

Antioxidant activity and kinetics on kiwi fruit (*Actinidia deliciosa*) ethanolic extracts by 2,2-diphenyl-1-picrylhydrazyl (DPPH·) method

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Abstract

Kiwi fruit (*Actinidia deliciosa*) are well-known and consumed products having antioxidant properties by means of specific compounds such as ascorbic acid, phenols and polyphenols (e.g., 6-hydroxy-7-(β-D-glucopyranosyloxi)coumarin, β-vanillic acid glucoside, caffeic, ferrulic acids and their glucosides, rutin, quercetin 3-O-β-D-rhamnoside, catechin, epicatechin, procyanidin tri- and tetramers).

The paper presents a study on the evaluation of antioxidant activity of kiwi fruit ethanolic extracts from various fruit parts using 2,2-diphenyl-1-picrylhydrazyl (DPPH·) method. Antioxidant activity was monitored for 15 minutes in the presence of 1 mM DPPH· solution and the DPPH· reaction rates were determined for two pseudolinear variation of this concentration (< 180 s and 3-15 minutes). Important antioxidant activity was obtained for kiwi fruit shell extracts obtained using 40-80% ethanol-water mixtures of 73.5-84.1%, while the antioxidant activity of the corresponding core extracts was in the range of 14.8-41%. On the other hand, the DPPH· reaction rates for the first pseudolinear range was significantly higher for shell extracts (0.2-0.5 μM/s) in comparison with the core extracts (< 0.1 μM/s). As a conclusion, the kiwi fruit shell extracts are more valuable for their antioxidant capacity, suggesting a much higher content of phenolic, polyphenolic, or enolic compounds.

Keywords: kiwi fruit, *Actinidia deliciosa*, antioxidant activity, DPPH·, kinetic study

1. Introduction

Kiwi fruits belong to *Actinidia* genus that comprise of more than sixty species. The most known are *Actinidia deliciosa* and *Actinidia chinensis*, but *A. setosa*, *A. latifolia*, *A. arguta*, *A. callosa*, *A. rubricaulis*, *A. rufa* and *A. tetramera* are also used for economical purposes. They are cultivated especially in Italy and Australia, as well as in Chile, Greece and France [1,2].

Kiwi fruits contain compounds having antioxidant properties such as ascorbic acid (Figure 1), which

have implications on risk reduction of atherosclerosis, cardiovascular and cancer diseases [3-6]. Other compounds having antioxidant properties that were identified in kiwi fruits are 6-hydroxy-7-(β-D-glucopyranosyloxi)coumarin, β-vanillic acid glucoside from the phenolic class (Figure 2), caffeic, ferrulic acids and their glucosides from the hydroxy-cinnamic acid class (Figure 3), rutin and quercetin 3-O-β-D-rhamnoside from the flavonoid class (Figure 4), catechin, epicatechin, procyanidin tri- and tetramers from procyanidin class (Figures 5 and 6).

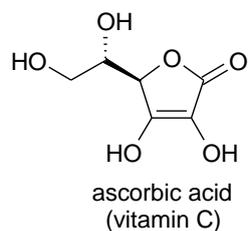


Figure 1. Structure of the ascorbic acid (vitamin C), the main antioxidant compound from kiwi fruit

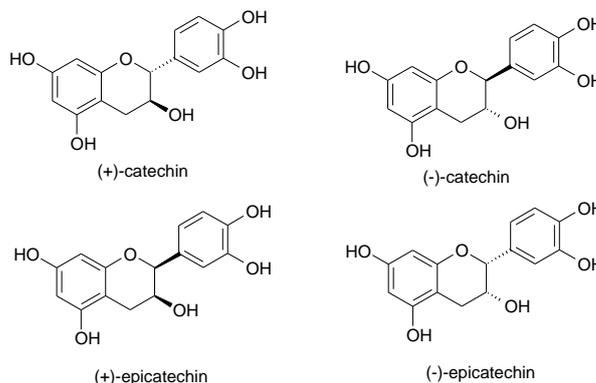


Figure 5. Structures of catechins and epicatechins from the kiwi fruits

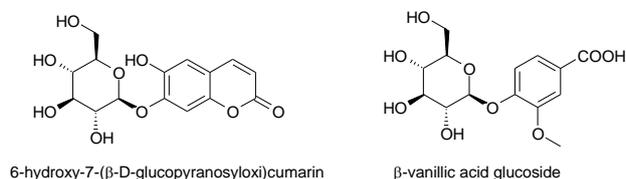


Figure 2. Structures of the main antioxidant compounds from kiwi fruits that belong to the phenolic class

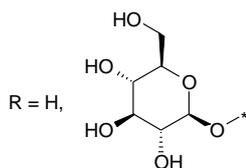
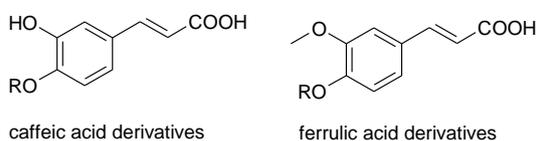


Figure 3. Structures of the caffeic and ferrulic acids and their derivatives from the kiwi fruits

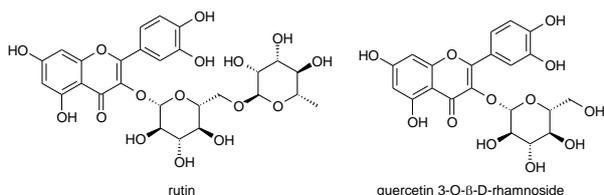


Figure 4. Structures of the main flavonoids from the kiwi fruits

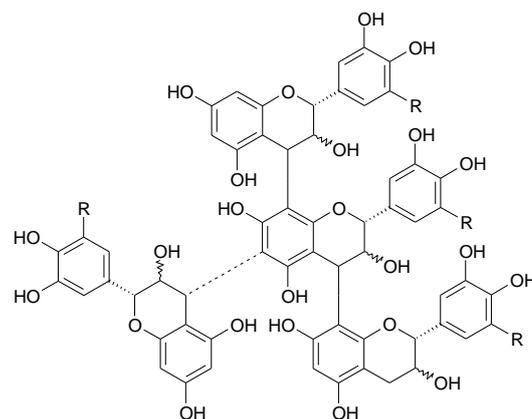


Figure 6. Examples of the tri/tetramers of procyanidins from the kiwi fruits

This study is focused on the antioxidant activity evaluation of kiwi fruit ethanolic extracts by means of DPPH· method as well as their comparative reaction rates on specific time ranges.

2. Materials and method

2.1. Materials. Kiwi fruits (*Actinidia deliciosa* var. *deliciosa*) were purchased from the local market (Timișoara, Romania) in the spring of the year 2015 and they were immediately used for solid-liquid extraction. The absolute ethanol used for extraction was purchased from Sigma-Aldrich (ACS grade) and DPPH· was obtained from Merck & Co., Inc. (purity >99%). All water-containing solutions were obtained using distilled water.

2.2. Obtaining of the kiwi fruit extracts. Kiwi fruit-containing compounds having antioxidant capacity are more hydrophilic compounds and they are easily soluble in hydrophilic mixtures such as water, ethanol and their mixtures. Consequently, various ethanol-water mixtures, which were in the range of 0-100%, were used for solid-liquid extraction of antioxidant compounds from shell and core kiwi fruits at room temperature (polyphenols are thermally labile compounds). Five concentrations for ethanolic solutions were selected: 0%, 20%, 40%, 60%, 80% and 100%. The fresh kiwi fruits were washed, dried, and seeds were separated before the sample preparation. The kiwi fruit shell (< 1.5 mm from the top) was manually separated from the core and the two kiwi parts (coded as “KS” for kiwi shell and “KC” for kiwi core) were grounded and subjected to extraction. Approximately 2 g of the sample was extracted with 20 mL solvent in a 100 mL Erlenmeyer-type extractor equipped with magnetically stirrer at room temperature (25 ± 2 °C) during 24 hours in dark place. The extract was then filtered at normal pressure and subjected to antioxidant activity monitoring using DPPH \cdot spectrophotometrically method. All determinations were performed in duplicate.

2.3. Antioxidant activity evaluation. Antioxidant activity of the kiwi fruit extracts was spectrophotometrically monitored using DPPH \cdot method. Thus, 500 μ L of kiwi fruit extract was mixed with 2 mL of absolute ethanol in the presence of 500 μ L of 1 mM DPPH \cdot ethanolic solution and spectrophotometrically monitored at 517 nm during 30 minutes. A CamSpec M501 spectrophotometer and 1 cm quartz cuvette were used for analysis. The negative and positive blank were absolute ethanol and 1 mM DPPH \cdot : ethanol solution (0.5:2.5, v/v), respectively. The DPPH \cdot concentration during analyses was determined using a calibration *Absorbance (@517 nm) versus Concentration (mM)* curve obtained for standard ethanolic DPPH \cdot solutions of 0.025-0.3 mM concentration range.

2.4. Statistical analysis. Classical statistical analysis of the data obtained from solid-liquid extraction was performed. Thus, the mean values of duplicates were determined and presented.

3. Results and discussion

3.1. Antioxidant activity of kiwi fruit extracts

The antioxidant activity of kiwi fruit extracts was evaluated using DPPH \cdot method. In the presence of phenolic or enolic compounds, this relatively stable radical reacts and the maximum absorbance of the initial compound at 517 nm has a hypsochromic shift after the reaction. The reactions between DPPH \cdot and antioxidant compounds are very complex, but the main steps involves the formation of an oxy-radical derived from the phenolic compound by means of DPPH \cdot radical, followed by the formation of an ortho-quinonic derivative (Figure 7). Other pathways involves the dimerization of the oxy-radical to a biphenyl semiquinone dimer or a diaryl ether semiquinone dimer. Further, these intermediates forms other radicals that stabilize later [7-9].

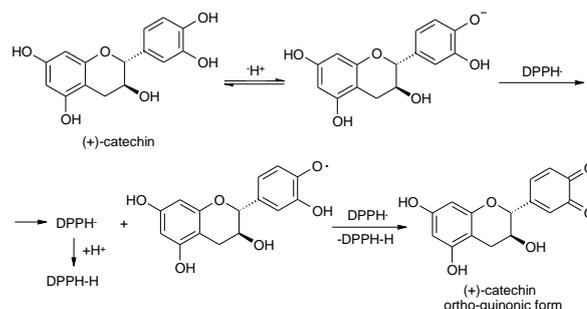


Figure 7. Possible reaction pathway for the interaction between catechin from kiwi fruit extracts and DPPH \cdot radical

The DPPH \cdot reaction was monitored for 15 minutes at 517 nm, when the absorbance decreases by an inverse logarithmic path. The most representative variation was observed for kiwi fruit shell extracts using water and diluted ethanol (20%, 40%, Figure 8) or concentrated ethanol (60%, 80% and 100%, Figure 9).

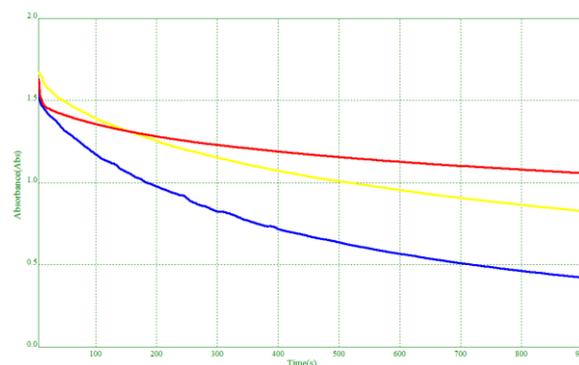


Figure 8. Absorbance (@517 nm) versus Time for the kiwi fruit shell extracts using water or diluted ethanol (water – red, ethanol 20% - yellow, and ethanol 40% - blue)

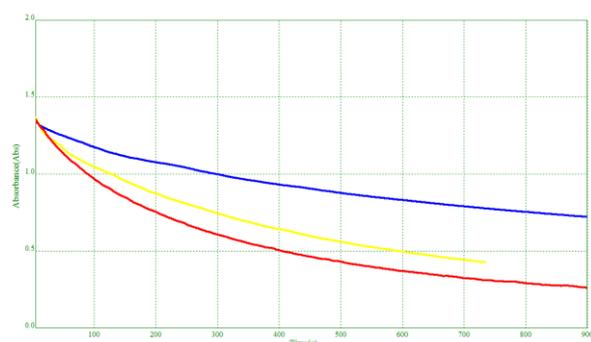


Figure 9. Absorbance (@517 nm) versus Time for the kiwi fruit shell extracts using concentrated ethanol (ethanol 60% – red, ethanol 80% - yellow, and absolute ethanol - blue)

Table 1. Antioxidant activity and mean DPPH· reaction rates for two pseudolinear variation of the DPPH· concentration in the presence of the kiwi fruit shell and core extracts (means of duplicate analysis; RSD <5%)

Code ^a	AO (%) ^b	v_1 ($\mu\text{M/s}$) ^c	v_2 ($\mu\text{M/s}$) ^c
KC000	22.4	0.063	0.018
KC020	24.5	0.041	0.012
KC040	38.8	0.100	0.037
KC060	14.8	0.097	0.044
KC080	26.4	0.087	0.032
KC100	41.0	0.052	0.015
KS000	35.0	0.200	0.051
KS020	49.3	0.300	0.098
KS040	74.2	0.400	0.100
KS060	84.1	0.500	0.100
KS080	73.9	0.400	0.100
KS100	55.8	0.200	0.083

^a “KC000”/”KS000” – “KC100”/”KS100”: kiwi fruit core/shell extracts using water or ethanol 20, 40, 60, 80, 100%

^b Antioxidant activity

^c mean DPPH· reaction rates in the presence of kiwi fruit extracts on two time ranges: 5-180 s and 180-900 s, respectively

Antioxidant activity (AO) of the kiwi fruit extracts was determined using the following equation:

$$AO = 100 - Abs_{.t} / Abs_{.t=0} [\%]$$

where $Abs_{.t}$ is the actual absorbance of the kiwi fruit extract and DPPH· solution mixture at 517 nm, while $Abs_{.t=0}$ is the absorbance of the same mixture at starting time (which was determined as a mean of a positive control, which contained DPPH· and ethanol instead extract).

The most important AO values were obtained for kiwi fruit shell extracts of 35-84%, with maximum values for ethanol 40-80% extracts (74-84%, Table 1). Antioxidant activity of core extracts were almost at half in comparison with shell extracts (AO between 15-41%, Table 1).

The kinetics of DPPH· reaction in the presence of kiwi fruit extracts requires a DPPH· standard curve.

Thus, five DPPH· solutions having concentrations between 25-300 μM were spectrophotometrically analyzed and the absorbance at 517 nm was plotted against concentration. The following standard curve was obtained:

$$Abs_{.517\text{ nm}} = 0.00626(\pm 0.00011) \cdot Conc. [\mu\text{M}]$$

$$n = 5, r^2 = 0.9987, s = 0.042, F = 3243$$

The DPPH· reaction rates were determined for the pseudolinear variation of the concentration in time, (i.e. 5-180 s and 180-900 s) using the relation [10-12]:

$$v_{DPPH\cdot} = -\Delta c / \Delta t [\mu\text{M/s}]$$

where Δc represents the variation of DPPH· concentration during the Δt time range.

DPPH· reaction rates for the first range were two or three times higher in comparison with those for the second time range. Moreover, the DPPH· reaction rates are significantly higher for shell extracts (0.2-0.5 $\mu\text{M/s}$) in comparison with the kiwi fruit core extracts (0.04-0.10 $\mu\text{M/s}$, Table 1). The highest DPPH· reaction rates were determined for kiwi fruit shell extracts obtained using ethanol 40-80% (0.4-0.5 $\mu\text{M/s}$, with a maximum of 0.5 $\mu\text{M/s}$ for the 80% ethanolic extract). Similar behavior was observed for the second time range: 0.05-0.10 $\mu\text{M/s}$ for the shell extracts and 0.015-0.044 $\mu\text{M/s}$ for the core extracts (Table 1). However, DPPH· reaction rate values are in good agreement with the AO of the extracts:

$$AO = 18.391(\pm 4.584) + 130.944(\pm 18.034) \cdot v_1$$

$$n = 12, r^2 = 0.841, s = 9.53, F = 53$$

4. Conclusion

The following conclusions can be drawn among the antioxidant activity and kinetics evaluation of kiwi fruit extracts by DPPH· method: (1) antioxidant activity of kiwi fruit extracts has optimum values of ~84% for 60% ethanolic extracts of the kiwi shell; (2) kinetic study on the DPPH· / polyphenolic compounds contained by kiwi fruits reveals higher reaction rates for kiwi fruit shell extracts suggesting that the main and most concentrated antioxidant compounds are more concentrated in the kiwi shell than in the core part; (3) statistically significant correlations between the overall antioxidant activity and DPPH· reaction rate for the starting range of interaction in the case of core and shell kiwi fruit extracts obtained using various ethanol-water mixtures have been established.

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