



## Patata Rossa di Colfiorito IGP (*Solanum tuberosum*, L.) and health-promoting potentialities: Do cooking techniques and storage affect chemical profile and antioxidant activity?

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### ABSTRACT

Acting as ‘antioxidants’, phenolic compounds have been shown to have a potential protective effect against a wide range of noncommunicable diseases. However, their content in plant-based products – and, in turn, their potential benefits – might be affected by the thermal procedures used in food cooking. In light of this, the aim of this work was a characterization of Patata Rossa di Colfiorito (PRC, an Italian PGI red-skinned potato) and an investigation of the impact of cooking and storage on its bioactive compounds, phenolic content, and antioxidant activity. After the harvesting ( $T_0$ ), samples were analyzed using instrumental analysis (e.g., HPLC-ESI-MS/MS) and a set of chemical assays (total phenolic content, 2,2-Diphenyl-1-picrylhydrazyl – DPPH, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) – ABTS, and oxygen radical absorbance capacity – ORAC assays). Analyses were also performed on boiled, pressure-cooked and one-month-stored ( $T_1$ ) products. A commercial red-skinned potato (RSP) was also analyzed.

Overall, compared with the raw product, both boiling and pressure-cooking led to a significant increase in bioactive compounds (raw: 168.15 mg kg<sup>-1</sup> of fresh matter, FM; boiled: 398.92 mg kg<sup>-1</sup> FM; pressure-cooked: 309.24 mg kg<sup>-1</sup> FM), total phenolic content and antioxidant activity measured by DPPH and ABTS assays.

PRC samples showed higher content in bioactive compounds, TPC values and antioxidant activity compared with commercial RSP (with some sporadic exceptions in ORAC values). Interestingly, one month of domestic storage did not affect the freshly harvested product's features.

In conclusion, these results highlighted the quality of this local product and its property to withstand thermal processing and storage. We believe that the results we have obtained should be taken into consideration by health professionals, at both local and national levels, and that PRC deserves to be further studied to investigate its potential human health benefits, as well.

### 1. Introduction

Patata Rossa di Colfiorito (PRC) is an Italian red-skinned potato, whose name was entered in the European Union Register of Protected Geographical Indications (PGI) in 2015 (European Commission, 2015). PRC tubers are characterized by an elongated oval and irregular shape (diameter: 35 mm, at least), a yellow flesh, and are completely covered

in a typical red skin (Supplementary Figure S1). Farming of PRC is permitted at a minimum altitude of 470 m above sea level, in an area of the Apennines Mountains, which covers part of the eastern Province of Perugia (Umbria Region) and part of the western Province of Macerata (Marche Region) (European Commission, 2014). This area is characterized by a plateau, where farmers have cultivated cereals and legumes for ages. Red-skinned potato is not a traditional crop in this area. Its

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cultivation started quite recently when a Dutch semi-late variety named *desirée* was introduced in the upland and rapidly adapted to its particular environmental conditions (Bevilacqua, 2012). The semi-late sowing allows the local farmers to perfectly integrate its agricultural cycle among the main practices of this area (cereal and hay harvest, and land preparation), decisively contributing to the socio-economic development of this territory (Faticenti, 2013). Soil and pedoclimatic conditions contribute to the characteristics of this product. The area has a mountain climate, with cold and snowy winters and cool summers with wide day/night temperature excursions. Precipitation is abundant at higher altitudes (generally, more than 1500 mm *per annum*) but decreases at lower altitudes. Moreover, the area is characterized by siliceous and permeable soils with a large presence of rocks and stones, whose features influence tubers' irregular shape, and their firm flesh. Locally, in addition to the usual uses (e.g., boiled, roasted, etc.), it is highly appreciated as an ingredient in the preparation of various local dishes, namely *focaccia*, *ciambelle* and *gnocchi*.

In general, potato is a highly nutritious, low-fat, low-sodium and gluten-free crop which represents a source of vitamins (vitamin C and B6), minerals (iron and potassium) and other bioactive molecules with potential human health benefits (Ms, 2023; Priya & Saiprasad, 2022; Singh et al., 2021). Based on a systematic review on its impact on non-communicable diseases, it was included in the Mediterranean Diet Pyramid proposed for the Italian population (3 servings a week, 1 serving = 100 g) (D'Alessandro et al., 2019). However, potato's chemical features and biological activity are dependent on genotype, environmental conditions, storage and cooking conditions (Priya & Saiprasad, 2022), and generalizations might sound inappropriate. In this context, the characterization of local ecotypes draws attention to the quality of specific products and provides accurate information to nutritionists and health professionals.

Among bioactive phytochemicals, phenolic compounds seem to play a crucial role in oxidative stress modulation, which represents the basis of many pathological conditions, as they act as 'antioxidants'. According to Halliwell and Gutteridge, an antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule" (Halliwell & Gutteridge, 2015). Phenolic compounds have been shown to have a potential protective effect against a wide range of noncommunicable diseases (NCDs), predominantly cardiovascular diseases, cancers, respiratory diseases and diabetes (Armas Díaz et al., 2023; Blekkenhorst et al., 2018; Cicero & Colletti, 2016; Del Bo' et al., 2019; Echeverría & Valenzuela, 2022; Howes & Simmonds, 2014; Serio et al., 2023; Surh, 2003).

Despite PRC's local popularity, and its officially recognized quality, it is still poorly investigated in terms of chemical features, including phenolic profile and antioxidant activity. In addition, as potatoes are thermally processed to obtain desirable textures and flavours before being consumed, and the extent of molecule loss during processing is also dependent on the cultivar/landrace's genotype (Priya & Saiprasad, 2022), the effect of cooking procedures is crucial in the context of this product's characterisation. In everyday scenarios, it's common for a portion of acquired tubers to undergo extended storage before consumption. Then, the primary objectives of this study were to identify bioactive compounds in PRC, characterize PRC in terms of total phenolic content (TPC) and antioxidant activity, explore the effects of conventional and pressure boiling methods, as well as domestic storage on the product's features, and compare these findings with the most economical equivalent product (commercial red-skinned potato, RSP) available in mass-market retailers.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All reagents used were of analytical grade. Methanol (MeOH) and sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) were obtained from Carlo Erba Reagenti Srl

(Milan, Italy). Cyanidin-3-glucoside chloride, delphinidin-3,5-diglucoside chloride, delphinidin-3-galactoside chloride, petunidin-3-glucoside chloride, malvidin-3-galactoside chloride, quercetin-3-glucoside and kaempferol-3-glucoside were purchased from PhytoLab (Vestenbergsgreuth, Germany). The remaining 31 analytical standards of the 38 phenolic compounds, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid (Trolox), Folin-Ciocalteu reagent, hydrochloric acid (HCl) (37 %), HPLC-grade methanol and gallic acid (GA) were purchased from Sigma-Aldrich Srl (Milan, Italy). Formic acid (99 %) was obtained from Merck (Darmstadt, Germany). Individual stock solutions of each analyte, at a concentration of 1000 mg L<sup>-1</sup>, were prepared by dissolving pure standards in HPLC-grade methanol and storing them in glass stoppered bottles at 4 °C, except for anthocyanins, which were stored at -15 °C until analysis. Standard working solutions at various concentrations were prepared daily by appropriate dilution of the stock solutions with HPLC-grade methanol. The ABTS Antioxidant Capacity Assay Kit was obtained from Bioquochem S.L. (Llanera, Spain). The OxiSelect™ ORAC Activity Assay Kit was bought from Cell Biolabs, Inc. (San Diego, CA, USA). Deionized water (>18 MΩ cm resistivity) was further purified using a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA).

### 2.2. Plant material, cooking procedures and domestic storage

PRC was propagated by vegetative methods, according to the usual technique (water supply set to 27,600 L water h<sup>-1</sup> - 140 h). Potato seeds - actually, whole tubers - were sowed in April 2018 (43°02'18.9"N 12°55'33.5"E). Tubers were harvested mechanically in September 2018, collected in large, aerated nylon sacks, and immediately delivered for analysis. PRC tubers were identified by experts in Agricultural Genetics at the University of Perugia (Unità di Genetica Agraria e Biotecnologie Genetiche, Dipartimento di Scienze Agrarie, Alimentari e Ambientali) (Supplementary Figure S1). Raw tubers were accurately washed, peeled, diced into 0.5 cm cubes and placed at -20 °C for 24 h. Then, 5 g of samples were homogenized (24,000 rpm, 20 sec) and freeze-dried for 24 h. After that, freeze-dried samples were placed in a conical flask and extracted with 10 mL of 70 % MeOH containing 0.1 % HCl (v/v) using a magnetic stirrer (ca. 13 h) at room temperature. After a sonication cycle (30 min), the mixture was placed in a plastic tube and centrifuged (10,000 × g, 20 min). Then, supernatants were collected, dried using a rotary evaporator (STRIKE 300, Steroglass S.r.L., Perugia, Italy), reconstituted with 10 mL 70 % MeOH containing 0.1 % HCl (v/v) and stored at -20 °C (for two weeks) until use.

To evaluate the effect of common and pressure boiling on phenolic content and antioxidant activity, unpeeled whole tubers were boiled in tap water (2 L) in a common pot for 40 min, or in a pressure-cooker for 20 min. Boiling and pressure-cooking times were chosen - after a series of attempts - as the shortest times which guaranteed tubers' cooking. After cooking, potatoes were peeled and underwent the same above-described procedure.

Sample preparation, cooking techniques and extraction procedures were also applied to the cheapest analogous product sold in mass market retailers, namely a commercial RSP (origin: France; diameter: 40-60 mm).

PRC was also analyzed one month (T<sub>1</sub>) after the harvesting (T<sub>0</sub>), to assess the effect of domestic food storage on product characteristics. Domestic storage conditions were reproduced by keeping tubers in a dark cupboard at room temperature for one month.

### 2.3. HPLC-ESI-MS/MS

HPLC-MS/MS investigations were conducted utilizing an Agilent 1290 Infinity series and a Triple Quadrupole 6420 from Agilent Technology (Santa Clara, CA). The instrument, equipped with an electrospray ionization (ESI) source operating in both negative and positive ionization modes, facilitated seamless single-run polarity switching.

MS/MS parameters for each analyte were optimized through flow injection analysis (FIA) employing 1  $\mu\text{L}$  of a 10  $\text{mg L}^{-1}$  individual standard solution, using Optimizer Software (Agilent). The separation of target compounds was achieved using a Synergi Polar-RP C18 analytical column (250 mm  $\times$  4.6 mm, 4  $\mu\text{m}$ ) from Phenomenex (Cheshire, UK), preceded by a Polar RP security guard cartridge (4 mm  $\times$  3 mm ID). The mobile phase employed a gradient elution mode at a flow rate of 0.8  $\text{mL min}^{-1}$  and consisted of a mixture of (A) water and (B) methanol, both containing 0.1 % formic acid. The mobile phase composition underwent the following variations: 0–1 min, isocratic condition, 20 % B; 1–25 min, 20–85 % B; 25–26 min, isocratic condition, 85 % B; 26–32 min, 85–20 % B. Solvents and solutions passed through a 0.2  $\mu\text{m}$  polyamide filter from Sartorius Stedim (Goettingen, Germany). The injection volume was 2  $\mu\text{L}$ . The column temperature was maintained at 30  $^{\circ}\text{C}$ , while the ionization source drying gas temperature was set at 350  $^{\circ}\text{C}$ . Gas flow, nebulizer pressure, and capillary voltage were 12  $\text{L min}^{-1}$ , 55 psi, and 4000 V, respectively. Detection was performed in the dynamic-multiple reaction monitoring (dynamic-MRM) mode, and the dynamic-MRM peak areas were integrated for quantification. The most abundant product ion was used for quantitation, and the others for qualification. The specific time window for each compound ( $\Delta$  retention time) was set at 2 min (Mustafa et al., 2022; Santanatoglia et al., 2023a; Santanatoglia et al., 2023b). The selected ion transitions and the mass spectrometer parameters for the analyzed compounds are reported in Supplementary Table S1.

Before the HPLC-ESI-MS/MS analysis, each sample was sonicated for 30 min (FALC, Treviglio) at 40 kHz (80 % amplitude, 25  $^{\circ}\text{C}$ ), transferred in a 25 mL tube and centrifuged twice. Finally, the supernatant was collected and filtered using a Phenex<sup>TM</sup> RC 4 mm 0.2  $\mu\text{m}$  syringeless filter, Phenomenex (Castel Maggiore, Italy) and addressed to HPLC-ESI-MS/MS analysis.

#### 2.4. Total phenolic content

Total Phenolic Content was determined using the procedure described by Moretti and co-workers (Acito, Palomba et al., 2022), based on the Folin-Ciocalteu method. Quantification rested on a calibration curve generated using gallic acid (GA) (10–500  $\mu\text{g mL}^{-1}$ ) as standard compound. Briefly, 25  $\mu\text{L}$  of GA or potato extract was allowed to react with 125  $\mu\text{L}$  0.2 M Folin-Ciocalteu reagent in a 96-well plate (10 min, room temperature). Subsequently, 125  $\mu\text{L}$  of 2 % (*m/v*) sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution was added and incubated for 30 min. Absorbance was recorded at 765 nm using an Infinite<sup>®</sup> 200 PRO microplate reader (Tecan Italia Srl, Milan, Italy), and results were expressed as mg of GA equivalents (GAE) per gram of fresh (FM) matter (mg GAE  $\text{g}^{-1}$  FM).

#### 2.5. DPPH assay

The radical scavenging capacity of the potato extracts was assessed by carrying out a previously described method, with minor modifications (Acito et al., 2023). Briefly, 25  $\mu\text{L}$  of potato extract or Trolox standard solutions (125–2000  $\mu\text{M}$ ) were allowed to react with 200  $\mu\text{L}$  of DPPH solution (methanolic solution, 350  $\mu\text{M}$ ) in a 96-well plate for 30 min at room temperature. Absorbance was then read at 517 nm using a Sunrise microplate reader (Tecan Italia Srl, Milan, Italy). The results were expressed as  $\mu\text{mol}$  Trolox equivalent (TE) per gram of FM ( $\mu\text{mol TE g}^{-1}$  FM).

#### 2.6. ABTS assay

In this study, the assay was performed on 96-well plates using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ABTS Antioxidant Capacity Assay Kit (Bioquochem S.L., Llanera, Spain), following the manufacturer's instructions. Absorbance was recorded at 734 nm using an Infinite<sup>®</sup> 200 PRO microplate reader (Tecan Italia Srl, Milan, Italy). The inhibition (%) of absorbance was calculated as

follows:

$$\text{Inhibition (\%)} = \left[ 1 - \left( A_f/A_0 \right) \right] \times 100$$

where  $A_0$  is the absorbance of the control (uninhibited radical cation), and  $A_f$  is the absorbance of the sample after 5 min of incubation. The results were expressed as  $\mu\text{mol}$  vitamin C equivalent (CE) per gram of FM ( $\mu\text{mol CE g}^{-1}$  FM), based on a calibration curve generated using an aqueous solution of ascorbic acid (0–600  $\mu\text{M}$ ) (inhibition of Abs of standards plotted as function of their concentrations).

#### 2.7. ORAC assay

The oxygen radical absorbance capacity (ORAC) assay was carried out on a 96-well plate using the OxiSelect<sup>TM</sup> ORAC Activity Assay Kit (Cell Biolabs, Inc. San Diego, CA, USA). Fluorescein was used as the fluorescent probe. Fluorescence was recorded at 37  $^{\circ}\text{C}$  every 5 min for a total of 60 min (excitation wavelength, 480 nm; emission wavelength, 520 nm) using an Infinite<sup>®</sup> 200 PRO microplate reader (Tecan Italia Srl, Milan, Italy). The standard curve was generated using Trolox (0–50  $\mu\text{M}$ ). The results were calculated by plotting the net area under the curve against the Trolox (standard) concentration and were expressed as  $\mu\text{mol}$  Trolox equivalent (TE) per gram of FM ( $\mu\text{mol TE g}^{-1}$  FM).

#### 2.8. Statistical analysis

Tests were performed on PRC coming from two different sacks (replicates) provided by producers. For each sack, the sample analyzed was obtained by mixing material coming from three tubers. Analogously, commercial RSP was analyzed using six tubers coming from two different sacks (replicates) purchased in the same shop. For each replicate, TPC and DPPH assays were carried out in triplicate (replications in 96-well plate), whereas ABTS and ORAC assays were carried out in duplicate (replications in 96-well plate). The results were expressed as mean values  $\pm$  standard error of the mean (SEM). Data were analyzed with One-Way ANOVA, and post hoc analysis was carried out with the Dunnett and Bonferroni tests. Values of  $p < 0.05$  were considered as statistically significant. All calculations were performed using SPSS software (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.). GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA) was used as the graphing software.

### 3. Results

#### 3.1. HPLC-ESI-MS/MS

Results are shown in Table 1. In PRC, the major bioactive compounds were: chlorogenic acid (238.77–139.28  $\text{mg kg}^{-1}$  FM), and neochlorogenic acid (119.29–17.91  $\text{mg kg}^{-1}$  FM). Other detected compounds included: caffeic acid (29.64–2.19  $\text{mg kg}^{-1}$  FM), rutin (12.48–0.25  $\text{mg kg}^{-1}$  FM), ferulic acid (3.74–0.97  $\text{mg kg}^{-1}$  FM) and ellagic acid (2.83–0.00  $\text{mg kg}^{-1}$  FM). With only a few exceptions, the highest amounts of bioactive compounds were detected in cooked samples, with a substantial increase when compared with the raw samples. Increases in chlorogenic and neochlorogenic acid upon boiling can be attributed to improved extractability of phenolic compounds due to the breakdown of cell walls at high temperatures (Joly et al., 2020). Additionally, the migration of these compounds from the peel to the internal tissues during cooking and potential chemical transformations under boiling conditions may also contribute to this increase (Sukrasno and Kusumardiyani, 2014). For example, in PRC at  $T_0$  chlorogenic acid (the most abundant bioactive compound) rose from 139.48  $\text{mg kg}^{-1}$  FM in the raw sample to 238.77  $\text{mg kg}^{-1}$  FM and 173.72  $\text{mg kg}^{-1}$  FM in boiled and pressure-cooked samples, respectively. For this compound, values at  $T_1$  are comparable to those observed at  $T_0$ . Analogous trends were also observed for neochlorogenic acid, the second most abundant

**Table 1**Contents of bioactive compounds (mg kg<sup>-1</sup> FM) in analyzed samples determined by HPLC-ESI-MS/MS.

Compound (mg kg <sup>-1</sup> FM)	Patata Rossa di Colfiorito (T <sub>0</sub> )			Patata Rossa di Colfiorito (T <sub>1</sub> )			Commercial red-skinned potato		
	Raw	Boiled	Pressure-cooked	Raw	Boiled	Pressure-cooked	Raw	Boiled	Pressure-cooked
Gallic acid	0.14 <sup>a</sup>	0.14 <sup>a</sup>	0.16 <sup>a</sup>	0.13 <sup>a</sup>	0.06 <sup>a</sup>	0.11 <sup>a</sup>	n.d.	0.05 <sup>a</sup>	0.05 <sup>a</sup>
Neochlorogenic acid	17.91 <sup>b</sup>	113.96 <sup>b</sup>	104.54 <sup>b</sup>	28.26 <sup>b</sup>	109.27 <sup>b</sup>	119.29 <sup>b</sup>	1.04 <sup>a</sup>	36.21 <sup>b</sup>	31.43 <sup>b</sup>
Delphinidin 3-galactoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Catechin	0.10 <sup>a</sup>	0.09 <sup>a</sup>	0.15 <sup>a</sup>	0.07 <sup>a</sup>	n.d.	0.04 <sup>a</sup>	n.d.	n.d.	n.d.
procyanidin B2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chlorogenic acid	139.48 <sup>c</sup>	238.77 <sup>c</sup>	173.72 <sup>c</sup>	139.28 <sup>c</sup>	206.88 <sup>c</sup>	164.92 <sup>c</sup>	21.47 <sup>b</sup>	99.12 <sup>c</sup>	180.19 <sup>c</sup>
<i>p</i> -Hydroxy benzoic acid	0.57 <sup>a</sup>	0.37 <sup>a</sup>	0.36 <sup>a</sup>	0.53 <sup>a</sup>	0.43 <sup>a</sup>	0.98 <sup>a</sup>	0.38 <sup>a</sup>	0.65 <sup>a</sup>	0.35 <sup>a</sup>
Epicatechin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cyanidin-3-glucoside	0.02 <sup>a</sup>	0.05 <sup>a</sup>	0.05 <sup>a</sup>	n.d.	0.02 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.
Petunidin-3-glucoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-Hydroxy benzoic acid	0.86 <sup>a</sup>	0.68 <sup>a</sup>	0.54 <sup>a</sup>	0.87 <sup>a</sup>	0.67 <sup>a</sup>	1.57 <sup>a</sup>	0.78 <sup>a</sup>	0.93 <sup>a</sup>	0.66 <sup>a</sup>
Caffeic acid	2.19 <sup>d</sup>	22.11 <sup>d</sup>	18.81 <sup>d</sup>	2.51 <sup>d</sup>	16.15 <sup>d</sup>	29.64 <sup>d</sup>	0.44 <sup>a</sup>	19.90 <sup>d</sup>	28.76 <sup>d</sup>
Vanillic acid	1.10 <sup>d</sup>	n.d.	n.d.	1.12 <sup>a</sup>	n.d.	n.d.	0.63 <sup>a</sup>	n.d.	0.46 <sup>a</sup>
Resveratrol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pelargonidin-3-glucoside	n.d.	0.01 <sup>a</sup>	n.d.	n.d.	0.02 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.
Pelargonidin-3-rutinoside	0.01 <sup>a</sup>	1.30 <sup>a</sup>	0.13 <sup>a</sup>	n.d.	1.08 <sup>a</sup>	0.40 <sup>a</sup>	n.d.	n.d.	0.22 <sup>a</sup>
Malvidin-3-galactoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Syringic acid	0.08 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	0.14 <sup>a</sup>	n.d.	n.d.
Procyanidin A2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>p</i> -Coumaric acid	0.09 <sup>a</sup>	0.36 <sup>a</sup>	0.23 <sup>a</sup>	0.09 <sup>a</sup>	0.42 <sup>a</sup>	0.61 <sup>a</sup>	0.07 <sup>a</sup>	0.10 <sup>a</sup>	0.32 <sup>a</sup>
Ferulic acid	0.97 <sup>a</sup>	3.61 <sup>c</sup>	1.99 <sup>c</sup>	1.69 <sup>d</sup>	3.15 <sup>e</sup>	3.74 <sup>e</sup>	1.72 <sup>c</sup>	3.34 <sup>e</sup>	6.05 <sup>e</sup>
3,5-Dicaffeoylquinic acid	0.40 <sup>a</sup>	1.82 <sup>a</sup>	1.32 <sup>c</sup>	0.59 <sup>a</sup>	2.93 <sup>e</sup>	2.04 <sup>e</sup>	n.d.	0.08 <sup>a</sup>	0.04 <sup>a</sup>
Rutin	1.07 <sup>a</sup>	12.48 <sup>d</sup>	4.03 <sup>c</sup>	3.26 <sup>d</sup>	0.25 <sup>a</sup>	1.02 <sup>a</sup>	0.12 <sup>a</sup>	0.02 <sup>a</sup>	0.80 <sup>a</sup>
Hyperoside	0.20 <sup>a</sup>	0.26 <sup>a</sup>	0.37 <sup>a</sup>	1.38 <sup>d</sup>	0.12 <sup>a</sup>	0.30 <sup>a</sup>	n.d.	n.d.	1.38 <sup>a</sup>
Isoquercitrin	0.07 <sup>a</sup>	0.11 <sup>a</sup>	0.14 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Delphinidin 3,5 diglucoside	0.05 <sup>a</sup>	0.09 <sup>a</sup>	0.12 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phloridzin	n.d.	0.10 <sup>a</sup>	0.03 <sup>a</sup>	0.01 <sup>a</sup>	0.03 <sup>a</sup>	0.04 <sup>a</sup>	n.d.	0.01 <sup>a</sup>	0.01 <sup>a</sup>
Naringin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercitrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Myricetin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Kaempferol-3-glucoside	0.01 <sup>a</sup>	0.05 <sup>a</sup>	0.04 <sup>a</sup>	n.d.	0.04 <sup>a</sup>	0.04 <sup>a</sup>	n.d.	n.d.	n.d.
Hesperidin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ellagic acid	2.83 <sup>d</sup>	2.56 <sup>e</sup>	2.32 <sup>e</sup>	2.48 <sup>d</sup>	n.d.	0.48 <sup>a</sup>	5.86 <sup>c</sup>	3.20 <sup>e</sup>	2.78 <sup>e</sup>
<i>trans</i> -cinnamic acid	n.d.	n.d.	0.19 <sup>a</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	0.07 <sup>a</sup>
Quercetin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phloretin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isorhamnetin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01 <sup>a</sup>	n.d.	n.d.
<b>Total (mg kg<sup>-1</sup> FM)</b>	<b>168.15</b>	<b>398.92</b>	<b>309.24</b>	<b>182.27</b>	<b>341.52</b>	<b>325.22</b>	<b>32.66</b>	<b>163.61</b>	<b>253.57</b>

n.d., not detectable. Relative standard deviation (RSD) for all compounds ranged from 2.26 to 7.87 %.

Within each cooking procedure, analytes not sharing same letters are statistically significantly different (ANOVA, *p*-value < 0.05).

bioactive compound.

Overall, in both PRC and commercial RSP, boiling and pressure-cooking led to an increase in the total content of the analyzed bioactive compounds. Furthermore, both before and after cooking procedures, and also after one month of domestic storage (T<sub>1</sub>), PRC showed higher content in bioactive compounds than commercial RSP.

### 3.2. Total phenolic content

Total Phenolic Content of PRC and commercial RSP are shown in Fig. 1 and Supplementary Table S2. In almost each red-skinned potato sample (both PRC and commercial one), both boiling and pressure-cooking led to a significant increase in TPC. At T<sub>0</sub>, PRC raw and cooked samples showed significantly higher TPC values than commercial RSP. In particular, in fresh raw PRC, TPC values were 0.25 mg GAE g<sup>-1</sup> FM, whereas in fresh commercial potato samples, TPC was 0.14 mg GAE g<sup>-1</sup> FM. In boiled PRC, TPC values were 0.29 mg GAE g<sup>-1</sup> FM, whereas, in boiled commercial RSP, TPC values were 0.15 mg GAE g<sup>-1</sup> FM. Pressure-cooked PRC (0.32 mg GAE g<sup>-1</sup> FM) showed significantly higher TPC values than commercial one (0.22 mg GAE g<sup>-1</sup> FM), as well. A one-month domestic storage was shown to significantly affect phenolic content only in pressure-cooked products (-13.75 %).

#### DPPH assay

DPPH values of PRC and commercial RSP are presented in Fig. 2 and Supplementary Table S3. In each sample (both PRC and commercial

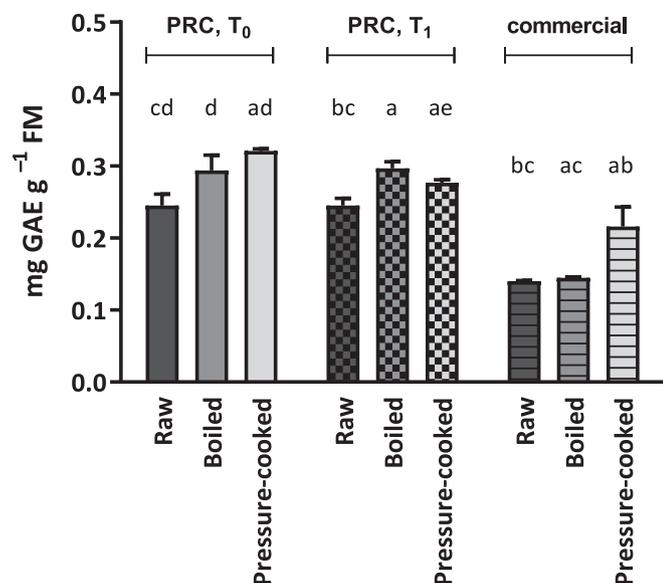
RSP), both thermal processing procedures led to a significant increase in DPPH values. At T<sub>0</sub>, PRC raw and cooked samples showed significantly higher antioxidant activity than commercial RSP. In particular, in fresh raw PRC, DPPH values were 0.46 μmol TE g<sup>-1</sup> FM, whereas in fresh commercial RSP were 0.09 μmol TE g<sup>-1</sup> FM. In boiled PRC, DPPH values were 0.96 μmol TE g<sup>-1</sup> FM, whereas in boiled commercial samples DPPH values were 0.32 μmol TE g<sup>-1</sup> FM. Pressure-cooked PRC (1.04 μmol TE g<sup>-1</sup> FM) showed significantly higher DPPH values than commercial one (0.61 μmol TE g<sup>-1</sup> FM), as well. After one month of domestic storage in the dark, neither raw nor cooked samples were affected in terms of DPPH values.

#### ABTS assay

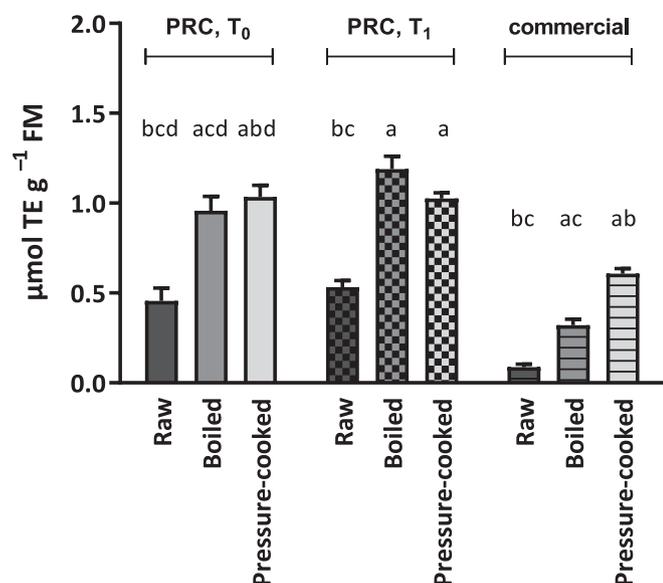
ABTS values of PRC and commercial RSP are summarized in Fig. 3 and Supplementary Table S4. PRC showed ABTS values of 0.89, 1.33 and 0.92 μmol CE g<sup>-1</sup> FM in raw, boiled and pressure-cooked samples, respectively. These values are significantly higher than those observed in commercial samples. Moreover, one month of domestic storage did not affect antioxidant activity measured with ABTS assay in PRC. Overall, ABTS values are higher in cooked products than in raw samples.

#### ORAC assay

ORAC values are shown in Supplementary Table S5. Antioxidant activity assessed by this assay was affected by cooking procedures in some samples (boiled one-month-stored PRC, boiled commercial RSP) whereas in other samples was not (PRC at T<sub>0</sub>). In PRC, one month of



**Fig. 1.** Total Phenolic Content of Patata Rossa di Colfiorito (PRC) IGP at T<sub>0</sub> and T<sub>1</sub>, and of commercial red-skinned potato at T<sub>0</sub>. The results are expressed as the mean ± standard error of the mean. Statistical significance:  $p < 0.05$ . <sup>a</sup> vs raw; <sup>b</sup> vs boiled; <sup>c</sup> vs pressure-cooked; <sup>d</sup> vs commercial red-skinned potato; <sup>e</sup> vs T<sub>0</sub>.



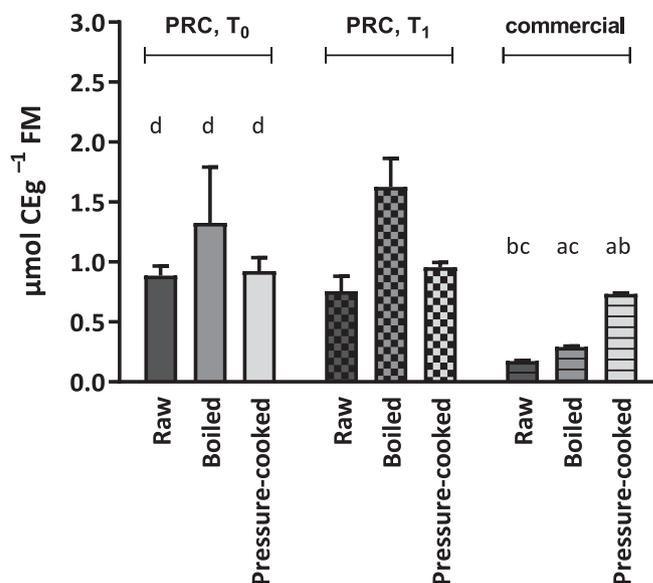
**Fig. 2.** Antioxidant activity of Patata Rossa di Colfiorito (PRC) IGP at T<sub>0</sub> and T<sub>1</sub>, and of commercial red-skinned potato at T<sub>0</sub> assessed by DPPH assay. The results are expressed as the mean ± standard error of the mean. Statistical significance:  $p < 0.05$ . <sup>a</sup> vs raw; <sup>b</sup> vs boiled; <sup>c</sup> vs pressure-cooked; <sup>d</sup> vs commercial red-skinned potato.

domestic storage did not affect antioxidant activity, but, on the contrary, led to a slight – but significant – increase in ORAC values.

#### 4. Discussion

The aim of this study was to carry out a preliminary description of PRC bioactive compounds, total phenolic content and antioxidant properties, not only as a raw product, but also after cooking and storing it in domestic conditions.

Chlorogenic acid was found to be the major bioactive compound, in line with previous works on potatoes (Cebulak et al., 2022; Furrer et al.,



**Fig. 3.** Antioxidant activity of Patata Rossa di Colfiorito (PRC) IGP at T<sub>0</sub> and T<sub>1</sub>, and of commercial red-skinned potato at T<sub>0</sub> assessed by ABTS assay. The results are expressed as the mean ± standard error of the mean. Statistical significance:  $p < 0.05$ . <sup>a</sup> vs raw; <sup>b</sup> vs boiled; <sup>c</sup> vs pressure-cooked; <sup>d</sup> vs commercial red-skinned potato.

2017; Makori et al., 2022; Vaitkevičienė et al., 2020). Several studies have indicated a correlation between chlorogenic acid consumption and a lower risk of metabolic syndrome and various chronic diseases via several promising health-related properties (antimicrobial, antioxidant, neuroprotective, cardiovascular protective, anti-hypertensive, gastrointestinal protective, renoprotective, hepatoprotective, glycemic control agent, anti-obesity agent, anti-inflammatory anticarcinogenic effects) (Kumar et al., 2020; Lu et al., 2020). Furthermore, also neochlorogenic acid and caffeic acid (i.e., other bioactive compounds detected in PRC) have shown interesting activities in previous studies. Several pieces of evidence have demonstrated that neochlorogenic acid exhibits antibacterial and anti-inflammatory activity, as well as an ability to modulate lipid metabolism (Bajko et al., 2016; Huang et al., 2015; Yu et al., 2021), whereas caffeic acid was shown to possess antioxidant, anti-inflammatory, anticancer, and neuroprotective effects (Alam et al., 2022). Interestingly, the concentrations of such compounds were higher in cooked potatoes than in raw ones. Overall, both boiling and pressure-cooking led to an increase in total bioactive compounds concentration, TPC and antioxidant activity measured by DPPH and ABTS assays. These results confirmed other studies reporting that cooked potatoes showed higher levels of some phenolics, TPC and/or antioxidant activity than raw ones (Navarre et al., 2010). This trend could be attributed to different mechanisms: firstly, heating treatments are assumed to improve the extractability of phenolic compounds from the cellular matrix, compared with uncooked samples (Blessington et al., 2010; Juárez et al., 2016). Second, a potential migration of phenolic compounds from the peel to the internal tissues of potatoes can occur during the cooking process (Mondy & Gosselin, 1988). Peel is recognized, indeed, as a source of phenolic and bioactive compounds and other phytochemicals (Albishi et al., 2013; Friedman et al., 2017; Silva-Beltrán et al., 2017). In this study, whole potatoes were cooked with an unbroken peel, and this expedient may have prevented the loss of compounds in cooking water, as reported in other works (Mulinacci et al., 2008; Navarre et al., 2010). Interestingly, vanillic acid was detected in potatoes cooked under pressure but not in those cooked under normal pressure. This observation can be explained by the fact that pressure cooking might promote the release or transformation of bound phenolic compounds more effectively than normal pressure

cooking (Yu et al., 2021). The higher temperature and pressure conditions during pressure cooking could facilitate the breakdown of complex phenolic structures, leading to the formation of vanillic acid (Wan et al., 2022). Additionally, vanillic acid might be present in bound forms that are more readily released under pressure cooking conditions (Noubigh & Abderrabba, 2016).

Other studies found opposite trends, namely a reduction of TPC and antioxidant activity after cooking procedures (Lachman et al., 2013; Kamrunnaher et al., 2019; Fang et al., 2022). However, in those cases, different from our study, potatoes were cut before boiling. This step might be at the basis of the observed losses.

Although we obtained clear results on the enhancement of PRC properties after both thermal treatments, our findings do not allow to draw any conclusion on the best cooking procedure. Interestingly, post-cooking improvement is maintained at T<sub>1</sub>, as well, testifying to an overall high quality of this local product.

Several reports have assessed TPC and antioxidant activity of other RSP samples. Geronimo and co-workers reported TPC values of 0.076 mg GAE/g<sup>-1</sup> FM for a steamed red-skinned and yellow flesh cultivar, *i.e.* ca. 4-fold lower than values we obtained for cooked PRC (Rumbaoa et al., 2009). In another study, TPC values that ranged from 1.56 to 2.09 and from 1.06 to 1.79 mg GAE g<sup>-1</sup> of freeze-dried weight were reported for four raw and boiled RSP cultivars, respectively (Xu et al., 2009). However, in addition to cooking techniques, TPC values strongly depend on potato genotype, extraction procedures and standard antioxidants used for calibration (Rumbaoa et al., 2009). Therefore, results from studies analyzing highly different cultivars, and, particularly, using highly different laboratory procedures, should be compared with caution.

PRC has been demonstrated to show interesting features in another work, as well. In this study (Marchettini et al., 2013), the content of acrylamide – classified in Group 2A (probably carcinogenic to humans) by the International Agency for Research on Cancer (IARC, 1994) – was analyzed in three different cultivars (PRC, Quarantina Bianca Genovese and Kennebec). Potato slices were fried (5 min at 170 °C) in different oils. When PRC was fried in extra virgin olive oil, acrylamide content was 304.19 ng g<sup>-1</sup>, in mixed seed oil was 286.47 ng g<sup>-1</sup>, in peanuts oil was 161.58 ng g<sup>-1</sup>. These values were always lower than those observed in Kennebec potatoes – the most common cultivar in Italy – especially when potatoes were fried in peanut oil (10-fold higher in Kennebec, 1616.15 ng g<sup>-1</sup> vs 161.58 ng g<sup>-1</sup>). This is quite remarkable, as there is an association between acrylamide intake and the risk of kidney cancer, and endometrial and ovarian cancers in never smokers (Pelucchi et al., 2015), and since consumption of fried potatoes is well diffused among the young population. Although frying does not represent the ideal cooking technique in terms of impact on consumers' health – boiling or pressure-cooking unpeeled tubers is to be preferred, with null or extremely low levels of acrylamide detected (Biedermann et al., 2002; Gökmen, 2023; Rifai & Saleh, 2020) – this information, along with the results obtained in our study, should be strongly taken into consideration by nutritionists and health communication professionals. Even though potato consumption may be limited or avoided in some particular populations (Acito, Natalucci, et al., 2023; Acito, Rondini et al., 2022; Beals, 2019), based on our results, we believe that, in general population, boiled or pressure-cooked PRC (cooked in the presence of the peel) could represent a precious alternative to commercial RSP that can be weekly consumed as the main source of carbohydrates within a meal. Moreover, given its gluten-free nature, it can be considered one of the most important healthy sources of carbohydrates for individuals with celiac disease.

A future research line that might be pursued could lie in the implementation of a set of *in vitro* assays with the aim of assessing the biological activity of this product's extract (Acito, Russo et al., 2022; Villarini et al., 2021).

## 5. Conclusions

In conclusion, these results highlighted the features of this product and described to what extent routine procedures affected its quality. Interestingly, with only a few exceptions, both boiling and pressure-cooking led to an increase in bioactive compounds, phenolic content and antioxidant activity. Compared with commercial RSP, PRC showed a higher content of bioactive and phenolic compounds and antioxidant activity. Moreover, PRC was also shown to keep these features unchanged also after one month of domestic storage. In light of the above, we believe that this information should be taken into consideration by nutritionists, at both local and national levels. This product's consume definitely deserves to trespass the regional context, and to be further studied to investigate its potential human health benefits, as well.

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## CRedit authorship contribution statement

**Mattia Acito:** Writing – original draft, Methodology, Investigation, Data curation. **Agnese Santanatoglia:** Methodology, Investigation, Data curation. **Cristina Fatigoni:** Investigation. **Milena Villarini:** Data curation. **Giovanni Caprioli:** Supervision, Data curation. **Gianni Sagratini:** Supervision, Data curation. **Iolanda Grappasonni:** Supervision, Funding acquisition. **Massimo Moretti:** Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its [supplementary materials](#).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jff.2024.106380>.

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