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THE *ACTINIDIA ARGUTA* 'HORTGEM THAI' AND *ACTINIDIA DELICIOSA* 'HAYWARD' KIWI FRUIT AS A SOURCE OF EXOGENOUS ANTIOXIDANTS

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Abstract: The aim of this study was to estimate the content of exogenous antioxidants in kiwi fruits available in Gdynia, Poland, and their ability to reduce free radicals. The test material consisted of mini kiwi fruit (*Actinidia arguta*) of the 'Hortgem Thai' cultivar and kiwi fruit (*Actinidia deliciosa*) of the 'Hayward' cultivar. The carotenoid pigments were determined by the Lichtenthaler method, vitamin C in accordance with PN-A-04019:1998, polyphenols by the Folin-Ciocialteu method and the ability to reduce free radicals using a synthetic DPPH radical. One-way analysis of variance was used to determine the effect of fruit species on the quality traits analysed. The significance of differences between the means was determined using Tukey's test. Relationships were considered statistically significant at $p < 0.05$. Correlation coefficients between the antioxidant activity of kiwi fruit and the content of exogenous antioxidants were also determined. The results showed that *Actinidia arguta* has significantly higher contents of exogenous antioxidants than *Actinidia deliciosa*. The antioxidant properties of this fruit were also found to be mainly due to its vitamin C content.

Keywords: kiwi fruit, antioxidant properties, vitamin C, polyphenols, carotenoids.

1. INTRODUCTION

Many essential biochemical reactions in the human body lead to the formation of highly reactive oxygen species and free radicals. Due to their ability to damage cells and tissues, the human body is equipped with antioxidant enzymes that protect it from the adverse effects of free radical reactions [Gryszczyńska and Iskra 2008].

However, the consumption of highly processed food and a detrimental lifestyle results in the formation of many more free radicals than the body can neutralise on its own. Excessive free radicals and reactive oxygen species are one of the causes of chronic non-communicable diseases. The risk of their occurrence can be reduced by increasing the intake of products providing the human body with exogenous antioxidants such as carotenoids, vitamin C and polyphenolic compounds [Pukszta and Platta 2017].

Exogenous antioxidants delivered to the human body with foods lower the risk of heart disease, cancer, allergies, and may also have anti-inflammatory effects. In addition, they can affect the activity of the redox system and the total antioxidant capacity of the body. Some important sources of exogenous antioxidants are: red wine, green tea, vegetables and fruit, including kiwi fruit [Gryszczyńska and Iskra 2008].

The best known species of this fruit is the fuzzy kiwi fruit (*Actinidia deliciosa*). There is also a lesser known species on the market – the hardy kiwi (*Actinidia arguta*), also called mini kiwi, baby kiwi or grape kiwi. It is distinguished by its small fruit, smooth edible skin and a taste reminiscent of a mixture of tropical fruits. Mini kiwis are grown on plantations in New Zealand, Canada, USA, Chile and Europe, including Poland [Krupa and Latocha 2007].

The aim of this study was to estimate the content of exogenous antioxidants in kiwi fruits available in Gdynia, Poland, and their ability to reduce free radicals.

2. MATERIAL AND METHODS

Test material consisted of retail available fuzzy kiwi fruit of the 'Hayward' variety (*Actinidia deliciosa*) and mini kiwi fruit (*Actinidia arguta*) of the 'Hortgem Thai' variety grown in New Zealand.

Contents of dry matter, carotenoid pigments, vitamin C, polyphenols and antioxidant activity in the test material were determined.

The dry matter content was determined by the method of thermal drying to constant weight in accordance with the PN-90/A-75101/03 standard.

The content of carotenoid pigments was determined using the Lichtenthaler method for the simultaneous determination of chlorophyll and carotenoid pigments. The essence of this method is to extract the pigments with an 80% acetone solution and then read the absorbance values using a spectrophotometer.

A total of 14 g of ground test material was homogenised with 50 cm^3 of anhydrous acetone for 3 minutes at 12,000 rpm. The homogenate obtained was filtered through a fluted paper filter. The precipitate that remained on the filter was then washed with 80% acetone to a volume of 100 cm^3. Test samples were prepared from the resulting pigment extract.

After 0.75 cm^3 of the 80% acetone solution was transferred to a 25 cm^3 volumetric flask it was then made up to the mark with the pigment extract. The prepared solutions were placed in a light-free area at 20°C for 3 hours. After the specified time, the absorbance of the samples was measured using a SEMCO S/Ec spectrophotometer at 470 nm, 649 nm and 665 nm. An 80% acetone solution was used as a reference.

a and *b* chlorophyll contents were determined using the following formulas:

$$C_a = 11.63A_{665} - 2.39A_{649} \text{ (mg/dm}^3\text{)}$$

$$C_b = 20.11A_{649} - 5.19A_{665} \text{ (mg/dm}^3\text{)}$$

The calculated *a* and *b* chlorophyll contents were converted into mg/100 g of the test material and used in the formula for the sum of carotenoid pigments in the test samples.

Carotenoid pigment content was calculated from the following formula:

$$C_X = \frac{1000 \cdot A_{470} - 1.82 \cdot C_a - 85.02 \cdot C_b}{198}$$

where:

A – absorbance;
C_a – *a* chlorophyll content (mg/100 g);
C_b – *b* chlorophyll content (mg/100 g);
C_X – total carotenoid content (mg/100 g) [Polak, Krzykowski and Kluza 2009].

Vitamin C content was determined by the spectrophotometric method in accordance with the PN-A-04019:1998 standard. This method consists in extracting the vitamin from the test sample with oxalic acid, quantitative oxidation in an acidic medium of ascorbic acid to dehydroascorbic acid with 2,6-dichlorophenolindophenol and extraction of the excess pigment with xylene.

From the crushed laboratory sample, 10 g of the test material was weighed and quantitatively transferred to a 100 cm^3 volumetric flask using an extraction solution. The contents of the flask were made up to the mark with the extraction solution, mixed and filtered, discarding the first few millilitres of filtrate.

A total of 5 cm^3 of the prepared filtrate was measured into a 50 cm^3 Erlenmayer ground-glass flask, then 5 cm^3 of buffer solution, pH 4.0, and 2 cm^3 of 2,6-dichlorophenolindophenol solution was added and mixed, and finally 10 cm^3 of xylene was added. The flask was then shaken for 10 seconds. After the layers separated, the xylene layer was collected into cuvettes and the absorbance was measured. Excess pigment was determined spectrophotometrically at λ = 500 nm.

The amount of cm^3 of 2,6-dichlorophenolindophenol solution that did not react was read off the calibration curve.

The vitamin C content expressed in mg/100 g was calculated using the following formula:

$$K = \frac{(V_0 - V_1) \cdot d}{m' \cdot V_2 \cdot m_0} \cdot 100$$

where:

K – vitamin C content (mg/100 g),
m_0 – test material weight (g),
m' – titre of the pigment solution (cm^3/1 mg vitamin C),
V_0 – volume of the 2.6-dichlorophenolindophenol solution added for the determination of the test sample (cm^3),
V_1 – volume of excess pigment solution, corresponding to the measured absorbance of the sample, read from the calibration curve (cm^3),
V_2 – volume of the test sample filtrate taken for the determination (cm^3),
d – capacity of the volumetric flask (cm^3) [PN-A-04019:1998].

The total content of polyphenolic compounds was determined by the Folin-Ciocalteu method, which consists in measuring the absorbance of a complex formed as a result of reduction of salts of phosphotungstromolybdenum acids, the so-called Folin-Ciocalteu reagent. Phenolic compounds present in the samples under study were oxidised and the salts of phosphomolybdic and phosphotungstic acids were reduced in an alkaline medium. The ability of polyphenols to colour react with the Folin-Ciocalteu reagent was exploited [Dmowski and Post 2018].

A total of 5 g of the test material was weighed and extracted with 30 cm^3 of 80% ethanol for one hour at room temperature in a light protected place. After the specified time, the mixture was centrifuged for 20 minutes at 1130 rpm. The centrifuged extract was transferred to a 50 cm^3 volumetric flask and made up to the mark with 80% alcohol. The extract obtained was used to determine the content of polyphenolic compounds and the ability to reduce free radicals.

A total of 6 cm^3 of distilled water, 0.5 cm^3 of the Folin-Ciocalteu reagent and 1.5 cm^3 of saturated Na_2CO_3 solution were added to the 0.1 cm^3 of ethanolic extract of the test samples. After a 30-minute incubation at 40°C, the absorbance of the solution was measured at λ = 765 nm.

The results of the determinations are presented as the amount of gallic acid equivalent in 100 g of test material (mg GAE/100 g) [Singleton and Rossi 1965; Peri and Pompei 1971].

Antioxidant activity was determined as the ability to quench the synthetic DPPH radical, expressed as a percentage of inhibition of the fruits tested.

A total of 0.02 cm^3 of an appropriately prepared extract of the test fruit was added to 1.5 cm^3 of ethanolic solution of DPPH. The samples were incubated at 30°C for 30 minutes. After that time, the absorbance was measured at λ = 517 nm against ethanol as a blank. The control sample was a mixture of 1.5 cm^3 DPPH solution and 0.02 cm^3 of ethanol. Each measurement was taken three times and the average absorbance value for the solution was calculated. The ability of the tested fruit to counteract the oxidation reaction, expressed as the percentage of inhibition of the DPPH radical, was calculated from the following relationship:

$$\%\ inhibition = 100 \cdot \frac{(A_0 - A_{sr.})}{A_0}$$

where:

A_m – mean absorbance value of the test solution containing the antioxidant,
A_0 – absorbance of the control sample [Zych and Krzepiłko 2010].

The results of the determinations are the arithmetic mean of three replicates for each fruit. One-way analysis of variance was used to determine the effect of fruit species on the quality traits analysed. The significance of the differences between the tested fruits was checked by Tukey's test. Correlations at the significance level of $p < 0.05$ were considered statistically significant.

The obtained results of the statistical evaluation are presented in a table with the affiliation to the average classes marked with a letter classification.

The relationships between antioxidant activity and the content of cartenoids, vitamin C and polyphenols were evaluated using Pearson's linear correlation coefficient.

All calculations were performed using Statistica 13.3 software.

3. DISCUSSION

The water content in the fruit ranged from about 74% to about 96% and thus the products are characterised by a reduced dry matter content [Pałacha 2008].

The Kiwi fruits being the subject of the study did not differ significantly in dry matter content. Comparison of dry matter content of the 'Hayward' kiwi fruit and the 'Hortgem Thai' mini kiwi fruit showed that higher dry matter content was found in the mini kiwi fruit. The dry matter content of that fruit averaged 15.41% while that of the fuzzy kiwi fruit averaged 14.23% (Tab. 1).

However, in order to objectivise the levels of exogenous antioxidants analysed, the results of the determinations were presented per fresh product weight (FW) and per dry matter (DM).

The content of dry matter, carotenoid pigments, vitamin C, total polyphenols in the fruit and their antioxidant activity are shown in Table 1, while the content of carotenoids, vitamin C and total polyphenols per dry matter are shown in Table 2.

Table 1. Dry matter content, exogenous antioxidants content, and antioxidant activity of kiwi fruit

Fruit	Dry matter [%]	Carotenoids [mg/100 g FW]	Vitamin C [mg/100 g FW]	Total polyphenols [mg GAE/100 g FW]	Antioxidant activity % inhibition
'Hayward' kiwi fruit	14.23 ±0.09a	0.87 ±0.05a	34.93 ±2.03a	34.36 ±2.89a	13.05 ±1.16a
'Hortgem Thai' mini kiwi fruit	15.41 ±0.76a	2.71 ±0.08b	53.95 ±2.63b	101.64 ±2.27b	20.14 ±1.65b

Results are presented as mean ± standard deviation, n = 3.
Mean values marked by different lower-case letters in the column are significantly different according to Tukey's test at $p < 0.05$.

Source: own study.

Table 2. Content of exogenous antioxidants in kiwi fruit per dry weight

Fruit	Carotenoids [mg/100g DM[	Vitamin C [mg/100g DM]	Total polyphenols [mg GAE/100g DM]
'Hayward' kiwi fruit	6.04 ±0.32a	245.47 ±14.24a	241.46 ±20.53a
'Hortgem Thai' mini kiwi fruit	17.59 ±0.49b	350.08 ±17.03b	659.57 ±14.76b

Results are presented as mean ± standard deviation, n = 3.
Mean values marked by different lower-case letters in the column are significantly different according to Tukey's test at $p < 0.05$.

Source: own study.

Carotenoids are effective lipophilic antioxidants displaying high activity against reactive oxygen species and free radicals. These properties contribute to the protection of the human body against many chronic non-communicable diseases. Frequent consumption of foods rich in these compounds is effective against the development of cancer and atherosclerosis [Rao and Rao 2007].

The human body cannot synthesise carotenoids by itself through biochemical processes. Therefore, they must be supplied to the body as part of the daily diet. More than 600 carotenoids have been identified so far [Gryszczyńska, Gryszczyńska and Opala 2011].

Sources of carotenoids in the human diet are mainly cultivated plants, where these compounds are found in roots, leaves, shoots, seeds, fruit and flowers. About 60 different carotenoids have been identified in fruit and vegetables consumed by humans, the most common being α- and β-carotene, β-cryptoxanthin, lutein, lycopene and zeaxanthin [Fraser and Bramley 2004].

The study showed that the 'Hortgem Thai' mini kiwi fruit contained significantly more carotenoids than the 'Hayward' kiwi fruit. The average content of these compounds in the mini kiwi fruit was 2.71 mg/100 g FW (17.59 mg/100 g DM) and 0.87 mg/100 g FW (6.04 mg/100g DM) in the 'Hayward' kiwi fruit (Tab. 1 and 2).

A higher content of carotenoid pigments in the mini kiwi fruit was also demonstrated by Latocha et al. [2010]. They observed the content of analysed compounds at the level of 0.57 to 0.64 mg/100 g FW in the mini kiwi fruit and 0.12 mg/100 g FW in the 'Hayward' kiwi fruit. A significantly higher content of carotenoid pigments in the mini kiwi fruit than in the fuzzy kiwi fruit was also indicated by Leontowicz et al. [2016]. According to the cited authors, the mini kiwi fruit, depending on the variety, contains from 5.21 mg to 14.65 mg of carotenoids/100 g DM., while the fuzzy kiwi fruit contains about 6.73 mg/100 g DM.

Carotenoid content is determined not only by the variety of the kiwi fruit but also by the climate and soil conditions of cultivation, as pointed out by Park et al. [2013]. They found significant differences in carotenoid content between the 'Bidan' kiwi fruit from organic and conventional cultivation. Also, the incomplete transformation of the chloroplast to the chromoplast during kiwi fruit ripening may be a differentiating factor in carotenoid pigment content [McGhie and Ainge 2002].

A comparison of carotenoid pigment contents in kiwi fruit used as the test material (Tab. 1) with other fruits presented in Table 3 leads to the conclusion that kiwi fruit is not a rich source of these pigments for the human organism.

Table 3. Carotenoid pigment content in selected fruits

Fruit	Carotenoid content [mg/100 g FW]
Apricot	219.6
Banana	12.6
Orange	21.1
Peach	30.9

Source: P.D. Fraser, P.M. Bramley, 2004, The Biosynthesis and Nutritional Uses of Carotenoids, Progress in Lipid Research, no. 43, pp. 228–265.

Vitamin C is an organic compound that has a positive effect on the human body. It is important for the proper functioning of the immune system, influences the condition of gums, teeth, and blood vessels, supports the production of collagen, accelerates wound healing, reduces cholesterol oxidation and prevents atherosclerotic lesions.

In addition, vitamin C helps to reduce the incidence of cancer. L-ascorbic acid is a powerful antioxidant which, by reducing free radicals, is responsible for improving the condition of the skin and delaying the appearance of wrinkles. As with

carotenoids, vitamin C must be supplied to the human body with the foods we eat. The primary sources of this vitamin for the human body are vegetables and fruit as well as products of plant origin [Janda, Kasprzak and Wolska 2015].

The kiwi fruit used as the test material differed significantly in its vitamin C content. The higher content was found in the 'Hortgem Thai' mini kiwi fruit, with an average of 53.95 mg of ascorbic acid per 100 g FW (350.08 mg/100 g DM). In contrast, the 'Hayward' kiwi fruit contained an average of 34.96 mg/100 g FW (245.47 mg/100 g DM) (Tab. 1 and 2).

The content of vitamin C in fruit is determined, among other things, by the variety of raw material of plant origin, place and method of cultivation, climatic conditions of a given region, degree of ripeness at harvest, and time and conditions of transport and storage [Janda, Kasprzak and Wolska 2015].

According to the 2020 nutrition standards for the Polish population, the human body requirement for vitamin C is at an average level (EAR) of 30 to 75 mg per day [Przygoda et al. 2020].

The results show that 100 grams of the 'Hayward' fuzzy kiwi fruit can provide from about 47% to 116% of the recommended daily vitamin C amount, while 100 grams of the 'Hortgem Thai' mini kiwi fruit provide from about 72% to 180%. It follows that kiwi fruit is a rich source of vitamin C for the human body. This is also confirmed by data presented in the literature. According to Latocha et al. [2010], the ascorbic acid content of the 'Hortgem Thai' mini kiwi fruit and the 'Hayward' kiwi fruit is similar, ranging from 86.1 to 106.6 mg/100 g FW and 116.6 mg/100 g FW respectively. Similar vitamin C content was also found in the 'Hayward' kiwi fruit by Okamoto and Goto [2005].

The vitamin C content of kiwi fruit was also the subject of a long-term study by Ferguson and MacRaea [1991]. According to the authors, the different species of kiwi fruit vary considerably in their L-ascorbic acid content, which ranges from about 20 mg to more than 1000 mg/100 g FW. Additionally, they found that freshly picked 'Hayward' kiwi fruit contained about 85 mg and mini kiwi fruit about 100 mg of vitamin C per 100 g fresh weight [Ferguson and MacRae 1991].

According to Nishiyama et al. [2004], the concentration of vitamin C in mini kiwi fruit grown in Japan can reach up to 140 mg/100 g FW.

For comparison, the vitamin C content in oranges is about 51 mg/100 g FW, in blackcurrants from 52 to 122 mg/100 g FW, in strawberries from 29 to 48 mg/100 g FW [Latocha et al. 2010], in grapefruits about 59 mg/100 g FW, in lemons 81 mg/100 g FW and in apples 12 mg/100 g FW [Sembratowicz and Rusinek-Prystupa 2015].

Differing in structure and molecular weight, polyphenols are the largest group among the natural antioxidants. They are formed from primary metabolites as a result of carbohydrate biosynthesis. Phenolic compounds have beneficial effects on the health and functioning of the human body [Rosicka-Kaczmarek 2004].

Polyphenols protect the body's cells from damage caused by free radicals, which can destroy cell membranes, genetic material and lipid and protein structures. They also exhibit anticancer, anti-inflammatory, antibacterial and antiviral properties. In addition, they prevent the formation of blood clots and the atherosclerosis of blood vessels, and also combine with collagen, thus reducing the risk of histamine intolerance [Bartosz 2003].

The content of polyphenolic compounds in the studied kiwi fruits showed significant differentiation. The 'Hortgem Thai' mini kiwi fruit had a higher polyphenol content, containing an average of 101.64 mg GAE/100 g FW (659.57 mg GAE/100g DM) (Tab. 1 and 2).

A similar relationship was also found by Leontowicz et al. [2016]. The mini kiwifruit used as their test material contained from 890 mg GAE/100 g DM to 1116 mg GAE/100 g FM, respectively, depending on the variety, while the 'Hayward' kiwi fruit contained 541 mg GAE/100 g DM.

The literature furthermore presents varying polyphenol contents for the mini kiwi and the 'Hayward' kiwi fruit. Latocha et al. [2010] found between 79 and 128.5 mg GAE/100 g FW in the mini kiwi fruit, and 76.5 mg GAE/100 g FW in the fuzzy kiwi fruit.

A significantly higher polyphenol content in the mini kiwi fruit was shown by Fisk et al. [2006]. They reported that the fruit contains up to 200 mg GAE/100 g FW. On the other hand, the content of these compounds recorded by Tavarini et al. [2008] in the 'Hayward' kiwi fruit was 60 mg GAE/100 g FW. An even lower polyphenol content in that fruit, at 41 mg GAE/100 g FW, was found by Du et al. [2009].

Differences in the content of phenolic compounds may be caused not only by the variety but also by the degree of maturity, growing conditions, year and origin of the kiwi fruit [Czapski 2015].

The results could also have been influenced by the Folin-Ciocalteu method used. The reagent used in this method also reacts with sugars, ascorbic acid, proteins, amino acids, copper and iron ions, which may influence the magnitude of the results obtained [Shahidi and Naczk 2004; Prior, Wu and Schaich 2005].

A comparison of the polyphenol content of the kiwi fruit used as the test material with other fruits (Tab. 4) shows that kiwi fruit, especially the mini kiwi fruit, forms a rich source of these compounds for the human body. Kiwi fruit provides more polyphenols than apples, grapefruits, lemons, peaches, green grapes or avocados.

The antioxidant properties of fruit, expressed as the ability to eliminate free radicals, are determined, among other things, by the content of compounds with a strong antioxidant nature, such as carotenoids, vitamin C and polyphenols. Their synergistic action results in a higher free radical reduction capacity achieved by the product than if these compounds were present separately [Latocha et al. 2010].

A study on the ability of the ethanolic extracts of the kiwi fruit that constituted the test material to inhibit synthetic DPPH radicals showed that the 'Hortgem Thai'

mini kiwi fruit had significantly stronger antioxidant properties than the 'Hayward' kiwi fruit. The ability of the 'Hortgem Thai' mini kiwi fruit to inhibit radicals averaged 20% while the ability of the 'Hayward' kiwi fruit averaged 13% (Tab. 1)

Table 4. Polyphenol content in selected fruits

Fruit	Polyphenol content [mg GAE/100 g FW]	Fruit	Polyphenol content [mg GAE/100 g FW]
Green apple	68.29	Cherry	114.56
Avocado	21.86	Banana	25.55
Blueberry	46.24	Pineapple	94.04
Grapefruit	60.35	Papaya	27.52
Lemon	61.47	Plum	73.04
Peach	27.58	Mango	35.02
Green grape	23.20	Orange	77.23

Source: E. Baranowska-Wójcik, D. Szwajgier, 2019, Characteristics and Pro-Health Properties of Mini Kiwi (Actinidia arguta), Horticulture, Environment and Biotechnology, vol. 60, pp. 217–225.

The antioxidant activity of products of plant origin is largely determined by the content of polyphenolic compounds. This was confirmed by a comparison of the polyphenol content and free radical reducing capacity of the 'Hortgem Thai' mini kiwi fruit and the 'Hayward' kiwi fruit. The mini kiwi fruit with a higher amount of polyphenolic compounds showed a higher capacity to reduce free radicals. A similar relationship was also found, for example, by Leontowicz et al. [2016] and Park et al. [2014].

Bearing in mind, however, that antioxidant properties are determined not only by polyphenols but also by vitamin C and carotenoid pigments, the relationship between the content of these compounds in kiwi fruit and their antioxidant activity was analysed. The analysis was based on Pearson's correlation coefficient (Tab. 5).

Table 5. Values of Pearson's correlation coefficients

		Antioxidant activity
Carotenoid pigment content	In fresh weight	0.9657
	In dry matter	0.9667
Vitamin C content	In fresh weight	0.9923
	In dry matter	0.9960
Polyphenol content	In fresh weight	0.9359
	In dry matter	0.9344

Source: own study.

The values of Pearson's correlation coefficients obtained indicate a positive and strong dependence of fruit antioxidant activity on the content of individual exogenous antioxidants. They also suggest that vitamin C was more decisive for the ability of kiwi fruit to reduce free radicals than other antioxidant components. A greater effect of vitamin C on the total antioxidant capacity of kiwi fruit has also been observed by other authors. Du et al. [2009] determined the correlation coefficient for the relationship between antioxidant activity and polyphenol and vitamin C content to be 0.81 and 0.83, respectively. Tavarini et al. [2008] also pointed out that the antioxidant activity of kiwi fruit is mainly determined by its vitamin C content (r=0.73). Krupa and Latocha [2007] also demonstrated that it was mainly vitamin C (r=0.91) that determined the antioxidant properties of kiwi fruit.

4. CONCLUSIONS

- *Actinida arguta* 'Hortgem Thai' had significantly higher contents of exogenous antioxidants such as carotenoids, vitamin C and polyphenols than *Actinida deliciosa* 'Hayward'.
- *Actinida arguta* 'Hortgem Thai' showed significantly higher free radical reduction ability than *Actinida deliciosa* 'Hayward'.
- The antioxidant activity of kiwi fruit was more influenced by the content of vitamin C than by other antioxidant components.
- The high content of exogenous antioxidants and the antioxidant properties of kiwi fruit justify its use in the diet as a rich source of these compounds for the human body.

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