Anthocyanin-rich extracts from purple and red potatoes as natural colourants: Bioactive properties, application in a soft drink formulation and sensory analysis

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A R T I C L E   I N F O

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Solanum tuberosum L.

A B S T R A C T

Aqueous extracts from seven coloured potato varieties (three red-fleshed, three-purple fleshed, and one marble-fleshed) were studied for their anthocyanin content, in vitro biological activities, colouring properties and their potential application in the food industry. Acylated glycosides or pelargonidin and petunidin aglycones were identified as the main anthocyanin forms in the red and purple varieties, respectively. The total anthocyanin content among varieties ranged from 478.3 to 886.2 mg/100 g extract. All the extracts presented in vitro antioxidant, antibacterial and antifungal activities, whereas no toxic effects were detected. Finally, two selected extracts were tested as colourants in a soft drink formulation and presented suitable sensory profiles as well as high colour stability during a 30-day shelf-life when compared with the commercial colourant E163. Therefore, the tested extracts could be used as natural food colourants and considered for substituting the existing synthetic colouring agents.

1. Introduction

There is a growing interest worldwide in the development of food colourants from natural sources as a consequence of perceived consumer preferences and the concerns about the use of synthetic compounds in food products (Albuquerque, Pinela, Barros, Oliveira, & Ferreira, 2020; Petropoulos et al., 2019). In response to that, the food and beverage industries have been seeking natural colouring agents that could substitute synthetic dyes and colouring additives and increase the beneficial health effects of the final product due to their high content in bioactive compounds (Petropoulos et al., 2019; Sampaio et al., 2020b; Schieber, Stintzing, & Carle, 2002).

Coloured root vegetable products can be alternative sources of colouring and bioactive compounds (Hossain, Rawson, Aguiló-Aguayo, Brunton, & Rai, 2015; Némí & Peksa, 2018). Among root vegetables, potato (Solanum tuberosum L.) is the most consumed worldwide, being a staple for 1.3 billion people and with an increasing popularity in the developing world (Stokstad, 2019). This nutritious vegetable also presents the highest genetic diversity among all cultivated species, with approximately 5000 registered varieties and a broad phenological variation in terms of flesh and skin colour (Petropoulos et al., 2019). Red and purple-fleshed potatoes are rich in phenolic compounds, particularly in anthocyanins, presenting about three times higher amounts of total phenolic compounds content than the widely consumed white- and yellow-fleshed tubers, as well as two to three times higher antioxidant activity (Petropoulos et al., 2019). Anthocyanins are suitable to be used as natural colouring compounds due to their bright attractive red and purple colours and water solubility that allows for their easy
incorporation into aqueous food systems (Rodriguez-Saona, Giusti, & Wrolstad, 2008; Rodriguez-Saona, Giusti, & Wrolstad, 1999). Nevertheless, their use can pose problems of colour instability when they are out of their natural environment, as affected by factors such as pH, temperature, light and oxygen or interactions with other coexisting components, among others (Santos-Buelga & González-Paramás, 2019). The anthocyanin molecules found in coloured potatoes are usually acylated with p-coumaric or ferulic acids. The acylation of the anthocyanin molecules improves pigment stability during processing and storage (Rodriguez-Saona, Giustil, & Wrolstad, 1998). This effect is chemically explained by the stacking of the acyl groups with the pyrilmium ring of the flavilium cation, which reduces the susceptibility of the nucleophile attack of water on the hydrophilic anthocyanin molecules and the formation of a colourless pseudobase and light yellow chalcone forms (Sasaki, Nishizaki, Ozeki, & Miyahara, 2014; Santos-Buelga & González-Paramás, 2019).

Natural anthocyanins have powerful colouring capacities and at acidic pH values only small concentrations of these compounds are required to obtain the desired red, pink and purple colours in food products (Mateus & Freitas, 2009). Moreover, these compounds are associated with several health effects that could improve the overall quality of the final product, while they could allow the design of new functional and natural products that are highly appreciated by the market (Quan et al., 2019; Wrolstad & Culver, 2012). The list of applications of anthocyanins as colouring agents is long, comprising products such as fruit preservatives (Bursav Kovacević et al., 2015), sugar confectionary (Mateus & Freitas, 2009), dairy products (Montibeller, de Lima Monteiro, Tupuna-Yerovi, Rios, & Manfrei, 2018; Pires et al., 2018), dry mixes (acid dessert mixes and drink powders) (Mateus & Freitas, 2009), frozen products (ice creams) (Mateus & Freitas, 2009), bakery products (Albuquerque et al., 2020; Da Silva et al., 2019a; Da Silva et al., 2019b) and beverages (Monteiro et al., 2017; Montibeller et al., 2018). Among all possible applications, soft drinks are suitable for an application study by incorporating their extracts in a pasteurised soft drink model. Moreover, considering the current market trends and consumer preferences for food products free of synthetic compounds we aimed at evaluating the potential use of the tested extracts as natural colouring agents in commercial products. Therefore, the sensory profile of the pasteurised soft drink was assessed by a panel experiment.

In this work we present an in-depth characterisation of the anthocyanin profile and the bioactivities (in vitro antioxidant, antibacterial and antifungal activities) of aqueous extracts from six varieties of red and purple-fleshed potatoes, as well as a marble-fleshed cultivar being cultivated in the experimental farm of the University of Thessaly in Velestino, Greece, according to commercial cultivation protocols during the spring-summer growing period of 2018. Seed tubers were planted manually on 3/4/2018 with distances of 75 cm between hills and 30 cm within each hill and approximately 10 cm depth (Petropoulos et al., 2020). Fertilizers were applied with base-dressing (400 kg/ha of 15–15–15 (N–P–K fertilizer) and two side-dressings (1st: 50 kg/ha of ammonium nitrate; 2nd 50 kg/ha of potassium nitrate). Irrigation was applied via a sprinkler irrigation system, while weeds and pests were controlled according to the best practice guides for the crop. Tubers were harvested manually on 27/7/2018. The fresh harvested tubers (Fig. 1) were transported to the Polytechnic Institute of Bragança, Portugal, where the samples were washed with cold water upon arrival to eliminate extraneous matter and refrigerated at 4 °C until analysis.

2.2. Extracts preparation

Washed potato tubers were manually peeled and immediately immersed in an aqueous citric acid solution (0.5 mol/L) to prevent browning (1 g fresh tuber/2 mL citric acid solution). After the immersion in the citric acid solution, the whole peeled tubers were gently blended (commercial juice blender, Breville Blend Active model VBL134) and filtered twice (Whatman No. 4 paper). The obtained acidified aqueous extracts (pH ~ 3) were freeze-dried (–40 °C, 0.08 bar, during 48 h, FreeZone 4.5 model 7750031, Labconco, Kansas, USA). All seven resulting extracts presented a yield of approximately 5% (mass of freeze-dried extract/mass of whole fresh potato). All steps in the extraction process were carried out in food grade facilities.

2.3. Determination of anthocyanin content and bioactivities

2.3.1. Chromatographic analysis of anthocyanins

The seven freeze-dried extracts were dissolved in water to a concentration of 50 mg/mL and filtered through 0.22 μm disposable syringe filters into amber vials for high performance liquid chromatography (HPLC) analysis. The analysis was performed using a Dionex Ultimate 3000 HPLC (Thermo Scientific) system equipped with a quaternary pump, an automatic injector (at 5 °C), a degasser, and an automated thermostat column compartment. The compounds detection was carried out with a diode-array detector (DAD), using 520 nm as preferred wavelength, and electrospray ionisation (ESI) coupled to a mass spectrometry (MS) detector (HPLC-DAD-ESI/MS), operating under the conditions described by Albuquerque et al. (2020). Data were collected and analysed using the Xcalibur® program (Thermo Scientific). The identification was performed from the chromatographic retention characteristics and UV–Vis and mass spectra, and comparison with data described in the literature. The quantification was performed using a 7-level calibration curve of peonidin-3-glucoside (y = 151438x – 3E^(-6)), R^2 = 0.9977, LOD = 0.20 μg/mL and LQ = 0.71 μg/mL, Sigma-Aldrich, St. Louis, MO, USA). Results were expressed as mg per 100 g of extract.

2.3.2. Antioxidant activity

The antioxidant activity of the seven extracts was assessed in terms of their potential to inhibit the production of thiobarbituric acid reactive substances (TBARS) in brain homogenates, a cell-based in vitro method previously described by Sampaio et al. (2020a). The formation of the complex malondialdehyde-thiobarbituric acid was measured at 532 nm and the results expressed as IC50 values (mg/mL of extract).

2.3.3. Cytotoxicity evaluation

The seven potato extracts were dissolved in water (4 mg/mL) and submitted to further dilutions. A cell culture (PLP2) was prepared using a freshly harvested porcine liver according to the method described by Correia et al. (2015). The sulphorhamodamine B assay was performed to evaluate any possible cytotoxicity of the extracts. Ellipticine (Sigma-Aldrich, St. Louis, MO, USA) was employed as a positive control and the...
results were expressed in GI\textsubscript{50} values.

2.3.4. Antibacterial and antifungal activities

With the aim to assess the potential use of the potato extracts as natural food preservatives, their antibacterial and antifungal activities were evaluated according to the procedure described by Corrêa et al. (2015). Three Gram-positive bacteria (Bacillus cereus (clinical isolate), Staphylococcus aureus (ATCC 11632) and Listeria monocytogenes (NCTC 7973)) and three Gram-negative bacteria (Escherichia coli (ATCC 25922), Enterobacter cloacae (ATCC 35030) and Salmonella Typhimurium (ATCC 13311)) were used to determine the potential antibacterial activity of the extracts. For the antifungal assays, six micromycetes were tested, namely Aspergillus fumigatus (human isolate), Aspergillus versicolor (ATCC11730), Aspergillus niger (ATCC 6275), Penicillium funiculosum (ATCC 36839), Trichoderma viride (IAM 5061) and Penicillium aurantiogriseum (food isolate). Two commercial food preservatives were used as positive controls: potassium metabisulphite (E224) and sodium benzoate (E211). Results were given as minimal inhibitory, bactericidal and fungicidal concentrations (MIC, MBC and MFC, respectively).

2.4. Validation of the anthocyanin-rich extracts as natural colourants

2.4.1. Soft drink formulation

Following the screening of the seven potato varieties based on their anthocyanin content and bioactive properties, two extracts were selected to be applied as colouring agents in a soft drink formulation: cv. Rosemary (red-fleshed potato variety) and cv. Purple (purple-fleshed potato variety). A typical raspberry flavoured soft drink formulation was prepared, composed of sparkling water, caster sugar, citric acid, sucralose and natural flavourings (raspberry and peach), in decreasing order of concentration. Three soft drink formulations were prepared with (1) the red anthocyanin-rich potato extract (Rosemary), (2) the purple anthocyanin-rich potato extract (Purple) and (3) the commercial colorant E163 (standardised in 1% anthocyanins content). The latter formulation (3) was used as control. The colourants were added to the respective formulations in order to obtain a similar concentration of anthocyanins (~8 mg/L solution), which visually matched the colour intensity of a commercial soft drink product used as reference. The commercial colorant E163 used as control in the soft drink formulations was purchased from Naturex (Derbyshire, UK), consisting of an anthocyanin glucosides’ extract derived from grapes and standardised in 1% of anthocyanins content by the supplier, preserved with potassium sorbate. All soft drink samples were prepared according to good hygiene and manufacturing practices in food grade facilities.

2.4.2. Pasteurisation

The three soft drink formulations (Rosemary, Purple and Control) were poured into glass containers (250 mL), which were sealed and placed in a water bath at 70 ºC for 20 min, following the in-pack pasteurisation method described by Ashurst (2011).

2.4.3. Colour stability and determination of pH

Following pasteurisation, the three soft drink formulations (Rosemary, Purple and Control) were stored at 4 ºC and their colour stability was assessed at 0, 7 and 30 days of storage. A PCE colourimeter (model CSM-3; PCE Instruments, Germany) was used to measure the colour by placing 80 mL of each formulation in a quartz glass box designed for measurements carried out on liquid samples. Using a silicon photodiode sensor and a measuring aperture of 8 mm, the following CIELab colour space readings were measured through the computerized system: L*: lightness from black (0) to white (100); a*: green (−) to red (+); b*: blue (−) to yellow (+); C*: chroma, relative saturation; and h*: hue angle in the CIELab colour wheel.

The pH of the formulated soft drinks was measured using a calibrated digital pH meter (portable food and dairy pH meter HI 99161, Hanna Instruments, Woonsocket, RI, USA). All pH measurements were carried out in triplicate. All three soft drink formulations presented a final pH of approximately 3.

2.4.4. Sensory analysis

A sensory analysis was carried out immediately after the soft drinks’ production and pasteurisation (Day 0). Sessions took place in the sensory suite facility of the Scottish Centre for Food Development and Innovation (SCFDI) at Queen Margaret University (Edinburgh, UK). The test panel participants were informed about the general aim of the study and the required procedures for handling personal data. All participants gave written informed consent prior to participation. Twelve experienced trained panellists were selected and subsequently received specific additional training on the sensory attributes relevant to the soft drink formulation developed in this project using a range of commercial products. Sensory tests were conducted under white light in each booth.

The soft drinks formulated with the two extracts obtained in the present study were compared with those of a control formulation containing the commercial colorant E163. Samples were presented to the panellists in coded clear plastic cups containing 20 mL of each formulation. Panellists were asked to cleanse their palate before the first sample and between samples using water and crackers. After training the following list of sensory attributes was generated and confirmed by the panel: colour intensity, cloudiness, sweetness, fruitiness, bitterness.
and sourness. A quantitative descriptive analysis (QDA) test was designed using the Compunse Cloud software (Compunse, Guelph, ON, Canada). The intensity of perception was scored for each attribute on a scale ranging from 1 (very low) to 9 (very high). Following the QDA questionnaire the panellists were asked to comment on the overall acceptability of each product based on their hedonic experience and indicate their preference among the three products.

2.5. Statistical analysis

Three samples were used for each assay, and all assays were carried out in triplicate. The results were analysed using the one-way analysis of variance (ANOVA) followed by Tukey’s HSD Test (p = 0.05). All data are expressed as mean values and standard deviation (SD). When less than three results were present in each individual analysis, t-Student test was used to determine the significant difference (p = 0.05). The sensory results were analysed using a two-way analysis of variance (ANOVA) followed by a Tukey’s post-hoc Honest Significant Difference (HSD) test using the Compunse Cloud software (p = 0.05).

### Table 1

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rt (min)</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt;(nm)</th>
<th>[M]&lt;sup&gt;+&lt;/sup&gt; (m/z)</th>
<th>MS&lt;sup&gt;2&lt;/sup&gt;(m/z)</th>
<th>Tentative identification</th>
<th>Rosemary</th>
<th>Red Emmaolie</th>
<th>Red Cardinal</th>
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<tbody>
<tr>
<td>1r</td>
<td>8.27</td>
<td>499</td>
<td>579 (100), 433 (15), 271 (45)</td>
<td>128.9 ± 0.3</td>
<td>Pelargonidin-3-O-rutinoside-5-O-glucoside</td>
<td>130 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>264 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>2r</td>
<td>20</td>
<td>501</td>
<td>433 (100), 271 (46)</td>
<td></td>
<td>Pelargonidin-3-O-rutinoside</td>
<td>109 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127.9 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>206.1 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>3r</td>
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<td>725 (100), 433 (5), 271 (16)</td>
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<td>Pelargonidin-3-O-p-coumaroylrutinoside-O-glucoside isomer</td>
<td>106.983 ± 0.000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>116 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>214 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>4r</td>
<td>29.86</td>
<td>505</td>
<td>741 (100), 433 (16), 271 (27)</td>
<td></td>
<td>Pelargonidin-3-O-cafeoylrutinoside-5-O-glucoside</td>
<td>109.7 ± 0.1</td>
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<tr>
<td>5r</td>
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<td>504</td>
<td>725 (100), 433 (10), 271 (13)</td>
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<td>Pelargonidin-3-O-p-coumaroylrutinoside-5-O-glucoside</td>
<td>235 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>332 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>6r</td>
<td>35.54</td>
<td>507</td>
<td>755 (100), 433 (21), 271 (41)</td>
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<td>Pelargonidin-3-O-p-feruloylrutinoside-5-O-glucoside isomer</td>
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<td>nd</td>
<td>202 ± 1</td>
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<tr>
<td>7r</td>
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<td>755 (100), 463 (15), 271 (38)</td>
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<td>Pelargonidin-3-O-p-feruloylrutinoside-5-O-glucoside</td>
<td>123.8 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>128.9 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>202 ± 1&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Total anthocyanin content</td>
<td>815 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>875.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>886 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
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<table>
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<tr>
<th>Peak</th>
<th>Rt (min)</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt;(nm)</th>
<th>[M]&lt;sup&gt;+&lt;/sup&gt; (m/z)</th>
<th>MS&lt;sup&gt;2&lt;/sup&gt;(m/z)</th>
<th>Tentative identification</th>
<th>Purple</th>
<th>Violetta</th>
<th>Kerfemarkter Blaue</th>
<th>Marble Black</th>
<th>Shetland Black</th>
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<tr>
<td>1p</td>
<td>7.46</td>
<td>530</td>
<td>479 (100), 317 (15)</td>
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<td>Petunidin-3-O-rutinoside-5-O-glucoside</td>
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<td>50.57 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.2 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>125.9 ± 0.2</td>
<td>158.2 ± 0.3</td>
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<tr>
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<td>10.04</td>
<td>515</td>
<td>479 (100), 317 (16)</td>
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<td>nd</td>
<td>43 ± 1</td>
<td>nd</td>
<td>113 ± 1</td>
<td>125.9 ± 0.2</td>
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<td>3p</td>
<td>25.34</td>
<td>525</td>
<td>625 (100), 479 (23), 317 (37)</td>
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<td>Petunidin-3-O-cafeoylrutinoside-5-O-glucoside</td>
<td>nd</td>
<td>43 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.5 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102 ± 0.1</td>
<td>125.9 ± 0.2</td>
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<td>27.66</td>
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<td>625 (100), 479 (34), 317 (46)</td>
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<td>Petunidin-3-O-cafeoylrutinoside-5-O-glucoside</td>
<td>nd</td>
<td>46 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.5 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>102.0 ± 0.2</td>
<td>158.2 ± 0.3</td>
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<tr>
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<td>530</td>
<td>771 (100), 479 (18), 317 (22)</td>
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<td>112.3 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>175 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>208 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113 ± 1</td>
<td>104.1 ± 0.2</td>
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<td>6p</td>
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<td>531</td>
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<td>101.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.7 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>117 ± 1</td>
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<td>130 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.3 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>106.8 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117 ± 1</td>
<td>104.1 ± 0.2</td>
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<td>Petunidin-3-O-p-coumaroylrutinoside-5-O-glucoside</td>
<td>nd</td>
<td>403 ± 0.1</td>
<td>nd</td>
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<td>158.2 ± 0.3</td>
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<td>Total anthocyanin content</td>
<td>478 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>513 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>533 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>481 ± 1</td>
<td>158.2 ± 0.3</td>
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nd – not detected. Standard Calibration Curve used for quantification: Peonidin-3-O-glucoside (y = 151438x – 3E + 06, R<sup>2</sup> = 0.9977, LOD = 0.20 μg/mL and LOQ = 0.71 μg/mL). In each row different Latin letters are significantly different according to Tukey’s HSD test (p = 0.05), for each potato group. *Mean statistical differences obtained by t-Student test.

3. Results and discussion

3.1. Aqueous extracts

#### 3.1.1. Composition of anthocyanins

The anthocyanins' profile, chromatographic characteristics, tentative identifications, and quantification of the seven aqueous extracts are shown in Table 1. All tentatively identified compounds were previously described in the literature for red and purple-fleshed potatoes (Alcalde-Eon, Saavedra, de Pascual-Teresa, & Rivas-Gonzalo, 2004; Kita, Bąkowska-Barczak, Hamouz, Kulakowska, & Lisinska, 2013; Oertel et al., 2017).

Seven peaks were detected in the red-fleshed varieties, six of which were identified as pelargonidin derivatives, based on the observation of their characteristic fragments in MS<sup>2</sup> spectra. Peak 1r ([M]<sup>+</sup> at m/z 741) was identified as pelargonidin-3-O-rutinoside-5-O-glucoside based on the HPLC-DAD-MS results and previous literature reports (Kita et al., 2013; Oertel et al., 2017), and was the major anthocyanin detected in the Red Cardinal extract. Peak 2r ([M]<sup>+</sup> at m/z 579) also showed an MS<sup>2</sup> fragment at m/z 271, corresponding to pelargonidin, bearing the loss of a hexose moiety (~162 u) and being assigned as pelargonidin-3-O-rutinoside. The remaining peaks were identified as acylated anthocyanins owing to the presence of a shoulder in the UV spectra of the compounds around 310–330 nm. Peaks 3r, 4r, 5r, 6r, and 7r were identified...
as acylated pelargonidin glycosides. Pelargonidin-3-O-p-coumaroyl-
rutinoside-5-O-glucoside (Peak 5p) was the main compound detected in
the Red Emmalie and Rosemary extracts. Peak 3r, with the same
molecular ion, might correspond to an isomer of peak 5r having the
glucoside residue located at position 7, which is expected to elute earlier
than the corresponding S-O-glucoside, as reported by Alcalde-Eon et al.
(2004) for similar petunidin isothers in Solanum stenotomum tubers. In
agreement with our findings, acylated pelargonidin glycosides have been
previously identified by other authors as the main pigments in red
potato tubers (Kita et al., 2013; Lewis, Walker, Lancaster, & Sutton,
1998; Oertel et al., 2017; Rodrigues-Saona et al., 1999).

All three red-fleshed varieties showed similar total anthocyanin content,
and the Red Cardinal variety presented the highest value (886.2 ± 1.9 mg/100 g extract). Considering the extraction yield of 5% (mass of
freeze-dried extract/mass of whole fresh potato), our results are in
agreement with those found by Kita et al. (2013) for red-fleshed potato
tubers. Moreover, Hamouz et al. (2011) also reported significant dif-
fences in total anthocyanin content among red- and purple-fleshed potato
cultivars.

Regarding the purple-fleshed varieties, eight peaks were detected,
seven of which were tentatively identified as petunidin derivatives
(Peaks 1p–6p and 8p) and one compound as an acylated peonidin
glycoside (Peak 7p). The molecular ions and fragmentation patterns of
peaks 4p to 6p were coherent with derivatives of petunidin-3-O-ruti-
oside-5-O-glucoside acylated with caffeic, p-coumaric and ferulic acids,
respectively, and peak 8p with petunidin-3-p-coumaroylrutinoside, all
of them previously identified in purple tubers of Solanum tuberosum
(Kita et al., 2013) and S. stenotomum (Alcalde-Eon et al., 2004). Similarly,
the characteristics of peak 7p coincide with those of petunidin-3-O-fer-
uloylrutinoside-5-O-glucoside described in purple tubers of S.
stenotomum (Alcalde-Eon et al., 2004). Peak 3p possessed the same
molecular ion [M]+ at m/z 933 as peak 5p, but a different fragmentation
pattern. Two compounds with this molecular ion were also reported by
Alcalde-Eon et al. (2004) in purple tubers of S. stenotomum that were
respectively identified as petunidin-3-O-p-coumaroyl-
rutinoside-7-O-glucoside and petunidin-3-O-p-coumaroyl-
rutinoside-5-glucoside, although in that case both compounds showed similar MS2 fragmentation. In the present study, peak 3p pro-
duced a main fragment at m/z 625 from the loss of 308 u that could
correspond either to a coumaroylrutinoside or a rutinosyl residue, and a
second fragment at m/z 479 (-146 u) from the further loss of either a
coumaroyl or a rutinosyl residue. This fragmentation rather seems to
suggest a compound where the coumaroyl would be located on a glucose
moiety instead on the rutinosyl moiety, e.g., petunidin-3-O-
rutinoside-5-O-coumaroyrjugoside or petunidin-2-O-
coumaroylglucosylrutinoside. Since no definite identity could be
assigned, this peak was just assigned as a positional isomer of peak 5p.
Finally, peaks 1p and 2p showed earlier retention times and their ab-
sorption spectra lacked a shoulder at 310–330 nm indicating that they
were not acylated. Peak 1p was assigned as petunidin-3-O-ruti-
oside-5-O-glucoside, previously identified by Kita et al. (2013) and
Lewis et al. (1998) in purple tubers of Solanum tuberosum. The MS2
fragmentation of peak 2p produced ions at m/z 479 (-292 u; loss of two
rutinosyl residues) and 317 (-162 u; loss of a glucosyl residue), so that
the compound could be assigned as petunidin-rutinoside-rutinoside, or
alternatively to petunidin-dirhamnoside-glucoside. Petunidi-
n-3-O-p-coumaroylrutinoside-5-glucoside (Peak 5p) was the main
anthocyanin found in the extracts obtained from Violetta, Kefermarkter
Blau and Shetland Black tubers. The same compound was reported by
Lewis et al. (1998) and Kita et al. (2013) as the main anthocyanin in
different varieties of purple potatoes, and by Alcalde-Eon et al. (2004) in
purple tubers of S. stenotomum. In our study, among the four tested
purple-fleshed genotypes, Kefermarkter Blau showed the highest total
anthocyanin content (533.4 ± 2.8 mg/100 g extract).

The variability in the anthocyanin content among the studied ex-
tracts could be mainly attributed to inherited differences of the potato
genotypes, as all samples were subjected to the same environmental
factors (growing conditions, planting location and climate), cultivation
practices, processing and storage conditions. Oertel et al. (2017) re-
ported a great diversity in polyphenol and anthocyanin profiles of red
and purple-fleshed potatoes and suggested differences in the enzymatic
reactions leading to the hydroxylation and methylation of B-ring of the
precursor dihydroflavonols, resulting in the biosynthesis of diverse and
species specific anthocyanin backbones. In the same context, Chaves-
Silva et al. (2018) attributed the differences in the genetic regulation of
biosynthetic pathways through the involvement of different structural
and regulatory genes, while Lachman et al. (2012) highlighted the effect
of genotype on the anthocyanin content of colour-fleshed potatoes.

3.1.2. Bioactive properties

The in vitro antioxidant, antibacterial and antifungal properties of the
red and purple aqueous extracts were also evaluated, and the results are
presented in Table 2. Additionally, a hepatotoxicity assay was carried
out to assess the safety of the extracts for incorporation into food
formulations.

Several studies have demonstrated the antioxidant properties of red
and purple potatoes, which have been linked to their high content of
anthocyanins (Jayawardana et al., 2012; Kita et al., 2013; Nemš et al.,
2015; Nemš & Peksa, 2018). Previous studies have reported positive results for in vitro antioxidant assays on coloured potatoes, namely the
2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), the 2,2-
diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing/antioxidant
power (FRAP) assays. Herein, a cell-based in vitro assay (inhibition of
production of TBAR substances) was applied to assess the antioxidant
properties of the tested colour-fleshed potato tubers.

As presented in Table 2, all seven studied aqueous potato extracts
were effective in diminishing the production of TBAR substances, which
result from lipid peroxidation-induced breakdown. Among the studied
varieties, the purple-fleshed cv. Violetta presented the highest anti-
oxidant capacity, since it required the lowest concentration of extract to
inhibit the lipid peroxidation process by 50% (IC50 value = 380 μg/mL)
compared to the control (Trolox, IC50 value = 139 ± 5 μg/mL). For the
red-fleshed varieties, cv. Rosemary presented the best result (IC50 value
= 417 μg/mL).

In agreement with our findings, Kita et al. (2013) previously reported
positive results for antioxidant activity assays carried out on fresh tubers
of red and purple-fleshed potatoes, where for example the purple variety
Salad Blue showed significant antioxidant activity (ABTS: 1.56 ± 0.2
mM/100 g dw; DPPH: 0.65 ± 0.3 mM/100 g dw; FRAP: 3.17 ± 0.3 mM/100
g dw). Similarly, Nemš et al. (2015) reported a positive antioxidant

Table 2

<table>
<thead>
<tr>
<th>Potato varieties</th>
<th>Antioxidant activity (TBARS)</th>
<th>Cytotoxicity (PLP2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50 values</td>
<td>GL50 values</td>
</tr>
<tr>
<td>Red flesh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosemary</td>
<td>417 ± 5.9d</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Red Emmalie</td>
<td>669 ± 4.4e</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Red Cardinal</td>
<td>592 ± 8.6f</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Purple flesh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple</td>
<td>426 ± 3.3e</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Violetta</td>
<td>380 ± 6.8f</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Kefermarkter Blau</td>
<td>485 ± 2.4d</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Marble flesh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shetland Black</td>
<td>548 ± 6.2e</td>
<td>&gt;400</td>
</tr>
</tbody>
</table>

IC50 values correspond to the sample concentration achieving 50% of antioxidant activity (against lipid peroxidation). GL50 values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2; maximum tested concentration: 400 μg/mL. Positive control; Trolox IC50 value = 139 ± 5 μg/mL (TBARS); Ellipticine GL50 value = 3.2 ± 0.7 μg/mL (PLP2). In each line different Latin letters are significantly different according to Tukey’s HSD test (p = 0.05).
activity for snacks enriched with coloured potato flour obtained from the same *Salad Blue* variety, employing *in vitro* assays (ABTS: 1.16 ± 0.07 μmol Trolox/g dw; DPPH: 0.60 ± 0.06 μmol Trolox/g dw; FRAP: 0.80 ± 0.07 μmol Trolox/g dw). Némys and Pékás (2018), who also incorporated dried coloured-fleshed potatoes into snacks, reported a beneficial effect on the inhibition of oxidative changes in the lipid profile compared to a control formulation over a 3-months storage period. Moreover, in a study conducted by Jayawardana et al. (2012), the addition of 2% coloured potato flakes into pork sausages suppressed lipid oxidation by 80% compared to the control. Thereby, the antioxidant properties of coloured potatoes could also have a beneficial effect in processed food products by extending their shelf-life.

In order to assess the safety of the studied extracts, an *in vitro* cytotoxicity assay was performed. Porcine liver was used as a model, which is justified by its similarity with the human liver in terms of cellular and physiological functioning (Correia et al., 2018). The results revealed that all seven aqueous extracts did not present toxicity against the porcine liver primary culture PLP2, as their GI50 values were higher than the highest tested concentration for all varieties (400 μg/mL) (Table 2). The verified absence of cytotoxicity suggests the safety of the tested extracts for their utilisation as natural colourants in food products.

The results for antibacterial and antifungal activities are presented in Table 3. All seven extracts presented relevant antibacterial and antifungal activities against all the tested bacteria and fungi strains. Cultivar *Red Cardinal* performed particularly well against specific bacteria, as the required MIC value of the extract against *Staphylococcus aureus* (2 mg/mL) was half the MIC value required by the commercial preservative E211, sodium benzoate (4 mg/mL). The same variety also performed better than the control preservative E224 (potassium metabisulphite) against *Bacillus cereus*, as it required half of the MIC and MBC values for this bacterium strain.

All seven aqueous extracts behaved equally or better than the control preservatives E211 and E224 for all the tested fungi strains. The best results against fungi were achieved by the extract from the *Kefermarkter Blaue* variety, as its antifungal capacity exceeded both commercial preservatives for all the tested strains. The obtained results of the antimicrobial properties indicate that all the studied extracts could be useful in retarding and/or suppressing the growth of food borne microbes, which make them suitable candidates for application in food products as natural preservatives.

Previous studies by other authors also reported positive antimicrobial activities from coloured potato extracts. For instance, Ombra et al. (2015) studied the purple-fleshed variety *Vitelotte Noire*, and found that its anthocyanin-rich extract was effective against the bacteria strains *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus*. Interestingly, those authors also found that the natural bioactive compounds from purple potatoes retained their biological activity (antimicrobial, antioxidant and anti-proliferative) during their passage through the gastrointestinal tract, suggesting that the consumption of these nutrient components could be beneficial in terms of disease prevention. Similar results were reported by Bontempo et al. (2013), who tested the purple-fleshed variety *Vitelotte* and observed significant activities against *Staphylococcus aureus* and *Rhizoctonia solani*.

### 3.2. Application of the extracts in soft drink formulations

Following the screening of the seven potato varieties on their anthocyanin profiles and bioactivities, two aqueous extracts were selected to be incorporated as natural colourants into soft drink formulations, namely one from the red-fleshed variety *Rosemary* and one from the purple-fleshed variety *Purple*. The two soft drink formulations were assessed for their sensorial attributes and stability after thermal processing (pasteurisation), in comparison with the *Control* colourant E163.

#### 3.2.1. Colour stability assessment

The results for the colour parameters L* (lightness), a* (green-redness), b* (blue-yellowness), C* (chroma/saturation) and h* (hue) measured over time (after pasteurisation) are presented in Fig. 2.

At Day 0, the control soft drink showed a slight distinct colouration when compared to *Rosemary* and *Purple* treatments, presenting lower a* and higher b* values, which means a more intense presence of blue tones

### Table 3

<table>
<thead>
<tr>
<th>Potato Variety</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red flesh</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosemary</td>
<td>MBC 4</td>
<td>2</td>
</tr>
<tr>
<td>Red Emmalie</td>
<td>MBC 8</td>
<td>4</td>
</tr>
<tr>
<td>Red Cardinal</td>
<td>MBC 2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Purple flesh</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple</td>
<td>MBC 4</td>
<td>2</td>
</tr>
<tr>
<td>Violetta</td>
<td>MBC 3</td>
<td>1</td>
</tr>
<tr>
<td>Kefermarkter Blaue</td>
<td>MBC 6</td>
<td>3</td>
</tr>
<tr>
<td><strong>Marble flesh</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shetland Black</td>
<td>MBC 4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E211</td>
<td>MBC 4</td>
<td>0.5</td>
</tr>
<tr>
<td>E224</td>
<td>MBC 1.0</td>
<td>2.0</td>
</tr>
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**Table 3** Antimicrobial and antifungal activities (mg/mL) of aqueous extracts from red, purple and marble-fleshed potato varieties.

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<td>1</td>
</tr>
<tr>
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<td></td>
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<td>2</td>
</tr>
<tr>
<td>Violetta</td>
<td>MBC 3</td>
<td>1</td>
</tr>
<tr>
<td>Kefermarkter Blaue</td>
<td>MBC 6</td>
<td>3</td>
</tr>
<tr>
<td><strong>Marble flesh</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shetland Black</td>
<td>MBC 4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E211</td>
<td>MBC 4.0</td>
<td>0.5</td>
</tr>
<tr>
<td>E224</td>
<td>MBC 1.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Table 3** Antimicrobial and antifungal activities (mg/mL) of aqueous extracts from red, purple and marble-fleshed potato varieties.

MIC: minimal inhibitory concentration (mg/mL); MBC: minimal bactericidal concentration (mg/mL); and MFC: minimal fungicidal concentration (mg/mL). Bacterial strains: S.a. (*Staphylococcus aureus*; ATCC 6538); B.c. (*Bacillus cereus*; food isolate); L.m. (*Listeria monocytogenes*; NCTC 7973); E.c. (*Escherichia coli*; ATCC 35210); S.t. (*Salmonella Typhimurium*; ATCC 13311), and E.a. (*Escherichia coli*; human isolate). Fungal strains: A.f. (*Aspergillus fumigatus*, human isolate); A.n. (*Aspergillus niger*; ATCC 6275); A.v. (*Aspergillus versicolor*; ATCC 11730). P.L. (*Penicillium funiculosum*; ATCC 36830); P.a. (*Penicillium aurantiogriseum*; food isolate); T.v. (*Trichoderma viride*; IAM 5061). Positive controls: E211- sodium benzoate and E224- potassium metabisulphite.
and less intensity of red ones. Despite all three formulations having the same concentration of anthocyanins, this difference in colour tone and intensity may be due to the distinct anthocyanin glycosides present in the two tested colouring agents (Table 1) and in the control E163 (grape anthocyanins). It should be noted that the colour profile of the three samples presented a great stability between Day 0 and Day 7 for all the analysed parameters. At Day 30 a significant decrease in a* values was recorded for all the tested formulations, which could be related to a reduction in the intensity of the red colour. A strong positive correlation between the total anthocyanins content and the chroma parameters in purple-fleshed sweet potatoes was also observed by Loypimai, Moongnarg, and Chottanom (2016). According to Ashurst (2011), in a comprehensive report on the stability and shelf life of fruit juices and soft drinks, product discoloration is a process commonly observed in almost any soft drink or fruit juice. This can be explained by a decrease in the concentration of the original anthocyanins as a result of their degradation and/or transformation in newly-formed derived pigments (Santos-Buelga & González-Paramás, 2019). The degradation of anthocyanins over time was also reported by Jie et al. (2013) and Loypimai et al. (2016) in purple-fleshed sweet potato and black rice extracts, respectively. Similarly, anthocyanin decay and the discoloration process was observed by Rodríguez-Saona et al. (1998) when monitoring the anthocyanin stability of frozen red-fleshed potato tubers over storage of a three-month period. Those authors attributed the observed anthocyanin content decay to possible enzymatic reactions.

According to the results herein obtained, the performances of the aqueous potato extracts over shelf-life could be considered satisfactory, as they were similar to the control commercial colourant (a grape extract containing potassium sorbate as preservative). This performance might be explained by the presence of acylated anthocyanins in potatoes. The acylation confers increased biochemical stability to anthocyanin forms, preventing their indiscriminate degradation by glycosidases, most of which are unable to act on acylated glycosides (Mateus & Freitas, 2009). Therefore, our results indicate the suitability of the red and purple potato aqueous extracts to be used as alternative natural colouring agents in beverages.

Fig. 2. CIELab colour values ($L^*$, $a^*$ and $b^*$) and cylindrical coordinates ($C^*$ and $h^*$) of the soft drink formulations Rosemary (red potato extract), Purple (purple potato extract) and Control (E163 commercial colouring), over a 30 days shelf-life period. $L^*$: lightness from black (0) to white (100); $a^*$: green (−) to red (+); $b^*$: blue (−) to yellow (+); $C^*$: chroma, relative saturation; and $h^*$: angle of the hue in the CIELab colour space. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.2. Sensory analysis

The scores obtained in the sensory analysis are shown in Fig. 3. The Rosemary, Purple and Control formulations were assessed by the 12 trained panellists for the following attributes: intensity of colour, cloudiness, sweetness, fruitiness, bitterness and sourness.

Regarding visual attributes, no statistical difference was reported between the Control formulation and Rosemary for intensity of colour and cloudiness. Purple, however, presented a significantly cloudier solution and a significantly lighter colour than both Rosemary and Control (p < 0.05). Rosemary and Control formulations were described by the panellists as presenting a light transparent pink colour while Purple was described as presenting a slightly opaque light pink colour.

No statistical difference was reported between the three formulations for the tested flavour attributes sweetness, fruitiness and bitterness. With regard to sourness, Control was rated significantly higher than Rosemary (p < 0.05). Nevertheless, no significant difference was found between Purple and Rosemary and between Purple and Control for this attribute. Additionally, no off-odours or off-tastes were detected in any of the tested formulations.

At the end of the evaluation session the 12 panellists were asked to choose their preferred formulation among the three ones tested. The soft drinks formulated with Rosemary and Purple extracts performed well, with 42% of the panellists choosing Purple and 25% choosing Rosemary as their preferred one, while 33% preferred the Control soft drink. Interestingly, some panellists highlighted in their comments the opaqueness described in Purple as a positive attribute, relating it to a “natural rather than synthetic appearance”.

The presented results regarding the sensorial analysis and the stability over time of the red and purple-fleshed potato formulations indicate promising potential for their future application in the food industry as substitutes of synthetic colouring agents.

4. Conclusion

The seven aqueous potato extracts tested presented high anthocyanin content and high antioxidant, antibacterial and antifungal
properties. Acylated pelargonidin glycosides were the main compounds found in the red varieties and acylated petunidin glycosides in the purple ones. Additionally, no cytotoxic effect was detected in the extracts up to the maximum tested concentration (400 μg/mL), indicating their safety to be incorporated in food formulations and substitute the existing synthetic colouring agents. The two extracts selected to be applied in a soft drink formulation showed suitable profiles in the sensory and shelf-life assessments when compared with the control commercial colourant E163. The aqueous extracts herein obtained by a simple one-step and cost-effective extraction method could be used as alternative natural food colourants, substituting synthetic compounds and satisfying the current market needs and consumer preferences for natural products. Nevertheless, future studies in pre- and post-harvest level could support the selection of colour-fleshed potatoes with high anthocyanin content. Furthermore, the assessment of different agronomic practices may increase the concentration of anthocyanins and the added value of this important vegetable crop.

CRediT authorship contribution statement


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.

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