.5077 ± 9.2695), followed by the orange peel extract (159.7059 ± 2.2823) and the mandarin peel extract (147.6003 ± 3.4991).

CONCLUSIONS

Following microscopic determinations, it was observed that there are more secretory pockets in the peel of the grapefruit fruit than in the peel of the orange fruit, and the secretory pockets of the grapefruit peel are much more concentrated in volatile oils than of the orange peel.

Following the identification of polyphenols and flavonoids in the alcoholic extracts of citrus peels (grapefruit, mandarin, orange) we have shown that these compounds can have important antioxidant capacity. The highest concentration of total polyphenols, flavonoids and the highest antioxidant capacity, regardless of the method used, is the ethanolic grapefruit peel extract, followed by the orange peel extract and then by the mandarin peel extract.

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