Kiwi fruit residues from industry processing: study for a maximum phenolic recovery yield

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Abstract A response surface methodology was used to study the conditions for a maximum recovery of phenolics from processing kiwi fruit residues. Ethanolic extracts were prepared with different conditions of pH (2, 5, 10), temperature (30, 50 and 70 °C) and time (10, 20, 30 min). Total phenolics, total flavonoids content and antioxidant activities by 2,2-diphenyl-1-picrylhydrazyl scavenging capacity and ferric reducing antioxidant power were determined. Samples from optimal extraction condition were injected HPLC-DAD system to access the phenolic profile and content. The best extraction conditions were pH solvent of 2, 70 °C of temperature and 20 min of extraction. Ten phenolics were identified: caffeic acid and its derivatives, chlorogenic acid and ferulic acid, (+)-catechin, (–)-epicatechin, rutin and quercitrin. These phenolics often reported as having important antioxidant, anti-inflammatory, antiaging and anticancer activities, turn this residues and excellent source of bioactive compounds to be used in agro-food, cosmetics or phytochemical industries.

Keywords Kiwi fruit; processing · Residues · Bioactive phytochemicals · Bio-based products

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Introduction

Phenolics are a group of hydroxylated molecules largely present in plants, gathered in different type of structures with a common aromatic ring (Działo et al. 2016), which have been associated with important antioxidant anti-inflammatory, antidiabetic, anti-aging, anticancer and antimicrobial (Johansson et al. 2014; Peng et al. 2016; Lemus-Mondaca et al. 2018; Lu et al. 2018; Ahangarpour et al. 2019; Caban et al. 2019; Cesari et al. 2019; Elfalleh et al. 2019; Zhu et al. 2019) activities. In recent years, the growing antibiotic resistance phenomena from bacteria to currents drugs and the need for having safe and environmental friendly antioxidant compounds in food industry, increased the looking for new materials to recover highly effective antioxidant compounds, including phenolics. Among those materials, pomaces from different fruits processing industry have been assayed to evaluate its own potential to be a source of natural antioxidant compounds. Kiwi fruit (*Actinidia deliciosa*) is from a perennial vine plant that contains high levels of bioactive compounds such as phenolics, vitamins C and E, and carotenoids (Dawes and Keene 1999, Fiorentino et al. 2009, Park et al. 2012; Pinelli et al. 2013) and recently, epidemiological studies have suggested that extracts from kiwi may exert antioxidant, anti-degenerative, antimicrobial and anti-tumoral activities (Kim et al. 2018). Although the chemical composition of kiwifruits are well known (Dawes and Keene 1999, Fiorentino et al. 2009, Pinelli et al. 2013), their byproducts from agro-food industry are less studied. Furthermore, every year, fruit and juice industry generates every year tones of kiwi fruit processing residues without any substantial usage, causing economic and environmental issues. Based in these two aspects, we set a study in which we evaluated the potential of pomaces from kiwi

fruit processing to be an easy and natural source of phenolics, contributing to find sustainable solutions for the excess of these residues. We assayed different conditions that can influenced the yield of phenolics extracted such as pH of solvent, temperature and extraction time.

Material and methods

Chemical reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), acetate sodium acetate, acid acetic glacial, 2,4,6-tripyrindyl-S-triazine (TPTZ), HCl, iron chloride, FeSO_4 , ethanol, methanol, acetonitrile, trifluoroacetic acid (TFA), sodium carbonate, gallic acid, caffeic acid, were purchased from Sigma-Aldrich (Taufkirchen, Germany). All organic solvents were HPLC grade. Ultrapure water from purified system (Isopad Isomante, Gemini BV, Pr. Beatrixlaan 301, 7312 DG Apeldoorn, Netherlands) was used. External standards caffeic acid, (+)-catechin, chlorogenic acid, ferulic acid, gallic acid, (–)-epicatechin, naringin, *p*-hydroxybenzoic acid, quercitrin, and rutin were purchased from Extrasynthese (Lyon Nord, Genay Cedex, France).

Vegetal material

Kiwi fruit pomaces were produced in laboratory of Agronomy department of University of Trás-os-Montes e alto Douro (UTAD), in the similar conditions of juices industry. Kiwi fruits were acquired directly at local producers in one of the most important Portuguese kiwifruit production regions, Felgueiras, North of Portugal (latitude: 41° 21' 58.32"N, longitude: – 8° 11' 57.41"W) at 317 m of altitude above sea level. In this region, the weather is typically Mediterranean with warm summers. According to the Köppen-Geiger climate classification (Kottek et al. 2006), the climate is the climate of the region is Csb. In 2018, the average minimum and maximum daily temperatures were 9.0 and 19.0 °C, respectively. According to producers, the kiwi fruits were harvested when soluble solids reached the average of 7.2° Brix grade. After collection, the fruits were submitted to juice extraction in a commercial blender and the resulting pomaces were collected. The pomaces were then freeze-dried (Scanvac Coolsafe 554 Pro, Labogene, Bjarkesvej 5, DK-3450 Allerød Danemark) for 72 h and milled in a commercial blender into a fine powder. The dried pomaces were immediately used for extraction procedure.

Experimental design and extraction procedure

The first step in this study was to define the optimum conditions for extraction of total polyphenols (TP), total flavonoids (TF), and DPPH scavenging capacity in kiwi pomaces. A response surface model (RSM) with a Box-Behnken design and a polynomial model with seventeen consecutive runs (Design-expert software, version 1, State Inc., Minneapolis, MN) using different conditions of temperature pH solvent (X_1), temperature of extraction (X_2) and time of extraction (X_3) were used (Table 1). A quadratic equation with three main factors (X_1 , X_2 , X_3), three two-interactive effects (X_1X_2 , X_1X_3 , X_2X_3) and three curvature effects (X_1^2 , X_2^2 , X_3^2) was calculated. Based in our previous studies (Saavedra et al. 2015; Aires et al. 2016, 2017), a concentration of 70% ethanol (v/v, ethanol: water) and 40 mg of sample per 1 ml of solvent, was used to perform the extraction, since those conditions were proved to be effective in extract phytochemicals from different plant matrices. All experiments were conducted randomly and a quadratic mathematic equation with 95% of confidence interval for each quantitative parameter was determined. All experiments were performed in triplicate to assure reproducible results.

Extraction of polyphenol compounds

Freeze-dried Kiwi pomaces was extracted with ethanol at different pH, temperature and extraction time as reported above. The mixtures were centrifuged (Eppendorf Centrifuge 5804 R, Hamburg, Germany) at 11,000 rpm, 15 min and 4 °C. The supernatants collected and filtered with Spartan filters (0.2 µm) to amber vials (Chromabond 2-SVW(A) ST-CPK, Sigma-Aldrich, Taufkirchen, Germany) and the precipitated rejected. These extracts were used to quantify TP, TF and in DPPH scavenging activity assay.

Total polyphenols content (TPC)

TPC of kiwi pomaces were determined by classic colorimetry (Singleton and Rossi 1965) performed in

Table 1 Independent variable values of kiwi pomaces extraction process and their correspondent levels

Independent variables	Symbol	Coded levels	
		– 1	+ 1
pH solvent	X_1	2	10
Temperature of extraction (°C)	X_2	30	70
Extraction time (min.)	X_3	10	30

96-microplate wells (Costar 3590, Corning, NY, USA). After the extraction procedure performed previously 20 μL of each extract was added to each microplate well followed by addition of 100 μL of Folin-Ciocalteu's phenol reagent (1:10 in dd H_2O) and 80 μL of 7.5% Na_2CO_3 . The microplates were then heated at 45° C for 15 min and the absorbance values were recorded at 765 nm wavelength in a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland), against blank (all reagents except sample). Simultaneously, a calibration curve with a commercial standard of gallic acid at different concentrations (starting from 0.0 to 1.0 mg mL^{-1}) was performed in order find an equation ($y = ax + b$) to quantify the TPC. The results were expressed as mg gallic acid equivalent (GAE) g^{-1} dry weight (dw).

Total flavonoids content (TFC)

TFC of kiwi pomaces were determined by classic colorimetry (Dewanto et al. (2002), but performed in 96 performed wells (Costar 3590, Corning, NY, USA). 25 μL of each sample extract prepared previously was added to each microplate well followed by 100 μL of ultra-pure water and 10 μL of 5% NaNO_2 . Then, the microplates were incubated at room temperature during 5 min in a dark environment. After this period 15 μL of 10% AlCl_3 was added to each well and the microplates were once again incubated at room temperature in a dark environment but for 6 min. Then, to each well, 50 μL of NaOH 1 M followed by 50 μL of ultra-pure were added and mixed thoroughly. The absorbance values were then recorded at 510 nm in microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland) against blank (all reagents and ultrapure water instead of extract). Simultaneously a calibration curve with commercial standard of catechin at different concentrations (1.0, 0.5, 0.25, ..., 0.0 mg mL^{-1}) was performed in order find an equation ($y = ax + b$) to quantify the TFC. The results were expressed as mg catechin equivalent (CAE) g^{-1} dw.

Measurement of in vitro antioxidant capacity

DPPH scavenging capacity

The DPPH scavenging activity of each extract was determined colorimetry (Siddhraj and Becker 2003) conducted in a 96-microplate wells. 285 μL of freshly DPPH radical solution (4 mg DPPH in 100 mL of 95% of ethanol) was prepared and added to each microplate well, followed by 15 μL of each extract. Simultaneously a blank sample (all reagents and extraction solvent instead of sample) added to

one well. The microplates, were then were incubated at room temperature, in the dark, and for 30 min. After this period, the absorbance values were recorded at 517 nm wavelength in a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland). The results were expressed as % DPPH radical scavenging activity using the following equation: % DPPH scavenging capacity = $(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{blank}}) \times 100$, where $\text{Abs}_{\text{blank}}$ means the absorbance value of blank and $\text{Abs}_{\text{sample}}$ the absorbance value of sample extract.

Ferric reducing antioxidant power (FRAP) activity

The FRAP activity as described previously (Benzie and Strain 1996), with small modifications and conducted in 96 wells microplates. Briefly, a fresh daily FRAP reagent solution was prepared by mixing sodium acetate buffer (300 mM, pH 3.6) with 10 mM TPTZ solution (40 mM HCl as solvent) and 20 mM iron (III) chloride solution in a volume ratio of 10:1:1, respectively. Then, in each microplate well 25 μL of kiwi pomace extracts were mixed with 275 μL FRAP solution reagent. The microplates were then stand in room temperature and dark environment for 5 min and after, the absorbance values were recorded at 593 nm in a microplate reader (Multiskan™ FC Microplate Photometer, USA). The FeSO_4 (Sigma-Aldrich, Taufkirchen, Germany) was used as control standard and the results (mean $\pm$ SE) were expressed as $\mu\text{mol FeSO}_4$ equivalents g^{-1} dw.

HPLC analysis of individual polyphenol compounds in optimum conditions

The samples with optimum extraction values of TPC, TFC, DPPH scavenging capacity and FRAP activity values were submitted to HPLC-diode array detector (DAD) in order assess the profile and content of polyphenols, using the procedure next described, adapted from Aires et al. (2013). 500 μL of each extract obtained previously was injected with an eluent composed by water with 0.1% of trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 0.1% TFA (solvent B). The elution was performed at a flow rate of solvent of 1 mL min^{-1} , with a gradient starting from 0% solvent B at 0 min, 0% solvent B at 5 min., 20% solvent B at 15 min, 50% B at 30 min, 100% solvent B at 45 min., 100% solvent B at 50 min, 0% solvent B at 55 min and 0% solvent B at 60 min. The injection volume was 10 μL of sample. Chromatograms were recorded at 254 and 280 nm for benzoic acids and flavan-3-ols, 320 nm for cinnamic acids, and 370 nm for flavonoids, with a C18 column (250 $\times$ 46 mm, 5 mm, ACE HPLC Columns, Advanced Chromatography Technologies Ltd, Aberdeen, Scotland,

UK). Individual polyphenols were identified using peak retention time, UV spectra, and UV max absorbance bands and trough comparison with external commercial standards (Extrasynthese, Cedex, France). External standards were freshly prepared in 70% methanol (methanol:water) at concentration of 1.0 mg mL^{-1} and running in HPLC-DAD simultaneously to the samples. The amount of each polyphenols was calculated using the internal standard (naringin) method. The results were expressed as $\text{mg } 100 \text{ g}^{-1} \text{ dw}$.

Statistical analysis

All determinations were done in triplicate and results were presented as mean and pooled standard deviation. Data were submitted to statistical analysis using the Design-Expert v.11 (20,121 East Hennepin Ave., Minneapolis, USA). The accuracy and general ability of model was evaluated by coefficient determination (R^2) and the statistical significance of the terms in regression equations was examined. In addition, an ANOVA analysis to the results were performed and a Pearson's correlation was done in order to evaluate the importance of TPC and TFC in the DPPH scavenging capacity and FRAP activity.

Results and discussion

Fitting model

The optimization of any extraction process is a crucial step to isolate and identify any type of compound from any type of food matrices. Therefore, to optimize the extraction of polyphenols from kiwi pomaces is fundamental to assay a preliminary study in which independent variables are tested such as solvent pH (X_1), temperature of extraction (X_2) and extraction time (X_3). In our study, 17 combinations of three variables (Table 1) were tested using a response surface model. The values of response variables employed in a multiple regression analysis (to fit quadratic equation) and the respective predicted model as well as the statistical parameters calculated after implementation of Box-Behnken design are listed in Tables 2 and 3. The results for TPC, TFC, DPPH and FRAP activities are presented in Table 4. According to the results, R^2 values for the regression model of TPC, TFC, DPPH and FRAP were significant with 0.9945, 0.9999, 0.9970 and 0.9999, respectively (Table 2), indicating that the model present a reasonable fit experimental data. Moreover, the results from ANOVA analysis (Table 3), showed a significant adequacy of the model with regard to TPC ($P < 0.001$), TFC ($P < 0.001$), DPPH ($P < 0.001$) and FRAP ($P < 0.001$) (Table 4) thus, showing the suitability of the model to predict the variation of

TPC, TFC, DPPH and FRAP in kiwifruit pomaces, accurately. The results obtained were in agreement with the results of Altemimi et al. (2015), showing that RSM can be suitable to evaluate the conditions for maximize the extraction of phenolics from natural matrices. These authors also showed a high correlation of antioxidant activities in spinach with the presence of phytochemicals, which were highly dependent of extraction conditions, reaching for the estimated model regression R^2 values higher than 0.95, similar to the R^2 values found in the current study.

Influence of extraction parameters on TPC, TFC, DPPH and FRAP

The results from ANOVA (Table 3) showed that all extraction factors (pH solvent, temperature of extraction and extraction time) affected significantly ($P < 0.001$) the contents of TPC and TFC. TPC extraction ranged statistically from 524.02 (assay number 7) to 7668.32 (assay number 14) $\text{mg GAE } 100 \text{ g}^{-1} \text{ dw}$. Dranca and Oroian (2016) reported the same influence of very similar extraction conditions (pH, temperature and extraction time) in total phenolic content and other phytochemicals such as total monomeric anthocyanins in eggplant. Our values were higher compared with those obtained by Pinelli et al. (2013) for kiwi fruits, pulps, seeds and juices. Based in our results and using three-dimensional (3D) and two-dimensional (2D) response surface plots obtained from values of RSM (supplementary data), we found a maximum yields of TPC and TFC in assay number 14, in which the extraction conditions were pH solvent of 2, 70°C of temperature and 20 min of extraction (Table 4). Extraction under different condition leads to a lower TPC and TFC yields. Similar analysis can be done for DPPH scavenging capacity and FRAP activity results (Table 4 and supplementary data). The highest antioxidant activities were achieved in the in the run 4 for DPPH and run 14 for the FRAP. The three-dimensional (3D) and two-dimensional (2D) response surface plots obtained from values of RSM (Table 4) showed that extraction conditions of assay number 4 with pH of solvent 5, extraction temperature of 70°C and 30 min of extraction where the conditions in which the DPPH were higher. While for FRAP activities the best extraction conditions were in the run 14 with pH solvent of 2, 70°C of temperature and 20 min of extraction. Although the DPPH scavenging activity present different optimal conditions, their average level is very similar to the presented by run 14, and we can consider that under run 14 we also have high antioxidant potential measured by DPPH. The difference in the results for these two antioxidant assays could reside on the type of compounds determined as well as in the effect of the temperature combined with

Table 2 Quadratic equations and statistical parameters calculated after running the Box-Behnken design

Regression coefficient	Quadratic equation	R ²	Adjust R ²	p value
TPC (Y ₁)	$4227.83 - 764.05X_1 + 3093.52X_2 + 57.96X_3 - 436.0X_1X_2 + 270.05X_1X_3 + 135.99X_2X_3 - 119.56X_1^2 - 448.10X_2^2 + 13.73X_3^2$	0.9945	0.9874	< 0.0001
TFC (Y ₂)	$467.39 - 69.89X_1 + 109.12X_2 + 20.16X_3 - 7.69X_1X_2 + 5.28X_1X_3 + 34.64X_2X_3 - 17.47X_1^2 + 0.00X_2^2 + 0.00X_3^2$	0.9999	0.9998	< 0.0001
DPPH scavenging activity (Y ₃)	$72.48 - 3.09X_1 + 5.71X_2 + 0.15X_3 + 2.50X_1X_2 + 1.70X_1X_3 + 1.41X_2X_3 - 0.77X_1^2 + 0.00X_2^2 + 0.00X_3^2$	0.9970	0.9932	< 0.0001
FRAP activity (Y ₄)	$51.19 - 7.15X_1 + 8.43X_2 - 0.60X_3 - 0.97X_1X_2 + 3.48X_1X_3 + 3.27X_2X_3 - 1.79X_1^2 - 0.00X_2^2 + 0.00X_3^2$	0.9999	0.0001	< 0.0001

extraction time on the extraction yield of antioxidant compounds. DPPH scavenging capacity assay, is a simple, fast and widely method that measures the ability of phenolic, ascorbic acid, carotenoid, cysteine, glutathione, tocopherol and polyhydroxy aromatic compounds in fruit, vegetables, liquids or solids, in a simple or complex biological systems to act as free radical scavengers or hydrogen (H) donors (Kedare and Singh 2011). While FRAP assay, is based on the capacity of small antioxidant molecules such as phenolic acids and flavonoids to reduce Fe^{3+} in to Fe^{2+} (Prior et al. 2005) and cannot detect the effect of compounds that act by radical quenching (H transfer) (Pulido et al. 2000). Therefore, DPPH assay can be used for complex food matrices with different type of compounds, whilst FRAP method is more suitable for simple food matrices with low diversity of compounds. Moreover, different temperatures and different extraction times may affect the type and amount of compounds released from food or plant matrices. Heating for longer periods may promote the extraction different antioxidant compounds but not phenolics (Mokrani and Madani 2016). Therefore, based in these results, under 70°C and extraction times higher than 20 min, other compounds than phenolics may be released from food matrices increasing its antioxidant, even if the content of phenolics decreased by heat degradation. The results from Pearson's correlation revealed a high relationship between TPC and DPPH (0.883, $P < 0.01$) and FRAP (0.871, $P < 0.001$), and between TFC and DPPH (0.932, $P < 0.001$) and FRAP (0.970, $P < 0.001$), showing a close relation between the antioxidant activities of kiwifruit pomaces and its contents of bioactive compounds.

Optimization and verification

Our results showed that an increasing in the pH of solvent from 2 to 10, implied a decreasing in both polyphenols extracted and antioxidant activities. While for the temperature and time of extraction, it is better to extract at 70 °C for 30 min to have higher content of antioxidant polyphenols. Under different conditions from those, the extraction yields of polyphenols and its antioxidant potential will be always lower. To verify the consistence of the model one additional experiment was performed by applying the conditions for the selected points, and as shown in Table 4 and Fig. 1, the experimental and predicted values were in close agreement. It is possible to observe a higher gain in yields of TPC and TFC, and in the levels of FRAP activity at optimum points of run 14, whilst in DPPH the gain was in the run 4 (Table 4). In the others points the predicted extraction yields and predicted antioxidant activities levels were very similar or lower than the experimental values. Therefore, it can be concluded

Table 3 ANOVA results of Box-Behnken model

Source of variation	Sum of Squares	df	Mean Square	F-value	p value
<i>Total phenolics content</i>					
Model	8.33	9	9.26	140.85	< 0.0001
X ₁ -pH solvent	4.67	1	4.67	71.06	< 0.0001
X ₂ -Temperature of extraction	7.66	1	7.66	1164.88	< 0.0001
X ₃ -Extraction time	26,871.70	1	26,871.70	0.4089	0.5429
X ₁ X ₂	7.60	1	7.60	11.57	0.0114
X ₁ X ₃	2.92	1	2.92	4.44	0.0731
X ₂ X ₃	73,976.12	1	73,976.12	1.13	0.3239
X ₁ ²	60,185.60	1	60,185.60	0.9158	0.3705
X ₂ ²	8.46	1	8.46	12.86	0.0089
X ₂ ³	793.16	1	793.16	0.0121	0.9156
Residual	4.60	7	65,722.43		
Lack of fit	4.60	3	1.53		
Pure error	0.0000	4	0.0000		
Cor total	8.38	16			
<i>Total flavonoids content</i>					
Model	1.44	9	16,002.61	10,305.01	< 0.0001
X ₁ -pH solvent	39,079.69	1	39,079.69	25,165.70	< 0.0001
X ₂ -temperature of extraction	95,251.42	1	95,251.42	61,337.96	< 0.0001
X ₃ -extraction time	3251.71	1	3251.71	2093.97	< 0.0001
X ₁ X ₂	236.44	1	236.44	152.26	< 0.0001
X ₁ X ₃	111.41	1	111.41	71.74	< 0.0001
X ₂ X ₃	4799.70	1	4799.70	3090.81	< 0.0001
X ₁ ²	1285.52	1	1285.52	827.82	< 0.0001
X ₂ ²	0.0000	1	0.0000	0.0000	1.0000
X ₂ ³	0.0000	1	0.0000	0.0000	1.0000
Residual	10.87	7	1.55		
Lack of fit	10.87	3	3.62		
Pure error	0.0000	4	0.0000		
Cor total	1.44	16			
<i>DPPH scavenging capacity</i>					
Model	384.30	9	42.70	261.55	< 0.0001
X ₁ -pH solvent	76.44	1	76.44	468.19	< 0.0001
X ₂ -Temperature of extraction	260.68	1	260.68	1596.72	< 0.0001
X ₃ -Extraction time	0.1738	1	0.1738	1.06	0.3365
X ₁ X ₂	25.01	1	25.01	153.18	< 0.0001
X ₁ X ₃	11.56	1	11.56	70.82	< 0.0001
X ₂ X ₃	7.92	1	7.92	48.49	0.0002
X ₁ ²	2.51	1	2.51	15.40	0.0057
X ₂ ²	0.0000	1	0.0000	0.0000	1.0000
X ₂ ³	0.0000	1	0.0000	0.0000	1.0000
Residual	1.14	7	0.1633		
Lack of fit	1.14	3	0.3809		
Pure error	0.0000	4	0.0000		
Cor total	385.44	16			
<i>FRAP activity</i>					
Model	1040.99	9	115.67	5566.55	< 0.0001
X ₁ -pH solvent	408.92	1	408.92	19,679.79	< 0.0001
X ₂ -Temperature of extraction	568.16	1	568.16	27,343.44	< 0.0001
X ₃ -Extraction time	2.88	1	2.88	138.81	< 0.0001

Table 3 continued

Source of variation	Sum of Squares	df	Mean Square	F-value	<i>p</i> value
X ₁ X ₂	3.75	1	3.75	180.66	< 0.0001
X ₁ X ₃	0.9005	1	0.90	43.34	0.0003
X ₂ X ₃	42.84	1	42.84	2061.78	< 0.0001
X ₁ ²	13.45	1	13.45	647.36	< 0.0001
X ₂ ²	0.0000	1	0.0000	0.0000	1.0000
X ₂ ³	0.0000	1	0.0000	0.0000	1.0000
Residual	0.1455	7	0.0208		
Lack of fit	0.1455	3	0.0485		
Pure error	0.0000	4	0.0000		
Cor total	1041.14	16			

that the model tested and its extraction conditions can be applied to extract higher yields of antioxidant phenolics from kiwifruits pomaces.

Characterization of polyphenols present in extracts of kiwi pomaces o by HPLC-DAD

Extract samples from the run 14, in which it was obtained higher yields of TPC and TFC, was analyzed in HPLC-DAD system to determine the main phenolics present (Fig. 2). The corresponding contents are summarized in Table 5. In kiwi fruit pomaces, from run 14 it was possible to identify 10 phenolics grouped in 4 major classes: two benzoic acids (*p*-hydroxybenzoic and protocatechuic acid), 4 cinnamic acids (caffeic acid and its derivatives, chlorogenic acid and ferulic acid), 2 flavan-3-ols ((+)-catechin and (−)-epicatechin) and 2 flavonols (rutin, quercitrin). This phenolic profile is in agreement with the profiles obtained by Pinelli et al. (2013) for kiwi fruits, but the average content in phenolics were higher in the samples studied in the current work. The major individual phenolic was caffeic acid (135.40 mg 100 g^{−1}), followed by *p*-hydroxybenzoic (54.47 mg 100 g^{−1}), (−)-epicatechin (45.16 mg 100 g^{−1}), ferulic acid (39.80 mg 100 g^{−1}) and (+)-catechin (32.61 mg 100 g^{−1}). All main five phenolics account 307.43 mg 100 g^{−1} dry weigh representing 86% of the total phenolics identified. The presence of these five phenolics in kiwi fruit pomaces is high important since they have been associated with important antioxidant, anti-inflammatory, antiaging and anticancer activities (Manach et al. 2004). For instance, caffeic and chlorogenic acids

have been associated with the reduction of human LDL lipoperoxidation induced by cupric ions (Nardini et al. 1995), as well as to be antioxidant (Sato et al. 2011) and anti-hypertensive by reducing blood pressure and some key enzymes involved in the pathogenesis of hypertension (Carmeli and Fogelman 2009). Moreover, Agunloye et al. (2019) found that caffeic and chlorogenic acids may cardio-protective effects due to its inhibition of angiotensin-1-converting enzyme (ACE) cholinesterase and arginase activities. Ferulic acid has been accounted for their antioxidant, anti-inflammatory and anti-diabetic activity (Piazzon et al. 2012). While, rutin have been found effective in the prevention and treatment of colorectal carcinogenesis via attenuation of pro-inflammatory cytokine production (Kwon et al. 2005).

Conclusion

The potential of kiwi fruit pomaces natural donor of phenolic compounds was evaluated. The RSM method showed a good reproducibility of data, and the results indicate that kiwi fruit pomaces from processing industry can be used easily and safely to extract easy caffeic acid and its respective derivatives, catechins and ferulic acid, which have been reported in previous studies as important anti-inflammatory, antioxidant, antiaging and anti-tumoral agents. The next step of experimentation should be the analysis of kiwi byproducts in an industrial and commercial scale to extract phenolics for nutraceutical, pharmaceutical, cosmetic or even other uses.

Table 4 Box-Behnken design arrangement of kiwi pomaces extraction coded with respective factors and responses of Total phenolics content (TPC), total flavonoids content (TFC), DPPH radical scavenging capacity and total antioxidant activity by FRAP activity assays^a

Std	Run	Variables ^b			TPC (mg GAE 100 g ⁻¹ dw)		TFC (mg CAE 100 g ⁻¹ dw)		DPPH (%)		FRAP (μM FeSO ₄ g ⁻¹ dw)	
		X ₁	X ₂	X ₃	Experimental Y _{e1}	Predicted Y _{p1}	Experimental Y _{e2}	Predicted Y _{p2}	Experimental Y _{e3}	Predicted Y _{p3}	Experimental Y _{e4}	Predicted Y _{p4}
9	1	5(0)	30(-1)	10(-1)	688.61	777.97	372.45	372.75	68.56	68.03	46.57	46.63
13	2	5(0)	50(0)	20(0)	4227.83	4227.83	467.39	467.39	72.48	72.48	51.19	51.19
15	3	5(0)	50(0)	20(0)	4227.83	4227.83	467.39	467.39	72.48	72.48	51.19	51.19
12	4	5(0)	70(1)	30(1)	7170.29	7080.92	631.61	631.30	79.22	79.74	62.35	62.29
14	5	5(0)	50(0)	20(0)	4227.83	4227.83	467.39	467.39	72.48	72.48	51.19	51.19
17	6	5(0)	50(0)	20(0)	4227.83	4227.83	467.39	467.39	72.48	72.48	51.19	51.19
2	7	10(1)	30(-1)	20(0)	524.02	238.61	279.55	278.59	60.10	60.41	34.91	34.79
1	8	2(-1)	30(-1)	20(0)	718.29	894.70	403.96	403.00	71.28	71.59	47.28	47.15
5	9	2(-1)	50(0)	10(-1)	5363.92	5098.14	504.26	504.92	76.14	76.35	57.57	57.63
4	10	10(1)	70(1)	20(0)	5730.06	5553.65	480.49	481.45	77.14	76.83	49.59	49.71
6	11	10(1)	50(0)	10(-1)	2833.91	3029.95	353.92	354.58	66.56	66.77	42.32	42.38
11	12	5(0)	30(-1)	30(1)	602.27	621.90	342.17	343.79	65.61	65.51	38.71	38.89
7	13	2(-1)	50(0)	30(1)	4870.00	4673.95	535.35	534.69	73.46	73.25	55.54	55.48
3	14	2(-1)	70(1)	20(0)	7668.32	7953.74	635.65	636.61	78.32	78.01	65.83	65.95
16	15	5(0)	50(0)	20(0)	4227.83	4227.83	467.39	467.39	72.48	72.48	51.19	51.19
10	16	5(0)	70(1)	10(-1)	6712.66	6693.03	523.32	521.70	76.53	76.63	57.12	56.94
8	17	10(1)	30(0)	30(1)	3420.18	3685.96	406.12	405.46	70.68	70.46	42.19	42.13

^aValues are expressed as mean of tree replicates in dry basis^bX₁: pH solvent; X₂: temperature of extraction (°C); X₃: extraction time (min.)

Fig. 1 Predicted response vs. actual response for total phenolics content (TPC) and Total flavonoids content (TFC), DPPH scavenging capacity, FRAP activity

Fig. 2 HPLC chromatograms of kiwifruit pomaces from assay number 14, measured at different wavelength (254 and 280 nm for benzoic acids and flavan-3-ols, 320 nm for cinnamic acids and 370 nm for flavonols)

Table 5 Content of phenolics compounds found in extracts from kiwifruit pomaces extracted at optimum conditions (pH 2, 70°C and 30 min)

Compound	Class type	Retention time (RT) (min)	UV (nm) detection	UV-DAD/VIS bands (nm)	Amount ^a (mg 100 g ⁻¹ dry weight)
<i>p</i> -Hydroxybenzoic acid	Benzoic acid	11.65	254	222, 255	54.47
Protocatechuic acid	Benzoic acid	14.64	254	257, 287	9.63
(+)-Catechin	Flavan-3-ol	15.07	280	278	32.61
Caffeic acid derivative	Cinnamic acid	16.19	320	303sh, 321	22.23
Chlorogenic acid (caffeoylquinic acid)	Cinnamic acid	16.93	320	298sh, 326	9.39
Caffeic acid	Cinnamic acid	17.84	320	291sh, 319	135.40
(-)-Epicatechin	Flavan-3-ol	18.80	280	280	45.16
Ferulic acid	Cinnamic acid	19.60	320	304sh, 326	39.80
Rutin (Quercetin-3- <i>O</i> -rutinoside)	Flavonol	21.64	370	266sh, 353	5.90
Quercitrin (Quercetin-3- <i>O</i> -rhamnoside)	Flavonol	23.76	370	266sh, 346	4.13

^aValues are expressed as mean of three replicates

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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