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Chemical composition and bioactive properties of byproducts from two different kiwi varieties

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Abstract

Kiwis are an example of fruits with excellent bioactive properties worldwide appreciated and consumed generating tons of waste. Thus, the objective of this work was to compare two varieties of kiwi: *Actinidia deliciosa* cv. "Hayward" (green) and *Actinidia* spp. (red) regarding the nutritional value of their pulps, chemical composition and bioactivities of each pulp and peel. The results revealed that pulps have a high water content and low amount of other macronutrients. Both parts of red kiwi presented the highest tocopherols content and red kiwi pulp presented the highest content in ascorbic acid. In general, the peels exhibited the highest antioxidant activity and green kiwi peels showed cytotoxicity and anti-inflammatory activity, which could be related to its higher content in phenolic compounds, especially B-type (epi)catechin dimer. Therefore, kiwi components currently underutilized may be indicated as a source of natural functionalizing ingredients with several benefits for human health.

Keywords: *Actinidia deliciosa* cv. "Hayward"; *Actinidia* spp; byproducts; nutritional and chemical characterization; antioxidant/antimicrobial/anti-inflammatory/cytotoxic properties

1. Introduction

Peels and seeds from fruits are annually discarded in large quantities in an industrial production that only uses pulps or juices. The use of these byproducts have been little explored, however, some studies have been attracting interest describing them as a good source of bioactive compounds, namely with high contents of carotenoids, triterpenes, and polyphenols (Santana-Méridas, González-Coloma, & Vioque, 2012). There are several biological activities namely antioxidant (Talukder, Talapatra, Ghoshal, & Raychaudhuri, 2016), anti-inflammatory (Li et al., 2014), antimicrobial (Soquetta,

Stefanello, Huerta, Monteiro, da Rosa, & Terra, 2016), and antidiabetic (Asgar, 2013), among many others (Latocha, Krupa, Wołosiak, Worobiej, & Wilczak, 2010), that have been extensively associated to these chemical compounds, especially regarding polyphenols. These facts together have led to consider the application of these residues from the food processing and agriculture systems, giving them an added-value by exploring their rich content in bioactive molecules, which until now have been little explored (Folletta, Jamieson, Hamilton, & Wall, 2019).

According to literature kiwi fruit is native to north-central China and the plant genus *Actinidia* contains about 60 species. The commercialization of these fruits appeared in the beginning of the 20th century and the cultivation "Hayward" is the most recognized and commercialized (Folletta et al., 2019). Kiwi fruits have a high conservation capacity and can be stored at the temperature of 0 ºC for many months without any decrease in quality (Krupa, Latocha, & Liwinska, 2011). Regarding its nutritional content, kiwi is described as being rich in dietary fiber, bioactive compounds, such as vitamins (C, E and A), phenolic compounds and minerals (Latocha et al., 2010). *Actinidia* fruits have been attracting a great deal of interest, mainly due to their highly described health benefits. This fruit is normally consumed *in natura*, but the food industry has been trying to innovate on its commercialization in diverse processed forms, such as frozen juice or pulp, sweets, ice creams, among other products (Zhu et al., 2013). Therefore, the peel of this fruit results in a byproduct that is still under-explored, but which has aroused a great interest, due to their high contents in bioactive molecules, such as phenolic compounds (1273 mg/100 g), when compared, for example, with orange peel (473 mg/100 g) or apple peel (329 mg/100 g) (Wojdyło, Nowicka, Oszmianski, & Golis, 2017). Therefore, this residue could be considered a rich source of add-value compounds, with great interest for different industrial sectors.

Although there are several studies in the literature that characterize kiwi fruits, it is considered that is necessary to carry out additional complementary studies to ensure the utilization of its total bioactivity (Wang et al., 2018). Nevertheless, more studies are needed in order to explore food byproducts by valorisation their application and therefore reducing food waste. In this sense, this study aimed to explore the bioactive properties of two different kiwi varieties, such as *Actinidia deliciosa* cv. "Hayward" (green kiwi pulp) and *Actinidia* spp (red kiwi pulp). The chemical characterization of the peel and pulp of both varieties was assessed and further evaluated regarding their antioxidant, antimicrobial, anti-inflammatory and cytotoxic activities, viewing to enhance the potential application of kiwifruits byproduct in the food industry.

2. Materials and methods

2.1. Preparation of the samples

Commercial samples of *Actinidia deliciosa* cv. "Hayward" (green kiwi pulp) and *Actinidia* spp. (red kiwi pulp) were provided by Kiwi Coop based in Oliveira do Bairro, Portugal. The peel was separated from the pulp and both were fruit parts were frozen, lyophilized and stored in a desiccator at room temperature (average 25 ºC), protected from light, until further analysis.

2.2. Chemical composition

2.2.1. Nutritional composition of kiwi pulps

The contents of protein, fat, carbohydrates and ash, were determined in the two varieties of kiwi pulps following the AOAC methods (AOAC International, 2016) and following a procedure previously reported by Barros et al. (2013). Total energy was calculated following the equation: Energy (kcal) = 4 × (g protein + g carbohydrates) + 9 × (g fat).

Free sugars. The pulps from the two kiwi varieties were evaluated regarding the sugar content and were extracted following a procedure previously described (Barros et al., 2013). The samples were then filtered through 0.2 μm Whatman nylon filters into a 1.5 mL vial for liquid chromatography analysis. The HPLC system was coupled to a refraction index (RI) detector and the free sugars were identified by comparison with standards and further quantified considering the internal standard and results were expressed in g per 100 g of fresh fruit (Barros et al., 2013).

Fatty acids. The fatty acids were extracted from the pulps of the two varieties and determined by gas chromatography coupled with a flame ionization detector (GC-FID, DANI model GC 1000, Contone, Switzerland) using a procedure previously described by Barros et al. (2013). The results were expressed as relative percentage of each fatty acid.

2.2.2. Organic acids

The organic acids were determined in both parts (peels and pulps) of the two kiwi varieties, according to a procedure previously described by Barros et al. (2013), using an Ultra-Fast Liquid Chromatography (UFLC, Shimadzu 20A series, Kyoto, Japan) and a photodiode array detector. The results were expressed in g per 100 g of fresh weight.

2.2.3. Tocopherols

Tocopherols were determined following a method previously described by Barros et al. (2013), using a HPLC (Knauer, Smartline system 1000, Berlin, Germany) coupled to a fluorescence detector (FP-2020; Jasco, Easton, USA) programmed for excitation at 290 nm and emission at 330 nm, using the IS (tocol) method for quantification. The results were expressed in mg per 100 g of fresh weight.

2.3. Bioactive evaluation

2.3.1 Extracts preparation

The extract was prepared by adding 2 g of dried sample to 50 mL of ethanol/water (80:20 v/v, for the anthocyanin extraction 0.5% of TFA was added to the extracting solvent) and left under stirring for 1 hour at room temperature. After filtration (Whatman No. 4 paper) the residue was extracted with an additional 50 mL of the same solution for 1 h under the same conditions. The combined extracts were evaporated at 40 °C in a rotary evaporator (Büchi R-210, Germany) in order to remove the organic solvent. For the non-anthocyanin extract, the aqueous phase was further frozen and lyophilized to obtain a dry extract.

For the anthocyanin compounds, the aqueous phase was purified using a C-18 SepPak® Vac 3 cartridge (Phenomenex). The activation was performed with 5 mL of ethanol and water, then 10 mL of the sample (50 mg/mL) was loaded into the cartridge. Afterword's, the sugars and the more polar compounds were removed by passing 15 mL of water and the anthocyanins were further eluted with 15 mL of acidified ethanol (0.1% of TFA). Afterwards, the ethanol was removed under vacuum until dryness and re-dissolved in 1 mL of acidified 80% aqueous ethanol (0.1% of TFA), filtered through a 0.22 μm disposable LC filter disk into a 1.5 mL amber vial for HPLC analysis (Rodrigues et al., 2012).

2.3.2. Identification and quantification of phenolic compounds

The phenolic compounds (non-anthocyanin and anthocyanin compounds) were separated, identified, and quantified using a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA), following the two previously described procedure for non-anthocyanin and anthocyanin compounds (Bessada, Barreira, Barros, Ferreira, & Oliveira, 2016; Gonçalves et al., 2017). The detection was performed with a DAD (280,

330, 370, and 520 nm, as preference wavelengths) and in a mass spectrometer (LTQ XL mass spectrometer, Thermo Finnigan, San Jose, CA, USA) working in negative mode for non-anthocyanin compounds and positive mode for anthocyanin compounds. Analytical curves (200-5 µg/mL) of the available phenolic standards were constructed based on the UV-Vis signal: caffeic acid ($y = 388345x + 406369$, $R^2 = 0.9939$); catechin ($y = 84950x - 23200$, $R^2 = 1$); epicatechin ($y = 10314x + 147331$, $R^2 = 0.9994$); quercetin 3-O-glucoside ($y = 34843x – 160173$, $R^2 = 0.9988$) and cyanidin-3-*O*-glucoside ($y = 134578x - 3E+06$; $R^2 = 0.9986$). Results were expressed in mg per g of dry extract.

2.3.3. Evaluation of antioxidant activity

To determine the corresponding IC_{50} values, the dry kiwi pulp and peel extracts were re-dissolved (2.5 mg/mL) in ethanol and successively diluted. Following the protocol described by Barros et al. (2013), the lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decrease in thiobarbituric acid reactive substances (TBARS). Following the protocol described by Lockowandt et al. (2019), the oxidative hemolysis inhibition assay (OxHLIA) was performed using sheep blood samples. For this assay, the results were expressed as the inhibitory concentration (EC_{50} value, µg/mL) able to promote a Δt haemolysis delay of 60 and 120 min. For both assays the Trolox was used as positive control.

2.4.4. Evaluation of anti-inflammatory activity

The anti-inflammatory activity was evaluated in mouse macrophage-like cell line RAW 264.7 according to the described methodology by Barros et al. (2013), after dissolving the kiwi pulp and peel extracts in water, at a concentration of 8 mg/mL. Dexamethasone

(50 μM) was used as a positive control and the results were expressed as EC_{50} values (μg/mL).

2.4.4. Evaluation of the cytotoxic activity

The kiwi pulp and peel extracts were dissolved in water at a concentration of 8 mg/mL and tested in four human tumour cell lines: MCF- 7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma), using the sulforhodamine B assay to measure the cell growth inhibition.

The hepatotoxicity was measured by using a freshly harvested porcine liver cell culture (acquired from certified slaughterhouses), being designated as PLP2 (Barros et al., 2013). For both assays a phase contrast microscope was used to monitor the growth of cell cultures, which were sub-cultured and plated in 96-well plates (density of 1.0 x 10^4 cells/well). Dulbecco's modified Eagle's medium (DMEM) supplemented with FBS (10%), penicillin (100 U/mL) and streptomycin (100 μg/mL) were used. Ellipticin was used as the positive control and the results were expressed as GI_{50} values (μg/mL).

2.4.5. Evaluation of antimicrobial activity

To access the antimicrobial activity, the kiwi pulp and peel extracts were dissolved in water at a concentration of 10 mg/mL. The antibacterial activity was evaluated according to a previously described methodology (Soković, Glamoćlija, Marin, Brkić, & van Griensven 2010) using two Gram-negative bacteria: *Escherichia coli* (ATCC 35210), *Enterobacter cloacae* (human isolate), and two Gram-positive bacteria: *Bacillus cereus* (food isolate), *Listeria monocytogenes* (NCTC 7973). The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined and streptomycin and ampicillin were used as positive controls.

Furthermore, the antifungal activity was evaluated following the protocol described by Soković and van Griensven (2006), using four reference species: *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839). The MIC and minimum fungicidal concentration (MFC) were determined and ketoconazole was used as the positive control. The microorganisms are deposited at Mycological laboratory, Department of Plant Physiology, Institute for biological research "Sinisa Stanković", University of Belgrade, Serbia.

2.5. Statistical analysis

The described assays were performed in triplicate and the results expressed in the mean ± standard deviation (SD) format. The data was analysed using the ANOVA test, to determine the significant differences between the samples, with p-value = 0.05 (SPSS v. 23.0; IBM Corp., Armonk, New York, USA).

3. Results and discussion

The nutritional composition results of *Actinidia deliciosa* cv "Hayward" (green kiwi pulp) and *Actinidia* spp. (red kiwi pulp) pulps are presented in **Table 1**. Both kiwifruit varieties present water as the main component representing a percentage greater than 80%. *A. deliciosa* cv "Hayward" pulp revealed a lower protein, ash and carbohydrate content compared to the *Actinidia* spp., which contrary reveled a much lower fat value. Thus, *Actinidia* spp. pulp also presented a higher energetic value. A study developed by López-Sobaler, Vizuete and Anta (2016), that aimed to characterize two varieties of kiwifruit (*A. deliciosa* and *A. chinensis*), showed similar energetic values (61 and 63 kcal, respectively) to those presented in the present work. In the same way, they also verified high contents

of moisture and low values of proteins. As in the present study, Hayward variety was also characterized as having the lowest protein content when compared to the Hort16A variety, also corroborating the results obtained herein. Moreover, D'Evoli et al. (2015) studied different genotypes of *A. chinesis*, showing higher protein contents in comparison to *A. delicious*. These facts are also in agreement with the results presented in our study. Another study revealed that in some varieties the cultivation conditions may also affect the content of proteins, namely Hayward cultivated in New Zealand presented a higher content in comparison to the same variety cultivated in China, thus, in turn, the variety Hort16A from different regions did not present significant differences (Ma et al., 2017). Currently kiwi fruits have come to be characterized as super-fruits since the low content of energy and the high amount of water, fiber, vitamin C among other nutrients confirm the high nutritional quality and recommended for the general population (Latocha et al., 2017). The fact that it has very low caloric values causes this fruit to be habitually incorporated into the diet plans of the weight control diets.

Fructose, glucose, sucrose and trehalose were the free sugars found in both pulps (**Table 1**). It is described in literature that the content in sugars tends to increase with the fruit maturation, so that in the same variety their contents may suffer some deviation (Latocha, 2017). Glucose, fructose and sucrose are the main sugars normally detected in all *Actinidia* fruits (Nishiyama, Fukuda, Shimohashi, & Oota, 2008), which is in agreement with the results presented in this study. The literature describes that the sugar content tends to increase with fruit maturation, so, in the same variety, the contents may suffer some differences (Latocha, 2017). In this study, red kiwi pulp is characterized by being one of the varieties with the highest sweet taste, which may be related to the higher amount of sucrose compared to green kiwi pulp. Nishiyama et al. (2008), verified that the variety will also have a direct influence on the total sugar content, in such a way that the

"Hayward" variety presented significantly lower total sugar content than "Abbott" and "Koryoku", but significantly higher than "Elmwood". In contrast, *A. arguta* presented sucrose as the major sugar followed by glucose and fructose (Latocha, 2017). The results presented in the herein study are in line with those presented in literature, highlighting glucose and fructose as the major free sugars for both tested varieties.

The individual fatty acid composition and the contents of saturated, monounsaturated and polyunsaturated fatty acid are shown in **Table 1**. The pulp of both varieties of kiwifruit, consists mainly of unsaturated fatty acids, due to the high contents of linoleic and α-linolenic acids. These results are in agreement with data reported in literature revealing a very similar fatty acid profile and content in the various kiwi fruit varieties, where unsaturated fatty acids prevail in a defined amount of 70 to 85% and where palmitic acid and α-linoleic acid were the major saturated and unsaturated fatty acid, respectively (Jin, Park, Park, Seung, & Ho, 2014). However, although both varieties have the same fatty acid profile in their composition, there were differences regarding the relative percentage of each individual compounds, which are reflected in the SFA, MUFA and PUFA contents. An example, are the higher amounts of SFA found in red kiwi pulp in comparison with the green kiwi pulp. This higher relative percentage in red kiwi pulp is due to the higher amounts of palmitic acid (14.3 ± 0.4 %), which is three times higher than the amount present in green kiwi pulp (4.6 ± 0.1). In contrast, polyunsaturated fatty acids prevail in green kiwi pulp, which is due to prevalence of linoleic and linolenic acids in this variety (2 fold higher in comparison to red kiwi pulp). These differences could also be explained by the fact that varieties and growing conditions are highly capable of causing significant changes in the chemical composition (Latocha, 2017).

Table 2 presents the composition in tocopherols and organic acids in the different kiwi varieties and parts (pulp and peels). α-Tocopherol was the most abundant vitamer in both

varieties and in both studied parts (peels and pulps). Moreover, the pulps only presented two isoforms (α- and γ-tocopherol), while the peels revealed three isoforms (α-, γ- and δ-tocopherol). Red kiwi pulp and peels was the variety that presented the highest content of tocopherols. A study developed by Chun, Lee, Ye, Exler, & Eitenmiller (2006), which compared the content of tocopherols between different fruits and vegetables revealed the presence of two isoforms (α- and γ- tocopherol) in kiwifruits. In addition, the authors mentioned that kiwifruits presented higher percentage of tocopherols in comparison to other fruits, such as grapes and plums. The literature associates the regular consumption of vitamin E of fruits and vegetables, the antioxidant activity capable of guaranteeing the reduction of the risk of chronic diseases (Kim et al., 2011). Taking into account the high amount of vitamin E in kiwi peels, these could be positively exploited by the food industry as a natural ingredient with functionalizing properties, capable of acting on the health of consumers.

Four organic acids were identified in all the studied samples (quinic, malic, citric and ascorbic acids) (**Table 2**), being the presence of these four organic acids previously reported in kiwi fruits (Nishiyama et al., 2008). Quinic acid was the major organic acid in all samples, with the exception of "Hayward" pulp, which presented citric acid as the main compound. *A. arguta* was also characterized by presenting a higher content of citric acid in comparison to quinic acid. The differences obtained between the different varieties could be due to cultivation conditions, such as soil fertility, climatic conditions and plant growth conditions, but also due to different extraction methodology and conditions (e.g.: time, temperature and solvent) (Park et al., 2015; Latocha, 2017). Kiwis are recognized as fruits exceptionally rich in ascorbic acid (vitamin C) and the amounts can vary considerably between different varieties (Nishiyama et al., 2008). The fact that vitamin C is described as being relatively unstable and therefore easily oxidized in the presence of

oxygen is one of the justifications for variations that may arise between the different varieties or studies (Okamoto and Goto, 2005). This fact may justify the significant differences between the pulps analyzed in the present study. However, the concentrations found in the two varieties meet the ones described for kiwifruits.

Marsh et al. (2003) carried out sensory studies of kiwi pulps revealing that citric, quinic and malic acids can cause different perceptions of acidity. For example, quinic acid is considered to have a greater impact on the perception of acidity than citric or malic acid, being responsible for the characteristic taste of "Hayward" variety. Likewise, *A. arguta* fruits have a low content of quinic acid, which may justify the fact that they are characterized as the sweetest variety (Nishiyama et al., 2008). Therefore, sugars and organic acids are considered the main factors responsible to determine fruits taste, being also reported that organic acids also guarantee the bacterial decay of fruits (Latocha, 2017).

The phenolic compositions of the hydroethanolic peel and pulp extracts of both *Actinidia* spp. are presented in **Table 3**. Twenty non-anthocyanin and one anthocyanin phenolic compounds were detected, among which twelve flavonoid derivatives and nine phenolic acids and derivatives. Of the identified compounds thirteen were identified in peels and sixteen in the pulps.

Compounds 10 and 17-20 were positively identified by comparison with commercial standards, all of them being also previously described in kiwifruits (Sun-Waterhouse, Wen, Wibisono, Melton, & Wadhwa, 2009; Pinelli, Romani, Fierini, Remorini, & Giovanni, 2013; Mena, Sanchez-Salcedo, Tassotti, Martinez, & Hernandez, 2016; Wojdylo et al., 2017). The remaining compounds were identified as caffeic acid glycoside derivatives (peaks 1-6, 8, 9 and 16) and B-type (epi)catechin dimer, trimer, tetramer and pentamer (peaks 7, 11-15), also previously described in other kiwi studies, which were

taken into consideration to tentative identify the compounds present in this study, by comparing the fragmentation pattern and UV-Vis spectra formerly reported (Pinelli et al., 2013; Watson, Preedy, & Zibadi, 2014; Wojdylo et al., 2017; Commisso et al., 2019; Sun-Waterhouse et al., 2009).

Li et al. (2018) presented the phenolic composition of green kiwi pulp (*A. deliciosa* cv. Hayward), being mainly composed by procyanidin B1 and B2, gallic acid, chlorogenic acid and quercetin-3-*O*-rhamnoside, thus revealing a very similar composition to the one present in this study. Flavan-3-ols appear to be the main class of phenolic compounds present in several varieties of kiwi fruits reported in literature (Wojdyło et al., 2017), which is also in accordance with the results presented in the varieties studied herein. Moreover, the presence of polymeric high molecular weight tannins, such as condensed tannins, in kiwi fruits have been pointed out as correlated with the characteristic astringency of kiwi skin (Kim, Beppu, & Kataoka, 2009). Flavonols, phenolic acids and anthocyanins were also identified in these fruits by other authors, thus being identified in lower amounts and usually following the mentioned order (Wojdyło et al., 2017). Quercetin and kaempferol derivatives have been the main flavonols identified in this fruit (Latocha, 2017), while *p*-hydroxybenzoic, *p*-coumaric, ferulic, gallic, and caffeic acids are the main phenolic acids, and (+)-catechin, and (+)-epicatechin the main flavan-3-ols (Kim, Beppu, & Kataoka, 2009; Latocha et al., 2010).

Epicatechin (peak 10) was the main phenolic compound present in all the samples, with the exception of red kiwi pulp where a B-type (epi)catechin dimer (peak 7) was the main molecule. Peels of “Hayward” variety presented the highest phenolic content, while among the pulps the red kiwi was the variety with highest content. Soquetta et al. (2016) described that the phenolic composition is distinct in the different kiwi parts and that normally the peels appear with higher content than the pulp, which is also in agreement

with the herein study. Moreover, Leontowicz et al. (2016) compared different kiwi varieties and extraction procedures regarding the phenolic composition, revealing that water extracts presented a higher content than ethanolic extracts.

Anthocyanins are well known for their antioxidant activity, but also due to the red, blue or purple color they give to many fruits. A study developed by Wojdyło et al. (2017) aimed to verify the differences in the content of anthocyanins among different Actinidia varieties, describing the presence of cyanidin-3-*O*-sambubioside as the main anthocyanin in both skin and pulps. These findings are also in agreement with our study, which also presented this anthocyanin as the main molecule in red kiwi pulp (**Table 3**).

The red kiwi pulp color characteristic of red kiwi varieties (*Actinidia* spp.) are attributed to the presence of different anthocyanins, namely delphinidin-3-*O*-(xylosyl)galactoside, delphinidin-3-*O*-galactoside, cyanidin-3-*O*-(xylosyl)galactoside, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-sambubioside and cyanidin-3-*O*-glucoside (Wojdyło et al. 2017; Peng, Lin-wang, Cooney, Wang, Espley, & Allan, 2019). The anthocyanins content may increase due to the fruit ripening stage, but also due to prolonged storage at low temperatures (90 days at 0°C) (Li et al., 2018).

The antioxidant activity was evaluated using two *in vitro* assays: inhibition of the formation of reactive substances of thiobarbituric acid (TBARS) and inhibition of oxidative hemolysis (OxHLIA). The results are shown in **Table 4** and displayed as EC_{50} values (value representing the sample concentration that provides 50% antioxidant activity). Considering that the lower EC_{50} value represent the higher antioxidant activity (Arbos, Freitas, Stertz, & Dornas, 2010), in TBARS and OxHLIA assays it was verified that both peels presented higher antioxidant activity than the pulps of both varieties. In both assays the best antioxidant activity was also expressed by the green kiwi pulp peel followed by the red kiwi pulp peel. In fact, both kiwi peels revealed the depletion capacity

of hemolysis for 60 and 120 min, despite the higher concentration required for the red kiwi pulp peel. These results may be related to the fact that the peels present in their composition, as previously described, a higher amount of phenolic compounds, which may be correlated with the bioactivity expressed in this part of the kiwifruits.

There are several reports discussing the antioxidant activity of kiwifruit, although describing different tested assays, namely DPPH (2,2-diphenyl-1-picryl-hydroxide) radical scavenging activity, reducing power and β-carotene-linoleate model system (Bernardes et al., 2011, Park et al., 2015), in comparison to the ones tested in this study, which only use cell homogenates and erythrocytes. Fiorentino et al. (2009) developed a comparative study where it was described that kiwi peel *in natura* presented a greater antioxidant activity than the pulp, revealing a potential of application of kiwi peels.

Many of the observed differences between the studied samples and the results presented in literature could be explained by many factors, such as the use of different kiwifruit varieties, antioxidant activity methodologies, extraction techniques and solvents applied (Ayala-Zavala, Wang, Wang, & González-Águilar, 2004).

The effect of the different kiwi extracts on the growth of the four human tumour cell lines (MCF-7, NCI-H460, HeLa, and HepG2) were determined and the GI_{50} values (concentrations that caused 50% of the cell growth inhibition) are detailed in **Table 5**. The results showed that only the peels of green kiwi variety presented positive results for all the tested cell lines (GI_{50} < 400 μg/mL), being able to inhibit the growth of MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma) cell lines in a moderate way, which could be correlated with the highest phenolic composition present in these samples. None of extracts demonstrate toxicity against PLP2 cell lines (GI_{50} > 400 μg/mL).

Kiwi fruits are considered to be a superfood, because they are rich in nutrients namely vitamins, minerals and beneficial compounds that are essential for good health. They have been used in traditional medicine since ancient times, due to their various beneficial effects to human health. Some Chinese therapies from which these fruits originate, have included kiwis in cancer therapy (Latocha, 2017). The inhibitory effect of different extracts of sham Actinidia against different human cancer cell lines, such as HepG2, HT29, Hep3B and HeLa have been confirmed in several *in vitro* studies, demonstration the cytotoxic properties associated with this fruit (Lim, Han, Kim, Lee, Lee, & Lee, 2016). Regarding the anti-inflammatory activity, as in the cytotoxicity effect, only the peels of the green kiwi variety presented this capacity. This is also in agreement with other reported studies in literature, such as the one developed by An, Lee, Kang, Heo, Cho, & Do et al. (2016), which also confirmed the *in vivo* anti-inflammatory potential of kiwi extracts. Gan, Zhang, Zhang, Chen, Liu, & Ma (2004) also demonstrated that kiwi juice has the ability to improve liver health by an *in vivo* study using mice.

In addition to the antioxidant, cytotoxicity and anti-inflammatory, kiwi fruits have also demonstrated to have an excellent antimicrobial activity against some pathogenic bacteria, namely, *Pseudomonas aeruginosa*, *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* (Kichaoi, El-Hindi, Mosleh, & Elbashiti, 2015). Thus, it was imperative to carry out an antimicrobial study (antibacterial and antifungal activity) of peels and pulps from the two studied varieties, which could confirm this potential. **Table 6** lists the results regarding the antibacterial and antifungal activities against a panel of four bacteria (*Escherichia coli*, *Enterobacter cloacae*, *Bacillus cereus*, *Listeria monocytogenes*) and four fungi (*Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium funiculosum*), selected according to their importance in public health.

The first part of **Table 6** corresponds to the antibacterial activity, where it can be verified that the green kiwi pulp showed the lowest MIC values for *Bacillus cereus*, together with the red kiwi peel for *Listeria monocytogenes* and *Enterobacter cloacae*, while the lowest MIC value for *Escherichia coli* was verified for red kiwi pulp. In the second part of **Table 6**, the results are presented regarding the antifungal activity where there is a great similarity between the different tested extracts. The red kiwi peel presented the lowest MIC value for *Aspergillus ochraceus*, while the green kiwi peel revealed the lowest MIC for *Penicillium funiculosum*.

These results are in agreement with previous studies describing the excellent antimicrobial potential presented by *A. deliciosa* (Tiwari, Tiwari, Patel, & Tiwari, 2017). A study carried out by Fisher and Phillips (2008), which aimed to study the antimicrobial potential of kiwi fruits highlighted Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) as being more vulnerable to the essential oil obtained from these fruits than Gram-negative bacteria (*E. coli*). This fact was justified by the presence of an external hydrophilic membrane with lipopolysaccharide molecules in Gram-negative bacteria, which acts as a barrier to hydrophobic compounds. Moreover, recently a study developed by Kichaoi et al. (2015) also revealed a good antimicrobial potential of different kiwi extracts (ethanol, methanol and water) against *S. aureus* and *E. coli*. Therefore, the results revealed herein are in accordance with those previously reported in literature.

4. Conclusions

Although kiwi pulps have been extensively studied over the past few decades, because of its health benefits, peels have also been attracting interest mainly due to its potential commercial value as promising natural ingredients, due to its rich content in bioactive compounds, as also due to its agroindustry waste reduction. Both variety peels revealed

a promising phenolic profile, emphasizing their bioactive potential, which could be exploited in different industrial fields, thus particular by the food industry as a natural preservative ingredient.

This work confirmed the attractive nutritional composition of these fruits, which highlights them in the literature as superfruits, besides demonstrating the potential applicability of the peel (mainly the green kiwi peel), which presented a rich phenolic content, were highlighted in the bioactivities tested.

Thus, there are very few studies regarding kiwi fruit peels and this work allowed to accomplish a complete characterization, in terms of chemical and bioactive characterization of two kiwi varieties (green and red kiwifruits), in order to exploit the potential of application of this resource considering this byproduct that is normally discarded with little value.

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Table 1. Nutritional composition (g/100 g fw), energetic value (kcal/100 g fw), free sugars (g/100 g fw) and fatty acids (relative percentage) composition of green kiwi pulp (PuKG) and red kiwi pulp (PuKR).

		PuKG	PuKR	*p*-value
Moisture		83.8 ± 0.1	82.3 ± 0.3	0.003
Fat		0.424 ± 0.001	0.031 ± 0.001	<0.0001
Protein		1.20 ± 0.07	1.54 ± 0.04	0.117
Ash		0.69 ± 0.04	0.78 ± 0.01	<0.0001
Carbohydrates		13.9 ± 0.1	15.4 ± 0.4	0.015
Energy		64.2 ± 0.5	67.4 ± 0.6	0.017
Free Sugars				
	Fructose	3.83 ± 0.05	3.39 ± 0.03	0.818
	Glucose	3.52 ± 0.09	3.09 ± 0.06	0.492
	Sucrose	1.44 ± 0.01	3.94 ± 0.06	<0.0001
	Trehalose	0.110 ± 0.004	0.080 ± 0.003	0.005
	Total	8.9 ± 0.1	10.49 ± 0.03	<0.0001
Fatty acids				
	C6:0	0.008±0.001	0.03±0.01	<0.0001
	C8:0	0.016±0.001	0.04±0.01	<0.0001
	C10:0	0.003±0.001	0.03±0.01	0,188
	C11:0	0.024±0.003	0.099±0.001	<0.0001
	C12:0	0.025±0.001	0.38±0.04	<0.0001
	C13:0	0.018±0.004	0.017±0.001	0,093
	C14:0	0.044±0.001	0.8±0.1	<0.0001
	C15:0	0.015±0.001	0.165±0.004	<0.0001
	C16:0	4.6 ± 0.1	14.3 ± 0.4	<0.0001
	C16:1	0.106±0.008	1.05±0.04	<0.0001
	C17:0	0.048±0.001	0.23±0.01	<0.0001
	C17:1	0.020±0.001	0.10±0.01	<0.0001
	C18:0	2.05 ± 0.02	2.9 ± 0.2	0.005
	C18:1n9	8.9 ± 0.2	7.94 ± 0.03	0.151
	C18:1n9	0.81±0.04	0.96 ± 0.02	<0.0001
	C18:2n6	14.44 ± 0.08	28.3 ± 0.5	<0.0001
	C18:3n6	0.024±0.002	0.012 ± 0.004	<0.0001
	C18:3n3	68.0 ± 0.1	35.0 ± 0.2	<0.0001
	C20:0	0.037±0.001	0.44 ± 0.01	<0.0001

C20:1	0.015±0.001	0.205 ± 0.004	<0.0001
C20:2	0.254±0.001	1.59 ± 0.04	<0.0001
C21:0	0.011±0.001	0.113 ± 0.001	<0.0001
C20:4n6	0.334 ± 0.002	4.71 ± 0.04	<0.0001
C22:0	0.043±0.003	0.27 ± 0.02	<0.0001
C22:1	0.081±0.001	0.04 ± 0.02	<0.0001
SFA	6.95± 0.09	19.77 ± 0.82	<0.0001
MUFA	9.9 ± 0.1	10.29 ± 0.08	0.002
PUFA	83.13 ± 0.04	69.93 ± 0.74	<0.0001

Results are presented as mean±standard deviation. Caproic acid (C6:0); Caprolic acid (C8:0); Capric acid (C10:0); Undecylic acid (C11:0); Lauric acid (C12:0); Tridecylic acid (C13:0); Myristic acid (C14:0); Pentadecylic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Margaric acid (C17:0); cis-10-Heptadecenoic acid (C17:1); Stearic acid (C18:0); Oleic acid (C18:1n9); linoleic acid (C18:2n6); γ-linoleic acid (C18:3n6); linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1); eicosadienoic acid (C20:2); Heneicosylic acid (C21:0); arachidonic acid (C20:4n6); Behenic acid (C22:0); Erucic acid (C22:1); SFA-Saturated fatty acids; MUFA-Monounsaturated fatty acids; PUFA-Polyunsaturated fatty acids.

Table 2. Tocopherols (mg/100 g fw) and organic acids (g/100 g fw) of green kiwi pulp (PuKG) and peel (PeKG) and red kiwi pulp (PuKR) and peel (PeKR).

	PuKG	PuKR	PeKG	PeKR	*p*-value
Tocopherols					
α-Tocopherols	1.7 ± 0.1^{d}	3.57 ± 0.01^{b}	2.40 ± 0.01^{c}	7.4 ± 0.2^{a}	<0.0001
γ-Tocopherols	0.040 ± 0.001^{c}	1.01 ± 0.01^{b}	0.060 ± 0.002^{c}	1.70 ± 0.04^{a}	<0.0001
δ-Tocopherols	n.d	n.d.	1.86 ± 0.05^{a}	1.75 ± 0.03^{b}	<0.0001
Total tocopherols	1.7 ± 0.1^{c}	4.58 ± 0.01^{b}	4.3 ± 0.1^{b}	10.8 ± 0.3^{a}	<0.0001
Organic acids					
Quinic acid	0.70 ± 0.02^{c}	1.91 ± 0.05^{a}	1.5 ± 0.1^{b}	1.6 ± 0.1^{b}	<0.0001
Malic acid	0.58 ± 0.02^{b}	$0.60 \pm 0,01^{b}$	0.82 ± 0.06^{a}	0.62 ± 0.03^{b}	<0.0001
Citric acid	1.56 ± 0.05^{a}	$1.27 \pm 0,03^{c}$	1.310 ± 0.003^{c}	1.38 ± 0.01^{b}	<0.0001
Ascorbic acid	0.062 ± 0.002^{b}	$0.103 \pm 0,002^{a}$	0.037 ± 0.002^{d}	0.0529 ± 0.0005^{c}	<0.0001
Total	2.90 ± 0.09^{c}	3.88 ± 0.09^{a}	3.7 ± 0.1^{ab}	3.67 ± 0.04^{b}	<0.0001

n.a.- non-applicable; n.d.-non-detected. The results were expressed mean±standard deviation. Statistical analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test and in each column different letters mean significant differences ($p<0.005$).

Table 3. Phenolic compounds composition of green kiwi pulp (PuKG) and peel (PeKG) and red kiwi pulp (PuKR) and peel (PeKR) extracts.

Peak	Rt (min)	λmax (nm)	$[M-H]^-$ /$[M+H]^+$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	References	Quantification (mg/g extract)			
							PuKG	PuKR	PeKG	PeKR
Non-anthocyanin compounds										
1	4.33	318	503	341(100),179(72),135(7)	Caffeic acid dihexoside	Commisso et al., 2019	0.52±0.02	n.d.	n.d.	n.d.
2	4.69	310	323	179(100),135(9)	Caffeic acid derivative	Pinelli et al., 2013	0.38±0.01	n.d.	n.d.	n.d.
3	5.09	320	341	179(100),135(7)	Caffeic acid hexoside	Sun-Waterhouse et al., 2009	0.56±0.02	n.d.	n.d.	n.d.
4	5.29	320	297	179(37),135(100)	Caffeic acid derivative	Pinelli et al., 2013	n.d.	1.23 ± 0.03^{c}	8.6 ± 0.2^{a}	2.14 ± 0.07^{b}
5	5.38	322	341	179(100),135(15)	Caffeic acid hexoside	Sun-Waterhouse et al., 2009	tr	n.d.	n.d.	n.d.
6	6.46	320	341	179(100),135(22)	Caffeic acid hexoside	Sun-Waterhouse et al., 2009	0.65 ± 0.03^{d}	3.3 ± 0.1^{b}	13.60 ± 0.03^{a}	2.22 ± 0.09^{c}
7	7.51	281	577	451(27),425(100),407(32),289(10)	B-type (epi)catechin dimer	Watson et al., 2014	n.d.	5.4 ± 0.2^{c}	35 ± 1^{a}	23.3 ± 0.6^{b}

8	7.63	321	341	179(100),135(8)	Caffeic acid hexoside	Sun-Waterhouse et al., 2009	1.69±0.04	n.d.	n.d.	n.d.
9	8.25	330	369	207(100),191(12)	Dimethyl caffeic acid hexoside	Wojdylo et al., 2017; Pinelli et al., 2013	0.35±0.01^{c}	n.d.	1.35±0.04^{a}	0.85±0.01^{b}
10	8.54	280	289	245(100),205(41),179(17)	Epicatechin	Wojdylo et al., 2017	11.8±0.6^{c}	0.59±0.02^{d}	163±4^{a}	110±2^{b}
11	10.65	281	865	739(87),713(52),695(100),577(72), 425(18), 289(3),287(12)	B-type (epi)catechin trimer	Watson et al., 2014	n.d.	4.5±0.2^{c}	24.6±0.7^{a}	17.6±0.5^{b}
12	11.78	281	1153	865(100),863(82),577(34),575(57), 289(3),287(3)	B-type (epi)catechin tetramer	Watson et al., 2014	n.d.	3.6±0.2^{c}	30.4±0.5^{a}	18.8±0.9^{b}
13	12.71	281	865	739(76),713(59),695(100),577(66), 425(15), 289(5),287(9)	B-type (epi)catechin trimer	Watson et al., 2014	n.d.	n.d.	14.9±0.7*	7.4±0.2*
14	13.26	281	1441	1153(20),865(34),863(47),577(38), 289(9), 287(29)	B-type (epi)catechin pentamer	Watson et al., 2014	n.d.	n.d.	12.4±0.5*	7.7±0.3*
15	13.8	281	1441	1153(43),865(23),863(31),577 (77), 289(7),287(17)	B-type (epi)catechin pentamer	Watson et al., 2014	n.d.	n.d.	12.9±0.6*	6.6±0.2*
16	14.53	320	411	369(5),207(100),191(27),179(5)	Acetyl-dimethyl caffeic acid hexoside	Sun-Waterhouse et al., 2009	n.d.	n.d.	3.33±0.04*	0.136±0.004*
17	17.64	351	609	301(100)	Quercetin-3-*O*-rutinoside	Wojdylo et al., 2017	0.358±0.003*	0.53±0.01*	n.d.	n.d.
18	18.76	352	463	301(100)	Quercetin-3-*O*-glucoside	Wojdylo et al., 2017	n.d.	0.64±0.01^{a}	0.406±0.001^{b}	0.42±0.01^{b}
19	21.11	340	593	285(100)	Kaempferol-3-*O*-rutinoside	Mena et al., 2016	0.347±0.001	0.464±0.001	n.d.	n.d.
20	22.31	351	477	301(100)	Quercetin-3-*O*-rhamnoside	Wojdylo et al., 2017	0.360±0.003^{c}	2.59±0.05^{a}	0.74±0.02^{b}	0.295±0.002^{d}
					Total non-anthocyanin compounds		17±1^{d}	23±1^{c}	322±8^{a}	197±3^{b}
Anthocyanin compounds										
21	16.81	516	581	287(100)	Cyanidin-3-*O*-sambubioside	Wojdyło et al. (2017)	n.d.	12.9±0.1	n.d.	n.d.

n.d. –not detected; tr - traces; The results were expressed mean±standard deviation; calibration curves: caffeic acid (y = 388345x + 406369, R^2 = 0.9939); catechin (y = 84950x - 23200, R^2 = 1); epicatechin (y = 10314x + 147331, R^2 = 0.9994); quercetin 3-*O*-glucoside (y = 34843x – 160173, R^2 = 0.9988) and cyanidin-3-*O*-glucoside (y = 134578x - 3E+06; R^2 = 0.9986). Different letters corresponded to significate differences ($p < 0.05$). *Means statistical differences obtained by a t-student test.

Table 4. Cell-based antioxidant activity of green kiwi pulp (PuKG) and peel (PeKG) extracts and red kiwi pulp (PuKR) and peel (PeKR) extracts evaluated by OxHLIA and TBARS assays.

	OxHLIA		TBARS
	60 min	120 min	
PuKG	291±6[b]	n.a.	406±16[a]
PuKR	545±37[a]	n.a.	377±26[b]
PeKG	183±14[d]	414±8*	76±5[d]
PeKR	252±20[c]	1439±37*	129±2[c]
p-value	<0.0001	<0.0001	<0.0001

The results are presented as EC_{50} values (µg/mL). n.a.: no activity. Trolox (positive control) EC_{50} values: 19.6 µg/mL (OxHLIA 60 min), 41.1 µg/mL (OxHLIA 120 min) and 23 µg/mL (TBARS inhibition). Results are presented as mean±standard deviation and were analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test and in each column different letters mean significant differences ($p<0.005$). *Means statistical differences obtained by a t-student test.

Table 5. Cytotoxicity, hepatotoxicity and anti-inflammatory activity of green kiwi pulp (PuKG) and peel (PeKG) extracts and red kiwi pulp (PuKR) and peel (PeKR) extracts.

	Cytotoxicity (GI_{50}, µg/mL)				Hepatotoxicity (GI_{50}, µg/mL)	Anti-inflammatory activity (IC_{50}, µg/mL)
	NCI H460	HeLa	MCF7	HepG2	PLP2	RAW 246.7
PuKG	>400	>400	>400	>400	>400	>400
PuKR	>400	>400	>400	>400	>400	>400
PeKG	300±16	246±7	223±8	166±7	>400	316±6
PeKR	>400	>400	>400	>400	>400	>400
Ellipticine	1.0±0.1	1.91±0.06	0.91±0.04	1.1±0.2	3.2±0.7	-
Dexametasona	-	-	-	-	-	16±1

Results are presented as mean±standard deviation. GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in human tumour cell lines or in liver primary culture PLP2.

Table 6. Antimicrobial activity of green kiwi pulp (PuKG) and peel (PeKG) extracts and red kiwi pulp (PuKR) and peel (PeKR) extracts.

Antibacterial activity (MIC and MBC values, mg/mL extract)								
	Gram positive bacteria				**Gram negative bacteria**			
	Bacillus cereus		*Listeria monocytogenes*		*Escherichia coli*		*Enterobacter cloacae*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
PuKG	3	4	2	4	2	4	2	4
PuKR	4	8	4	8	1	2	4	8
PeKG	4	8	4	8	2	4	4	8
PeKR	4	8	2	4	1.5	2	2	4
Streptomycin	0.10	0.20	0.20	0.30	0.20	0.30	0.20	0.30
Ampicillin	0.25	0.40	0.40	0.50	0.40	0.50	0.25	0.50

Antifungal activity (MIC and MFC values, mg/mL extract)								
	Aspergillus niger		*Aspergillus ochraceus*		*Aspergillus versicolor*		*Penicillium funiculosum*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
PuKG	4	8	4	8	4	8	8	>8
PuKR	4	8	4	8	4	8	8	>8
PeKG	4	8	4	8	4	8	4	8
PeKR	4	8	2	4	4	8	4	8
Ketoconazole	0.20	0.50	0.15	0.20	0.20	0.50	0.20	0.50

MIC values corre- spond to the minimal sample concentration that inhibited the bacterial growth; MBC or MFC correspond to the minimum bactericidal or fungicidal concentrations, respectively.

Research highlights

Pulp and peels of two kiwifruit varieties (green and red kiwi pulp) were explored.

Green kiwi pulp peel (PeGK) presented the highest content in phenolic compounds

Red kiwi pulp revealed the presence of cyanidin-3-*O*-sambubioside.

The peels exhibited the highest antioxidant activity.

PeGK was the only extract that showed cytotoxicity and anti-inflammatory activity.

Graphical abstract

Chemical composition and bioactive properties of byproducts from two different kiwi varieties

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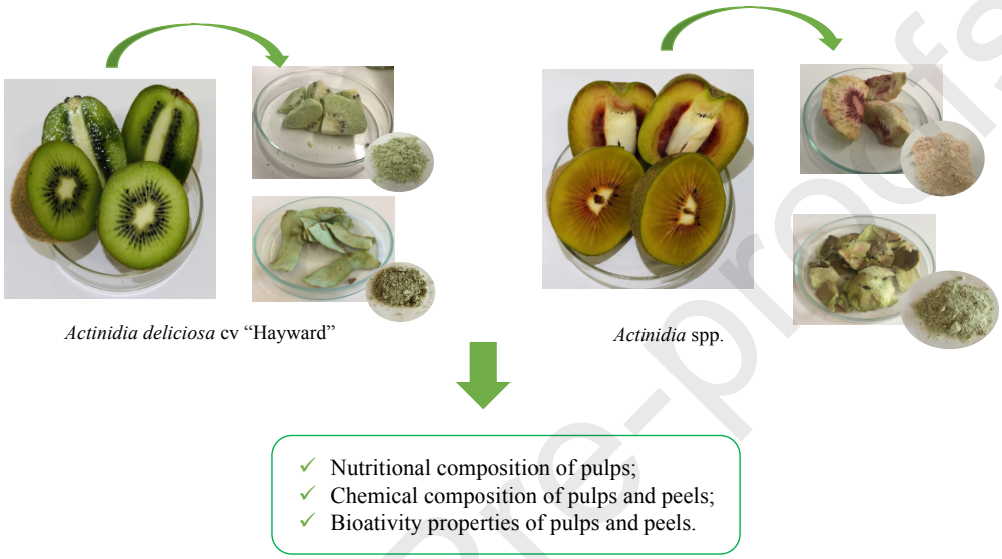

Kiwi peels currently underutilized may be indicated as a source of natural functionalizing ingredients with several benefits for human health.