



Microwave hydrodiffusion and gravity pretreatment of *Camellia japonica* flowers for the extraction of bioactives

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Abstract

The valorization of *Camellia japonica* flower varieties through the green extraction of their bioactive components is addressed. Microwave hydrodiffusion and gravity (MHG) can be an efficient and rapid pretreatment to dry the petals; for example, in the Royal Velvet variety, the energy consumption was 16 times lower compared to air drying. The hydrolates obtained in this stage represented only 0.5 mg gallic acid equivalents/g flower, but some of the fractions contained up to 10 times higher and a Trolox equivalent antioxidant capacity (TEAC) value equivalent to 0.4 g Trolox/g extract. The dried solids were extracted using 96% ethanol to yield around 30% solubles, which was 50% higher than the extraction yield from the air-dried samples. The MHG dried solids were treated with supercritical CO₂ to yield up to 2.4% of an extract containing phenolics and lipids with a favorable omega-3 to omega-6 ratio. The residual solids from supercritical extraction were further processed with microwave heated pressurized hot water for 5 min at various temperatures. The 200 °C extract achieved a solubilization of 50% of the material, obtaining a product with 22% phenolic content, almost 30% of the antiradical capacity of Trolox, a minimum inhibitory concentration (MIC) value of 8 mg/mL against *Staphylococcus aureus*, and contained mainly organic acids, tannins like (epi)catechin gallate and B-type (epi)catechin oligomers, and prodelphinidins like (epi)gallocatechin-*O*-hexoside and (epi)catechin-(epi)gallocatechin gallate. The obtained extracts were proposed for incorporation onto starch films. The results confirm the potential of non-toxic solvents to achieve a complete valorization of this resource.

Keywords Camellia · Petals · Green extraction · Antioxidant · Antimicrobial

Introduction

Flowers can be regarded as a traditional food or an ingredient of natural therapies in different civilizations worldwide. In addition to their nutrients, proteins, and essential amino acids, the presence of phytochemicals, such as polyphenols and carotenoids, may confer anti-inflammatory, antibacterial,

and antioxidant properties. Color is an important attribute in the food and cosmetic industry, and both natural and synthetic food colorants are required, but the demand for those from natural origin has increased due to the negative health effects of some synthetic colorants. Flowers can be a good source of color compounds, anthocyanins, and antioxidant compounds, but they are highly perishable and have to be

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immediately dried to prevent fungal deterioration [1]. Adequate selection of the drying conditions is required since the stability of anthocyanins is influenced by temperature, oxygen, light intensity and quality, presence of copigments and metallic ions, and enzymatic and nonenzymatic reactions during drying. Rapid and efficient drying methods are being explored; i.e., vacuum drying allowed lower degradation of anthocyanins from poppy flowers compared to air drying [1], microwave drying is a rapid method for practical uses, and also infrared drying proved suitable for *Bletilla striata* flowers [2]; infrared assisted drying of rose petals at 500 W for 18 min reduced by half the time compared with forced convective drying and showed higher retention of color, ascorbic acid, and anthocyanin content [3]; and also, infrared drying of *Rosa rugosa* flowers improved the drying rate and energy efficiency and maintained aroma, structure, and nutritional value with shorter drying time [4]. Microwave hydrodiffusion and gravity (MHG) has been successfully proposed for simultaneously obtaining an aqueous extract and for drying the solid material from flowers [5], seaweeds [6, 7] and mushrooms [8].

Edible flowers can be regarded as sources of bioactives with a number of health benefits [9, 10] and tea flowers are a good example due to their antioxidant, immune-stimulating, anti-inflammatory [11] and antibacterial [12] actions, as well as the antiproliferative activity against different human cancer cells [13]. The nutritional and bioactive potential of camellia flowers is well known, i.e., due to the content of arachidic acid [14], malic acid, which could be used as a natural antimicrobial agent [14] as well as different phenolic compounds, including phenolic acids (gallic acid, protocatechuic acid), flavonoids (quercetin, kaempferol), and anthocyanins [15]. They should be further explored as a source of ingredients and additives for food, nutraceutical, and cosmetic applications [14].

Green extraction of phenolic compounds from camellia flowers can be achieved by ethanolic extraction [9], by enzymes, or by pressurized solvent extraction [10]. Also, soluble sugars and insoluble polysaccharides could be hydrolyzed and extracted [10]. The utilization of these technologies offers advantages in environmental aspects both for the analytical applications required for characterization studies and quality control of the products and for industrial processing. This approach would conform with the green extraction principles to (i) valorize renewable underutilized resources, (ii) use green solvent, (iii) in efficient compact and flexible processes, (iv) allow shorter times and lower energy consumption, (v) obtain safe products, and (vi) coproducts instead of wastes [16].

The novelty of the present study lies in the use of MHG for the aqueous extraction of bioactives and drying of distinct varieties of *Camellia japonica* petals, as shortened

processing times may preserve anthocyanin stability. The further solvent extraction either by conventional ethanolic extraction or by supercritical CO₂ extraction is expected to offer increased phenolic compounds and anthocyanin yields due to the additional cell wall degradation caused by microwave exposure. A third stage based on pressurized hot water extraction was proposed to fully address the valorization of this renewable resource. Finally, starch films using anthocyanin-rich ethanolic extracts were formulated, and a further analysis of the highest phenolic content sample as to LC-MS for profiling and antimicrobial activity was conducted.

Materials and methods

Raw material

Camellia japonica flowers from different varieties (Fig. 1) were collected in private gardens in Ourense and at the International Camellia Garden of Excellence at Pazo Quinteiro da Cruz in Ribadumia (Pontevedra, Spain) in Spring 2024. Flowers were processed as soon as possible or dried in an air oven at 40 °C. Discarded potatoes with irregular and low size from *Agraia* variety grown in Galicia (Spain) were used as raw material for the starch extraction.

Extraction

Conventional solvent extraction

Dried flowers were extracted with 0.1 M HCl in 96% ethanol solution at a 75 liquid to solid ratio (w/w) for 24 h. Then, the liquid phase was separated by filtration, and the solvent was removed in a rotavapor.

Microwave hydrodiffusion and gravity (MHG)

Microwave hydrodiffusion and gravity was used as a drying technique alternative to oven drying at 40 °C. In addition, the bioactive fractions drained (10 mL of the sample was collected each time that quantity was drained) during this process were collected and evaluated for phenolic content and antioxidant properties. MHG extraction experiments were conducted at least in duplicate to process fresh petal batches on a multimode microwave extractor (NEOS-GR, Milestone Srl, Italy). Fifty grams of fresh flowers was placed on the 1.5-L open vessel operating under conditions used for other similar materials [17]. The collected drained samples were stored in a fridge (4 °C) in darkness until further analysis.



Fig. 1 Different *Camellia japonica* varieties used in the present study

Supercritical CO₂ extraction

The MHG dried flowers (10 g) were packed with glass beads into a 1-L extractor vessel (Thar Designs SFE-1000F-2-C10, Pittsburgh, USA). The precooled CO₂ was pumped by a P-200A piston pump (Thar Design Inc., Pittsburgh, PA, USA) at 25 g CO₂/min, and the modifier was pumped by a HPLC pump (Scientific Systems, Inc., USA, model Series III). Extraction conditions were 30 MPa, 40 °C, 5% ethanol, and 135 min, based on literature and preliminary experiments [11, 18]. The extract was collected at ambient temperature in the first separator, then washed with 96% ethanol, which was further removed in the rotavapor.

Pressurized hot water extraction using microwave heating

MHG dried camellia flower material obtained after supercritical CO₂ extraction was further subjected to pressurized hot water extraction using a microwave-assisted system (Anton Paar Monowave 450 microwave reactor, Austria), equipped with a MAS 24 automatic sampler. Distilled water was used as the solvent at a liquid to solid ratio of 30:1 (w/w). The extraction was conducted at a power of 850 W,

maintaining the selected temperatures, in the range from 120 to 200 °C, for 5 min with a stirring speed of 850 rpm. The cooling temperature was set to 50 °C. Upon completion of the extraction at the target temperature, the suspension was rapidly cooled and vacuum filtered to separate the liquid and solid phases.

Starch extraction

Peeled potatoes were crushed, mixed with water (1:2 w/w), and agitated in a beaker for an hour. After filtration, the operation was repeated on the residual solid. The mixed liquid phases were then placed at 4 °C for one night. The following day, the precipitated starch was separated from the aqueous phase and dried overnight at 40 °C in a convective oven.

Film formulation

Control films were prepared with starch at 2% (w/v) and glycerol at 40% starch dry weight (dw). Starch samples were stirred at 500 rpm at 60 °C for 15 min. The plasticizer is added halfway through heating once the biopolymer is

solubilized. The forming solution (5 mL) is poured into a small petri dish (2-cm diameter) previously to be placed in an air-dried convection oven for 24 h until constant weight. Films with starch at 2% (w/v), glycerol at 40% starch (dw), and pigment at 0.1% were developed.

Analytical methods

Proximal composition

The moisture content of the solids and the dry content of the soluble extracts were measured after drying at 105 °C in an air oven (P Therooven, JP Selecta, Spain) until constant weight. The ash content was determined after calcination at 575 °C in a muffle furnace (ELF, Carbolite, UK). Elemental analysis was performed on a Thermo Flash EA 1112 analyzer. The protein content was calculated applying the conversion factor (6.25) on the total nitrogen content, determined by Kjeldahl. The monosaccharide content was determined using an Aminex HPX-87H column for glucuronic acid, glucose, and arabinose in an Aminex CPX-87H column for xylose, mannose, and galactose. The total phenolic content has been estimated with the Folin-Ciocalteu method [19]. In test tubes, 1.875 mL of distilled water, 0.125 mL of the Folin reactive diluted at 1:1 with distilled water, and 0.25 mL of Na₂CO₃ at 20% were added to 0.25 mL of the sample and mixed. After 1 h in the dark, the absorbance was measured at $\lambda = 765$ nm. The standard curve was prepared using gallic acid at 0.00625–0.1 mg/mL. Soluble protein content was determined by Bradford method using bovine serum albumin (Sigma, Spain) as the standard.

Ultra-high-performance liquid chromatography with photodiode array detector and mass spectrometry (UHPLC-DAD-ESI-MS)

UHPLC-DAD-ESI/MS analyses were performed using an Ultimate 3000 (Dionex Co., San Jose, CA, USA) system equipped with an auto-sampler, a quaternary pump, an Ultimate 3000 diode array detector (Dionex Co., San Jose, CA, USA), and an automatic thermostatic column compartment. It was coupled to a Thermo LTQ XL ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) fitted with an electrospray ionization (ESI) source. Instrument control and data acquisition were carried out with the Thermo Xcalibur Qual Browser data system (Thermo Scientific, San Jose, CA, USA). Nitrogen above 99% purity was used, and the gas pressure was 520 kPa (75 psi). The MS was operated in negative ion mode, acquiring full scan spectra across a mass range of m/z 100–2000. ESI needle voltage was set at 4.80 kV and an ESI capillary temperature of 275 °C. The compounds were separated using a Hypersil GOLD C18 column (100 mm length; 2.1-mm i.d.; 1.9- μ m particle diameter,

end-capped from Thermo Scientific, USA) at 30 °C. Elution was performed using a linear gradient of solvent A (0.1% formic acid in water) and solvent B (acetonitrile), at a flow rate of 0.200 mL/min. The gradient program initiated at 5% B increased to 40% at 14.72 min, from 40 to 100% over 1.91 min, maintained at 100% for 2.19 min, and then returned to the starting conditions. UV-Vis spectral data for all peaks were collected in the range of 200–700 nm, while the chromatographic profiles were recorded at 280 nm. Compound identification was based on comparison of retention times, UV absorption spectra, and mass spectral data with values previously reported in the literature. MS and MS/MS data were processed using the Thermo Xcalibur Qual Browser data system (Thermo Scientific, USA).

Fat content

Lipid content was determined using a mixture of chloroform and methanol [20, 21]. One gram of milled material was added to 20 mL of chloroform-methanol solution (2:1 v/v). Centrifugation (10 min, 966 rpm, 15 °C) was thereafter performed. The mixture was filtered, and 5 mL of distilled water was added. A second centrifugation was conducted to separate the organic and inorganic phases. After that, the organic phase was recovered and evaporated to determine the lipid content of the sample. This was followed by transesterification to profile its composition [22]. HCl (2.5%) was added to methanol at 85 °C for 90 min to trans-methylate lipids. Gas chromatography (GC-MS QP, 2010; Shimadzu, Kyoto, Japan) was performed to analyze fatty acid methyl esters. Measurements were assessed at least in triplicate.

Anthocyanin content

Monomeric anthocyanin content was determined following Lee et al.'s protocol [23]. Two buffers made with 0.025 M KCl and 0.4 M CH₃COONa were prepared, and pH was respectively adjusted at 1 and 4.5 with HCl. The pigment extract was diluted with the buffer at pH 1 until the absorbance at 520 nm was lower than 1. A blank was made with distilled water. Then, the sample was diluted with this same dilution factor with the buffer at pH = 1 and with the buffer at pH = 4.5. After 20 min, the absorbance of both samples was measured at 520 and 700 nm. The anthocyanin content was determined following Eq. 1:

$$\text{Anthocyanin Content (mg/g)} = \frac{A \times MW \times DF \times 10^3}{e \times 1} \quad (1)$$

with $A = (\text{Abs}_{520 \text{ nm}} - \text{Abs}_{700 \text{ nm}})_{\text{pH } 1} - (\text{Abs}_{520 \text{ nm}} - \text{Abs}_{700 \text{ nm}})_{\text{pH } 4.5}$
 $\epsilon = 26900$

MW = 449.2 g/mol (MW of cyanidin – 3 – O – glucoside)

DF = Dilution factor

Stability measurements

A fraction of the pigments formulated with starch was placed at room temperature, another one in the fridge. The evolution of the anthocyanin and phenolic compounds was followed for 4 weeks, measuring spectrophotometrically the contents every week.

Antioxidant activity

The antioxidant properties were evaluated as antiradical capacity and as reducing power. The antiradical properties against ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) were measured by the Trolox equivalent antioxidant capacity (TEAC) assay [24]. Briefly, the liquid samples (10 μ L) were placed in a test tube, and the ABTS solution (1 mL) was added. The mixture was incubated for 6 min at 30 °C in a water bath, and then the absorbance was read at 734 nm with a standard curve prepared with Trolox (Sigma-Aldrich, Denmark). The data were expressed as TEAC value.

The scavenging activity against DPPH (α,α -diphenyl- β -picrylhydrazyl) of the samples (50 μ L) was evaluated with DPPH solution recently prepared [25]. The reduction in absorbance after 16 min was measured at 515 nm, and the percentage inhibition was calculated for different sample concentrations.

The ferric reducing antioxidant power (FRAP), based on the reduction of the ferric 2,4,6-tripyridyl-s-triazine (TPTZ) complex in acidic conditions [26] was measured by mixing the sample or standard (0.1 mL) with the reagent (3.0 mL). The absorbance was read at 593 nm for 6 min, and ascorbic acid was used as the standard. All the above analyses were performed in triplicate.

Antimicrobial activity

For the determination of the antimicrobial activity, *Staphylococcus aureus* isolated from Coimbra Hospital resistant to methicillin (MRSA) was chosen. The microdilution assay described by ISO 20776-1 (2019) [27] was performed. The minimum inhibitory concentration (MIC) is the extract's lowest concentration at which no bacterial growth was visible (no visible turbidity on the microplate). Stock bacterial cultures were maintained at 4 °C in tryptic soy agar (TSA; Liofilchem, Roseto degli Abruzzi, Italy). Before each assay, three bacterial colonies were transferred to 30 mL of tryptic soy broth (TSB; Liofilchem, Roseto degli Abruzzi, Italy) and incubated for 24 h at 37 °C with stirring (120 rpm). Then, 300 μ L of the formerly grown suspension was transferred to another TSB bottle and incubated under the previously described conditions, when the stationary phase of approximately 10^9 colony-forming units per mL (CFU/mL)

is reached. After that, the overnight bacterial culture was diluted in TSB adjusted to 0.5 McFarland Standard, which corresponds to $\sim 10^8$ CFU/mL. Finally, 100 μ L of Mueller-Hinton broth (Liofilchem, Roseto degli Abruzzi, Italy) and 100 μ L of extract were poured on each well and incubated in a 37 °C oven for 24 h. To ensure that the color of the extracts themselves does not affect the test, one extra plate without bacteria was used.

The contents of each well were serially diluted in PBS and drop-plated onto TSA plates in duplicate (10 μ L each drop) in order to quantify the number of bacterial cells. Four dilutions were plated onto each plate, and the decision of which ones to plate was based on if the extract's concentration was above or below the MIC (dilutions 10^0 to 10^3 if it was above MIC and 10^3 to 10^6 if below). The colonies were counted at the most suitable dilution following an 18 h incubation period at 37 °C, and the viable bacterial density was calculated as log CFU/mL. For every condition, three separate assays were conducted, and the results were averaged.

Color features of dried petals

The color characteristics of dehydrated *Camellia japonica* Royal Velvet flowers, processed through conventional oven drying and microwave hydrodiffusion and gravity (MHG) drying, were evaluated using the CIELab* color space. This variety was used as a representative example. Measurements were carried out with a CR-400 colorimeter (Konica Minolta, Japan). The color coordinates and values were assessed based on the CIELab* color system. In this model, L^* indicates the lightness level, ranging from 0 (black) to 100 (white); a^* describes the position on the red-green axis, with positive values indicating redness and negative values indicating greenness; and b^* reflects the yellow-blue axis, where positive values correspond to yellowness and negative values to blueness. Additional color metrics, including hue angle (h°), chroma (C), and saturation (S), were also calculated (Eqs. 2–4). The total color difference (ΔE^*) and hue difference (ΔH^*) were determined following the Eqs. using the oven-dried samples as the reference. A minimum of ten measurements were recorded for each sample.

$$h(\circ) = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

$$S = \frac{C}{L^*} \quad (4)$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (5)$$

$$\Delta H^* = \sqrt{(\Delta E^*)^2 - (\Delta L^*)^2 - (\Delta C^*)^2} \quad (6)$$

Starch analysis

Total starch content, amylose/amylopectin percentage, and damage content of the employed starch were determined using two Megazyme enzymatic kits (Wicklow, Ireland) following standard procedures [28]. The starch crystalline structure assessment was conducted on a diffractometer using CuK α radiation (λ , 0.154 nm) working at 20 mA and 40 kV. Starch was scanned through the 2 θ (diffraction angle) (from 2 to 50°, 4°/min) at room temperature. The relative crystallinity degree (%) was estimated as $I_c/(I_a + I_c)100$ following the method explained elsewhere [29];

Film analysis

Rheological properties of both the film-forming solutions and the corresponding films were measured using a controlled-stress rheometer (MCR302, Anton Parr). A sand blasted parallel plate (25 mm) was used as measuring geometry. Film-forming solutions were placed between the plates with a 1-mm gap, whereas the formed starch-based films were evaluated at a 0.25-mm gap. All samples were rested for 5 min prior to the rheological testing. Steady-state shear measurements were conducted to determine the apparent viscosity profile of the film-forming solutions at 25 °C, and small amplitude oscillatory shear measurements were made to monitor the viscoelastic features of the films in terms of G' (elastic modulus) and G'' (viscous modulus).

Scanning electron microscopy

Dehydrated petals and the corresponding raw material obtained after conventional and microwave hydrodiffusion and gravity treatment were assessed using a FEI Quanta 200 scanning electron microscope (Thermo Fisher Scientific, USA) operating at 12.5 kV. The structure of the formulated starch-based films was analyzed using the same device.

Statistical analysis

All the above measurements were performed at least in triplicate. Experimental data were assessed using one-factor analysis of variance, ANOVA, by means of a statistical software (PASW Statistics v.22, IBM SPSS Statistics, New York, USA). Whenever differences between means

were distinguished, a post hoc Scheffé test was conducted (95% confidence, $p < 0.05$). The antimicrobial activity of the extracts was assessed by comparing the differences in the bacterial concentration between each treatment and the corresponding controls. The significances of the differences among the tested conditions were assessed using one-way analysis of variance (ANOVA).

Results and discussion

Chemical composition of the flowers

Table 1 shows the major components of the *Camellia japonica* Royal Velvet flowers as a representative example of the species. The most abundant were carbohydrates, accounting for 59.5% dry weight. This value is in the range reported for other varieties, with 40.5% soluble sugars, which include fructose, glucose, and sucrose [10]. Other components were ash ($1.5 \pm 0.3\%$), protein ($0.5 \pm 0.1\%$), and lipids ($0.25 \pm 0.03\%$), and the ethanol extractables accounted for 18.6%. The mineral content was comparable, but the protein content was significantly lower than for other varieties from the same geographical area [30]. Low lipid content was previously reported [14], with arachidic acid as the most abundant saturated fatty acid; palmitoleic acid the major monounsaturated fatty acid; and minor amounts of stearidonic acid, gamma-linolenic acid, and linoleic acid.

Table 1 Average proximal composition of *Camellia japonica* flowers, Royal Velvet flowers used in this study and ranges reported in literature [10, 14, 30]

Component	Content (% dw)	
	This study	Literature
Ash	1.5 ± 0.3	0.4–3.75
Lipids	0.25 ± 0.03	0.31
Protein (N $\times 6.25$)	3.1 ± 0.1	4.4–6.3
Extractives	18.6 ± 1.2	
Carbohydrates	59.5	66.0
Fructose (OS)	19.5 ± 0.4	16.6–22.5
Glucose (MS, OS)	25.2 ± 0.5	13.1–24.7
Galactose (OS)	2.2 ± 0.2	3.7
Arabinose (OS)	6.4 ± 0.1	5.05
Xylose (OS)	0.1 ± 0.0	
Mannose (OS)	2.1 ± 0.1	2.4
Sucrose	2.9 ± 0.1	2.3–3.2
Galacturonic acid (OS)	1.1 ± 0.2	1.6

Extractives analyzed in 96% ethanol

MS, monomeric saccharides; OS, oligomeric saccharides

Statistical analysis was performed by rows ($n = 3$, $p < 95\%$)

Remarkable levels of polyunsaturated fatty acids (especially ω -3 fatty acids) were also reported [30]. Tocopherols and tocotrienols were identified by these authors, as well as carotenoids, with β -carotene and lutein among them. Malic acid, citric acid, and levulinic acid were the most abundant organic acids [14].

Microwave hydrodiffusion and gravity

Figure 2 depicts the three heating profiles tested in initial experiments performed with *Camellia japonica* Royal Velvet variety: sequence 1 (150 W, 20 min + 100 W, 30 min + 50 W, 70 min), sequence 2 (125 W, 10 min + 100 W, 20 min + 50 W, 70 min), sequence 3 (100 W, 10 min + 100 W, 20 min + 50 W, 70 min).

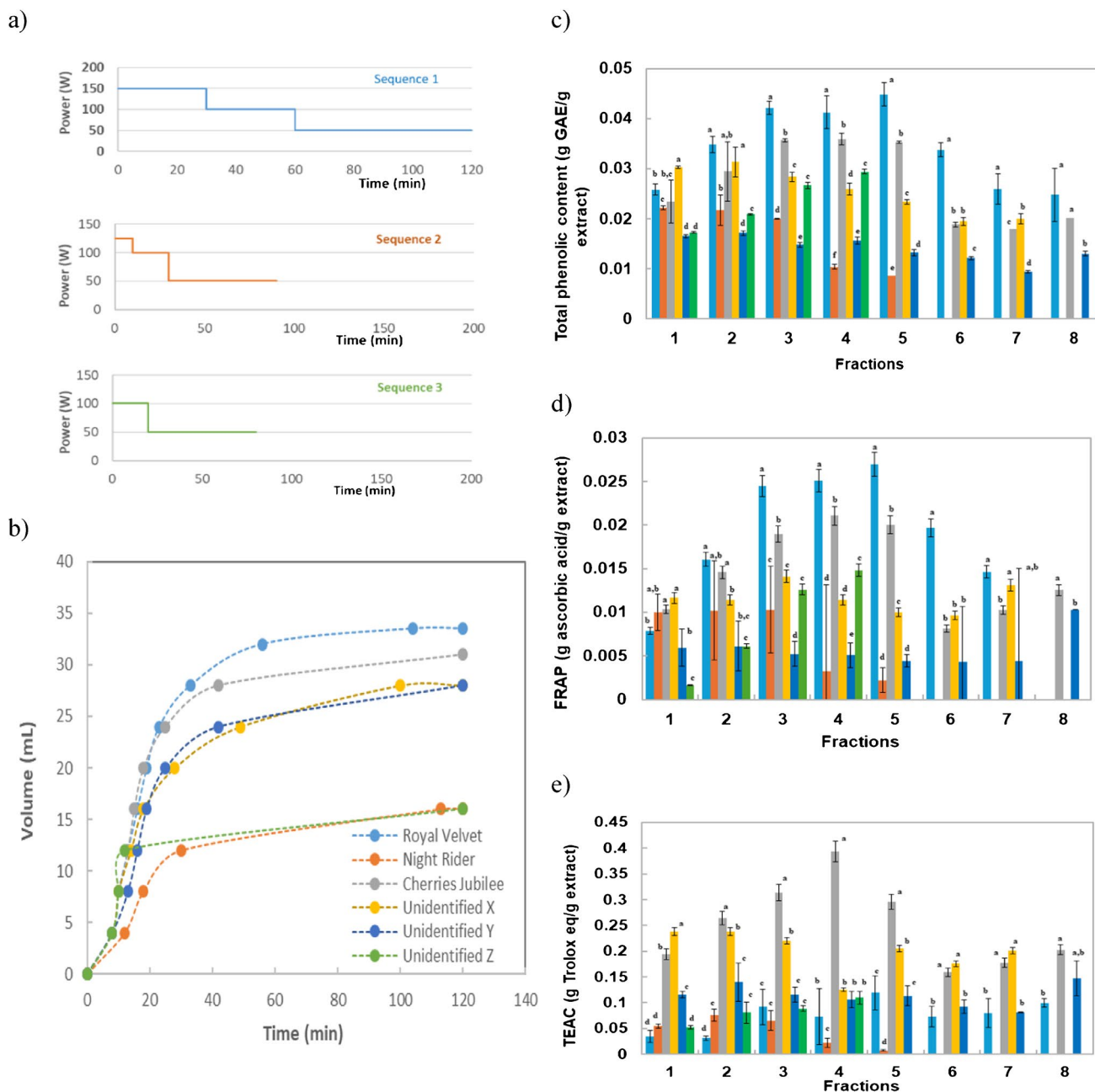


Fig. 2 **a** Heating sequences evaluated with *Camellia japonica* var. Royal Velvet and for sequence 3. **b** Drainage kinetics during microwave hydrodiffusion and gravity for petals from var. Royal Velvet (●), var. Night Rider (○), var. Cherries Jubilee (●), unidentified X (●), unidentified Y (●), and unidentified Z (●) varieties. **c** Total phenolic content expressed as g gallic acid equivalents (GAE)/g extract. **d** Reducing

power (g ascorbic acid/g extract). **e** ABTS radical scavenging capacity, expressed as TEAC value (g Trolox/g extract) in the collected fractions during microwave hydrodiffusion and gravity. Note here that error bars were not included in those plots where they were lower than the symbol size. Statistical analysis was made by type of flower for each fraction ($n=3$, $p<95\%$)

W, 60 min), and sequence 3 (100 W, 30 min + 50 W, 60 min). They allowed moisture reduction under 10% without sample burning. Sequence 3 was further selected because it was the shortest one and was adapted to thinner petals.

Hydrolate

The volume drained during MHG treatment and periodically collected during the third heating profile is plotted in Fig. 2b for the different *C. japonica* varieties tested in the present study. Up to 35 mL from 50 g fresh flowers were obtained from the Royal Velvet variety, whereas around 30 mL was collected for Cherries Jubilee and unidentified Y and Z varieties, whereas only 16 mL was recovered from Night Rider and for Unidentified X, representing 30–70% of the initial material and 70–75% of the initial moisture. This value is higher than those obtained from other flowers, in the range 43 to 48 mL/100 g fresh material [5, 31, 32] operating at 25–200 W. The final samples contained from 9 to 12% moisture (Table 2). The moisture difference between the values determined in the solid and in the collected extract corresponds to the water that has been evaporated since this system operates in open configuration.

The initial latency period could be due to the low heating rate and the fact that the water is still in the intact cells. The second period allowed collection of more liquid volume, and finally, a decreasing rate period leads to low liquid phase recovery due to the low water content in the solid. This behavior has also been described for petals from *Ulex europaeus* [5], from *Erica australis* [17] and *Cytisus scoparius* [5] but was less noticeable on other flower morphologies, such as those from *Acacia dealbata* [32].

The low extraction yield of the drained extract in MHG was expected, as already found for mushroom, yielding less than 1% of the weight of the initial material, but was useful as an initial stage to dehydrate the biomass [8]. The phenolic concentration in the drained extract was low, with a

maximum in the range 0.01–0.045 g gallic acid equivalents (GAE)/g for the initial/medium fractions (3rd–5th). Data in Fig. 2c show the value of the phenolic content in each fraction of the different varieties. Significant differences among batches can be observed, confirming the difficulty of this technique to provide reproducible results from this material. The notable standard deviations of this technique have also been observed with petals from *U. europaeus* flowers [5] and *E. australis* [31].

The sum of the phenolics extracted in the different fractions accounted for 0.17–0.48 mg/g dry flower (Table 2). These yields are comparable or higher than those obtained for other flowers, 0.27 mg/g *Erica australis* dry flowers operating at 50 W for 130 min [31] or 0.15 mg GAE/g *Acacia dealbata* dry flowers [31] and 0.23 mg/g *Cytisus scoparius* dry flowers [5]. Similarly, the reducing and antiradical properties were correspondingly higher. As a general trend, the antioxidant properties increase with the phenolic content as expected and already reported for other flower extracts [10, 30]. Glucose was the most abundant component in the hydrolates with slight differences among varieties in the range 17.9–23.1%, followed by fructose, in the range 13.9–18.2% and lower values, in the range 0.6–1.2% were found for sucrose. The highest saccharidic content was found in the MHG hydrolate from the Unidentified Variety X, whereas those from the Red Velvet and Cherries Jubilees variety showed the highest phenolic extraction yield.

Solids

Drying kinetics The initial moisture content of the petals was in the range 70–75% for all tested varieties and the final moisture content of the MHG dried solids was in the range 9.0 to 11.8% (Table 2), suitable for storage purposes. The solids could be dried in 40–120 min, which represented a substantial reduction compared to that required during convective air drying (Fig. 3). The petals from the Royal

Table 2 Characteristics of liquid and solid phases obtained after MHG from different *Camellia japonica* varieties

Variety content (% or mg/g)	Royal Velvet	Night Rider	Cherries Jubilees	Unidentified variety X	Unidentified variety Y	Unidentified variety Z
Liquid phase						
Glucose (%)	20.2 ± 0.3 ^c	21.3 ± 0.4 ^b	19.6 ± 0.3 ^c	23.1 ± 0.1 ^a	18.5 ± 0.3 ^d	17.9 ± 0.4 ^c
Fructose (%)	16.2 ± 0.2 ^b	18.1 ± 1.2 ^a	17.3 ± 0.4 ^{a,b}	18.2 ± 0.2 ^a	15.4 ± 0.3 ^c	13.9 ± 0.6 ^d
Sucrose (%)	1.2 ± 0.6 ^a	1.4 ± 0.6 ^a	1.1 ± 0.7 ^a	1.2 ± 0.1 ^a	0.9 ± 0.4 ^b	0.6 ± 0.3 ^b
Total phenolics (mg/g dry flower)	0.48 ± 0.14 ^a	0.17 ± 0.01 ^d	0.45 ± 0.01 ^a	0.37 ± 0.02 ^b	0.23 ± 0.02 ^c	0.20 ± 0.01 ^c
Solid phase						
Final moisture (%)	9.5 ± 0.3 ^b	10.2 ± 0.3 ^b	9.1 ± 0.3 ^{b,c}	11.8 ± 0.2 ^a	12.0 ± 0.1 ^a	9.0 ± 0.3 ^c

Total phenolics in the sum of collected fractions

Statistical analysis was performed by rows ($n=3$, $p<95\%$)

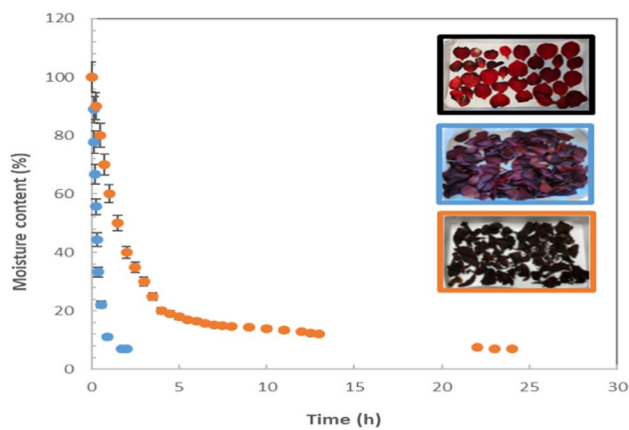


Fig. 3 Drying kinetics of *Camellia japonica* Royal Velvet variety in air oven and in MHG. The images depicted represent the flowers before (black), after oven drying (blue) and after (orange) the MHG procedure

Velvet variety dried through the conventional oven technique required 24 h at 40 °C with an energy consumption of 8540 ± 130 kJ/kg. The unidentified Y variety required 21 h and 30 min. Drying by application of MHG using sequence 3 required 80 min and demanded 520 ± 70 kJ/kg, with a reduction of 93.91%.

The reduction in time and energy consumption during flower drying with MHG was already reported [31, 32]. Lu et al. [2] reported that microwave drying on the *Bletilla striata* flowers, requiring 0.87 h, was faster than other drying technologies, such as those based on infrared, natural, hot air, vacuum, and freeze drying. However, flavonoids and polysaccharides were better retained than after freeze drying, which showed the best color, phenolic, and anthocyanin protection.

Microstructure The scanning electron microscopy confirmed a similar aspect regardless of the flower variety; in all cases, the solid microstructure exhibited a significant cell shrinkage (Fig. 4). Such an effect was expected, and examples reported for flowers from other species, i.e., in *Erica australis* flower tissue, confirmed that this effect was more marked with increasing irradiation power [5]. Microwave-dried *B. striata* flowers exhibited a more compact cell state and wrinkles after microwave drying [2]. It is well known that these microstructural changes accelerate the drying rate due to mechanical shock and internal cell destruction; in addition, it can enhance the extraction of solutes from the cells and cell walls [2].

Color features of *Camellia japonica* var. Royal Velvet flowers following oven and MHG drying Petal color is a key characteristic, as it provides valuable insight into the pigments and

chemical compounds present in a given species. Major flavonoid pigments like anthocyanins, along with other important phytochemicals, are closely associated with the coloration of plant tissues [33]. The texture and color are greatly influenced by the drying method [1, 4]. Figure 5 shows the effect of the dehydrating process using an air-convective oven drying and MHG on color parameters L^* , a^* , and b^* established by the International Commission on Illumination (CIE). L^* is a luminosity measurement, a^* with positive values indicating redness, and b^* with a positive value denotes yellowish color [35]. The comparison of CIELab* color values reveals notable differences in the surface color of *Camellia japonica* var. Royal Velvet petals dehydrated using both techniques. Petals treated with MHG exhibited significantly higher lightness ($L^* = 42.86$) compared to those dried in an oven ($L^* = 29.84$), indicating better preservation of brightness. Additionally, the a^* value was markedly higher in MHG-treated petals (43.85) than in oven-dried ones (17.48), suggesting enhanced retention of red pigments such as anthocyanins. Although both methods yielded positive b^* values, oven-dried petals showed a slightly stronger yellow hue ($b^* = 17.94$) than those treated with MHG ($b^* = 15.00$). Overall, MHG drying was more effective than conventional oven drying in preserving or enhancing the brightness and red pigmentation of the petals, while also minimizing color degradation. However, the total color differences detected among the fractions from *E. australis* were small [31].

The comparison of color magnitudes derived from the CIELab space for pink camellia flowers var. Royal Velvet dehydrated using oven drying and microwave hydrodiffusion and gravity (MHG) (Table 3) reveals significant differences in color preservation. The MHG-treated sample exhibits a much higher chroma (46.35 vs. 25.05) and saturation index (1.08 vs. 0.84), indicating more vivid and intense coloration compared to the oven-dried sample. Although both treatments retain a hue angle within the red spectrum, MHG results in a slightly redder tone. The total color difference (ΔE^*) between the two samples is 29.58 (> 3), far exceeding the threshold for perceptibility [34], which confirms a highly noticeable visual difference. Additionally, the hue difference (ΔH^*) of 15.86 suggests a distinct shift in color tone. Overall, MHG demonstrates greater effectiveness in maintaining the original color qualities of pink camellia flowers, making it a more suitable method for uses where visual appearance is essential.

Solvent extraction after MHG

Drying is required to provide samples stable during storage and, in addition, the structural degradation of the cell walls caused by microwave irradiation could enhance the further extraction of bioactives with a conventional

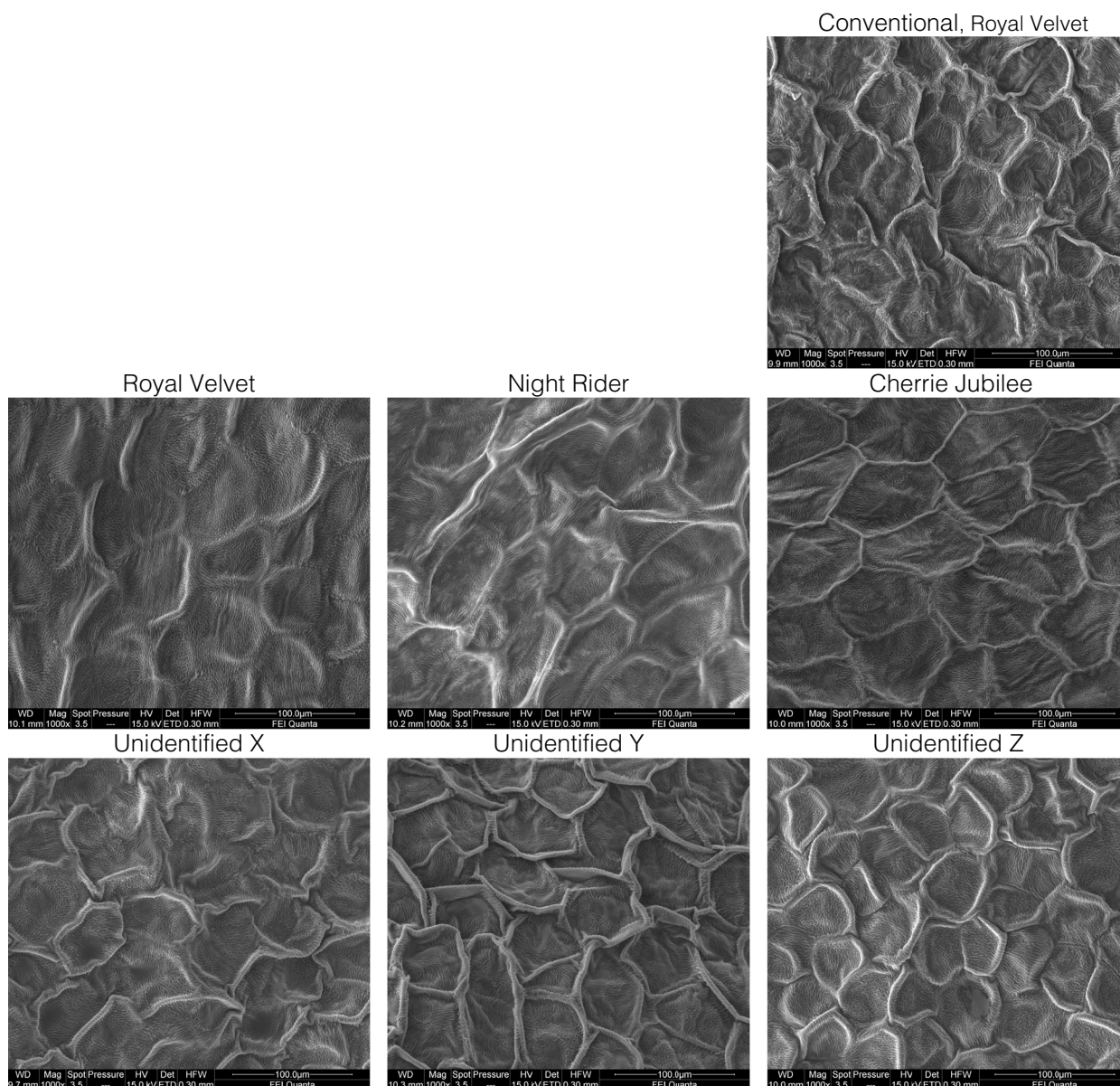


Fig. 4 Scanning electron microscopy of different varieties of *Camellia japonica* petals after treatment by microwave hydrodiffusion and gravity

(ethanol) or an alternative (supercritical carbon dioxide) solvent. Based on the results from the previous section, the MHG stage was proposed as a drying and pretreatment stage before solvent extraction of phenolics and anthocyanins. The ethanol extraction was carried out using both samples of previously dried petals (MHG extracted and oven dried), since the drained hydrolate separated during MHG operation did not contain appreciable amounts of these compounds, and in fact exhibited a clear color.

Ethanol extraction

Ethanol extraction from the MHG dried Royal Velvet solids yielded 28.4% of solubles, representing a 52.7% increase with respect to the value obtained using this procedure after air drying. Furthermore, the total phenolic content of the extract, with 0.254 g/g extract and a TEAC value equivalent to 1.47 g Trolox/g extract, compared favorably with other reported conventional extraction,

Fig. 5 Surface color parameters (L^* , a^* , b^*) of dehydrated *Camellia japonica* var. Royal Velvet flowers subjected to air-convective oven drying and microwave hydrodiffusion and gravity (MHG) treatment. Statistical analysis was performed between treatments ($n = 3$, $p < 95\%$)

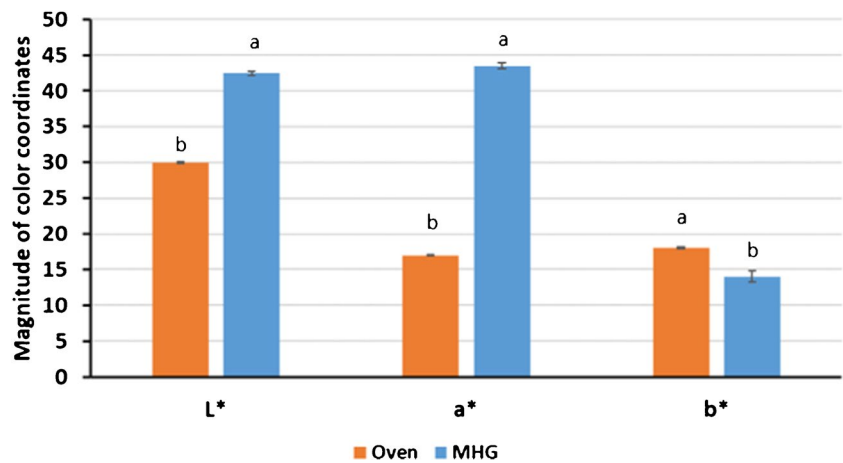


Table 3 Surface color magnitudes of *Camellia japonica* var. Royal Velvet after MHG processing

Color parameter	Oven	MHG
h°	0.8 ± 0.00^a	0.33 ± 0.02^b
C^*	25.05 ± 0.09^b	46.35 ± 0.36^a
S^*	0.84 ± 0.00^b	1.08 ± 0.01^a
ΔE^*	-	29.58 ± 0.2
ΔH^*	-	15.86 ± 0.14

Letters indicate statistical differences (in rows) between means ($n=3$, $p < 95\%$)

i.e., the flavonoid content 0.127 g/g extract in 70% ethanol extracts of *Camellia japonica* [13]. The better performance of microwave drying than other drying techniques was observed for ethanolic extracts of *Clitoria ternatea* L. petals regarding the antioxidant properties and pigment retention in the extracts [35].

The extraction of anthocyanins was also favored in this stage. The extraction time was fixed from the results of ethanol extraction kinetics from the unidentified variety X, which exhibits the lowest anthocyanin content (Fig. 6a), then a process in two stages was used to compare the different varieties. Data in Fig. 6b confirmed the highest content in Night Rider variety, in the range of values reported for other varieties [31].

Supercritical CO₂ extraction

Supercritical CO₂ extraction of *Camellia japonica* flowers from MHG (Table 4) yielded an extract with a modest recovery of 2.4%. The extract demonstrated notable antioxidant capacity, as evidenced by a TEAC value of 36.63 ± 0.07 mg/g and a FRAP value of 8.57 ± 0.07 mg/g. The extraction time was selected at 2 h, with the objective of recovering different bioactives. Several examples can be found on the successful supercritical CO₂ extraction of

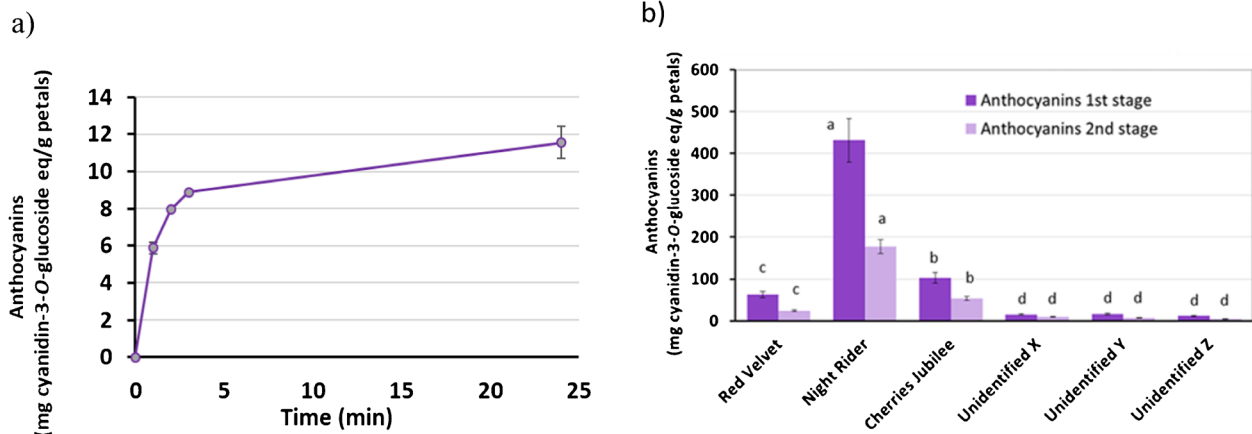


Fig. 6 **a** Kinetics of anthocyanin extraction from in *Camellia japonica* var. X and **b** extraction yields for the different varieties in first and second ethanolic extraction stages after MHG. Statistical analysis was performed by extraction stage for all tested varieties of flowers ($n = 3$, $p < 95\%$)

Table 4 Supercritical CO₂ extraction yield, antioxidant activity, and fatty acid composition of *Camellia japonica* var. Royal Velvet following microwave hydrodiffusion and gravity (MHG) processing

Performance	Yield and content (%)	Fatty acid	Content (%)
Extraction yield	2.4	Palmitic acid (C16:0)	46
TEAC value (mg Trolox eq/g)	36.63 ± 0.07	Stearic acid (C18:0)	22
FRAP value (mg ascorbic acid eq/g)	8.57 ± 0.07	Linoleic acid (C18:2)	25
		Linolenic acid (C18:3)	7

anthocyanins and other phenolic compounds, i.e., from black carrots extracted at 30.4 MPa at 52 °C during 40 min with aqueous ethanol as modifier [36], from industrial residue of juçara, using a static period of 7 min followed by a dynamic one of 39 min, with acidified 50% ethanol at 20 MPa and 60 °C [34] or from tea flower at 30 MPa with the same flow rate of CO₂ and ethanol at 30 MPa [11].

The fatty acid composition of the extract was predominantly composed of saturated fatty acids, with palmitic acid (C16:0) accounting for 46% and stearic acid (C18:0) for 22% of the total lipid content. Among the unsaturated fatty acids, the linoleic acid (C18:2) represented 25%, whereas linolenic acid (C18:3) contributed 7%. These results indicate that while the lipid fraction is dominated by saturated components, the presence of bioactive unsaturated fatty acids, combined with the strong antioxidant potential, highlights the value of *C. japonica* flower extracts obtained via supercritical CO₂ as a promising source of functional ingredients for several applications.

Compared to other plant parts, particularly the seeds, which exhibit a significantly higher lipid content ranging from 16.1 to 31.9%, the flowers have a relatively low total lipid concentration [37]. The fatty acid (FA) profile obtained in this study exhibits notable differences compared to the findings of Pereira et al. [30] who analyzed eight *Camellia japonica* flower varieties using n-hexane in Soxhlet extraction for 3 h at 68 °C with a lipid content ranging from 0.85 to 1.55 g/100 g dw. In their study, saturated fatty acids (SFAs) represented 24.39 to 33.90% of total lipids, with palmitic and stearic acids being the most prevalent, reported in the ranges of 19–25% and 1.8–3.1%, respectively. In contrast, our results showed a significantly higher SFA content, accounting for 68% of the total fatty acids, with palmitic acid at 46% and stearic acid at 22%. Additionally, certain minor SFAs reported by Pereira et al. [30], including myristic acid, arachidic acid, and behenic acid, were not detected in our extract obtained through supercritical CO₂ extraction. Moreover, monounsaturated fatty acids (MUFAs) were not detected in our samples extracted using supercritical CO₂, whereas Pereira et al. [30] reported MUFA levels between 3.8 and 4.6%, depending on the variety. As for polyunsaturated fatty acids (PUFAs), our analysis revealed a total of 32%, comprising 25% omega-6 and 7% omega-3 fatty

acids. These values are notably lower than those reported by Pereira et al., who found omega-6 levels ranging from 44.3 to 52.9% and omega-3 from 15.9 to 22.7%. Regardless of the variety and environmental conditions, the observed differences in fatty acid composition are likely attributed to the extraction method, with Pereira et al. [30] employing n-hexane as a solvent assessed for characterization purposes, whereas our study utilized a greener approach through supercritical CO₂ extraction, which holds potential for various industrial and functional applications.

Linoleic acid was identified as the predominant fatty acid across all *C. japonica* varieties examined by Pereira et al. [30], a finding that aligns with our results obtained via supercritical CO₂ extraction from a pink camellia variety. Notably, the linoleic acid content observed in our study was substantially higher than in common reference oils, such as olive oil (7.4%) and peanut oil (21.6%) [38]. This is particularly relevant considering the extensively studied bioactive effects of linoleic acid isomers, which have been linked to anticancer, anti-obesity, antidiabetic, and blood pressure-lowering properties [39]. Furthermore, linoleic acid is an essential omega-6 fatty acid widely used in the food industry as a functional ingredient [40]. The camellia samples analyzed in this study also exhibited a favorable omega-6 to omega-3 ratio (approximately 3:1), falling within the nutritionally recommended range [41], thus supporting their potential as a source of well-balanced PUFAs.

Pressurized hot water extraction using microwave treatment

Considering the high thermal sensitivity of anthocyanins, the hydrothermal processing is expected to have potential for the extraction of other bioactives from petals, and a variety with low anthocyanin content was selected. Figure 7 shows the effect of extraction temperature on the total yield, which accounted for around 50% of the petals at 160–200 °C, and the recovery of bioactive compounds from *Camellia japonica* flowers using microwave-assisted pressurized hot water extraction (PHWE), also evaluated in terms of total phenolic content (TPC), antioxidant capacity (FRAP and TEAC), and anthocyanin content. The TPC increased progressively with temperature, ranging from 139.7 mg GAE/g extract

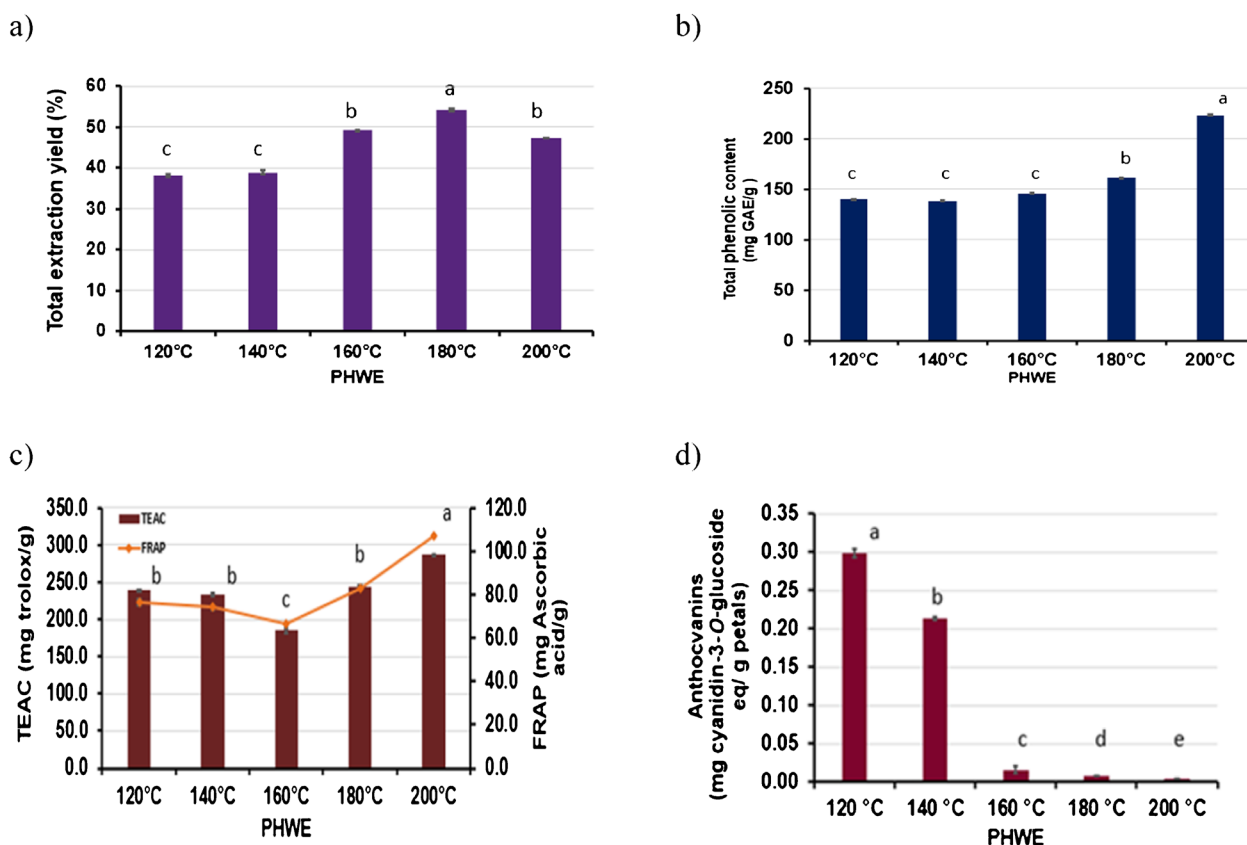


Fig. 7 **a** Total extraction yield (%), **b** total phenolic content, **c** antioxidant activity (TEAC and FRAP), and **d** anthocyanin content of *Camellia japonica* var. X extracts obtained at different final heating

temperatures (120–200 °C) using pressurized hot water extraction (PHWE). Statistical analysis was performed on tested temperatures ($n=3$, $p<95\%$)

at 120 °C to a maximum of 223.3 mg GAE/g at 200 °C (Fig. 7b). This enhancement is likely due to improved solubilization of phenolic compounds and disruption of cell structures at elevated temperatures. Antioxidant capacity, as assessed by both FRAP and TEAC assays, followed a similar trend. FRAP values rose from 76.4 to 107.1 mg equivalent ascorbic acid/g extract, whereas TEAC (Fig. 7c) increased from 239.0 to 286.7 mg equivalent Trolox/g extract across the same temperature range, indicating a strong correlation between phenolic content and antioxidant potential. As expected, the anthocyanin content (Fig. 7d) showed a marked decline with increasing temperature, dropping from 0.30 mg/g petals at 120 °C to undetectable levels at 200 °C. This suggests that anthocyanins are highly thermosensitive and degrade under high-temperature conditions. Overall, these results highlight a trade-off between maximizing antioxidant compounds and preserving heat-labile anthocyanins, since higher temperatures favor phenolic recovery but compromise pigment stability.

Previous studies conducted on eight varieties of *Camellia japonica* flowers reported significantly lower total phenolic content compared to the values obtained in the present work

using PHWE for 5 min at temperatures ranging from 120 to 200 °C [31]. In those studies, TPC levels ranged from 78.47 to 108.64 mg GAE/g extract, following heat-assisted extraction at 50 °C for 1 h in a thermostatic water bath with continuous agitation using 60% methanol as the extraction solvent. This contrast highlights the efficiency of PHWE in enhancing phenolic compound recovery from *Camellia japonica* petals. Several studies have reported comparatively lower total phenolic content in *Camellia japonica* flowers, with variations often attributed to flower color. Kanth et al. [42] found TPC values of 4.8 mg GAE/g dry weight (DW) in white flowers, 6.2 mg GAE/g DW in red, and 19.6 mg GAE/g DW in pink varieties. In contrast, Trinh et al. [10] achieved significantly higher TPC levels, ranging from 56.7 to 107.6 mg GAE/g DW, when enzymatic treatments were applied, highlighting the important role of extraction methods and sample preparation in maximizing phenolic compound yield.

Regarding total anthocyanin content reported herein at 120 and 140 °C after pressurized hot water extraction, values fall within the range illustrated in the study of Pereira et al. [30]; whereas in flowers for the pink ecotype the content

ranged from 0.02 to 1.8 mg cyanidin-3-O-glucoside/g sample, the highest value corresponding to flowers with more intense pink colors. Xiaowei et al. [43] reported higher anthocyanin levels in *Camellia japonica* flower extract, with an average content of 0.82 mg anthocyanin/g sample. The extraction was performed using an ethanol-based solution, leaching times ranging from 2 to 5 h, and temperatures between 30 and 60 °C.

In addition, the hydrothermal treatment can also be suitable to extract the polysaccharide fraction from petals, which could also exhibit interesting biological properties, as reported for flowers from other *Camellia* species [44]. Therefore, a potential sequence that could be explored in further studies could be based on the microwave hydrodiffusion and gravity process optimized in the present work, to obtain an hydrolate and pretreated solids to be further used for hydrothermal processing to extract anthocyanins, phenolics, and oligosaccharides. Data in Fig. 8 summarizes the flow diagram of the cascade processing proposed in this study. The remaining solid waste could be explored for biocomposite materials. A similar combination of supercritical CO₂ (sc-CO₂) extraction and pressurized liquid extraction

with ethanol and water has been successful for Araçáúna extraction of volatiles, phenolics, and anthocyanins [45]. This combination was also valid for roselle anthocyanins; in this case, sc-CO₂ was chosen to recover the phenolic compounds and the second step was with subcritical water at 137 °C to extract anthocyanins [18].

Antimicrobial activity

The evaluation of antimicrobial activity in floral extracts could be of interest due to the diverse phytochemical composition of flowers, some of these compounds associated with antibacterial properties [46–48]. *Staphylococcus aureus* is frequently used as a model Gram-positive pathogen in antimicrobial assays due to its clinical relevance in skin and soft tissue infections [49]. Screening floral extracts against *S. aureus* provides valuable insight into their potential for therapeutic applications.

In order to find the adequate concentration of samples to use in the assays, an initial screening was conducted, using the highest anthocyanin and phenolic content samples of the hydrothermal treatment at 120 °C and 200 °C, respectively.

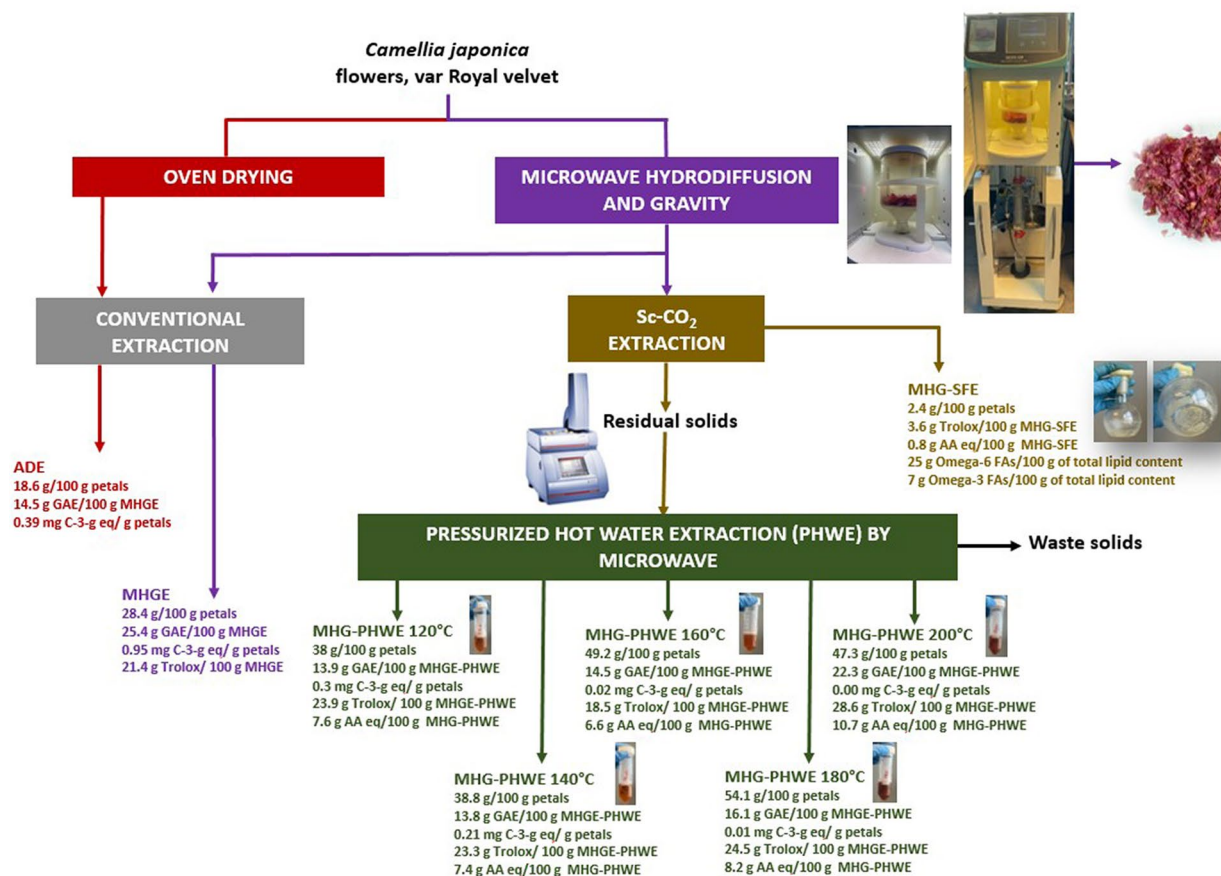


Fig. 8 Extraction yield and properties of the extract obtained from petals of *Camellia japonica* var. Royal Velvet using the technologies evaluated in this study

Concentrations between 2–8 and 0.5–8 mg/mL were used, respectively. A MIC value of 8 mg/mL was found for the 200 °C extract. Interestingly, 0.5 mg/mL increased bacterial growth. MIC value could not be determined in the 120 °C sample, but significant inhibition was found as the concentration of the extract increased.

In a further experiment, conducted using the samples from hydrothermal treatment at the different temperatures, and evaluated at 4–12 mg/mL. The MIC value was determined as 12 mg/mL for the 140 °C and 180 °C extracts. Significant inhibition was seen as the concentration increased (Fig. 9). Comparing with the literature, chamomila flower ethanolic extract was subjected to this analysis against methicillin-resistant *S. aureus*. An MIC of 6.25 mg/mL was obtained [50]. Sharma et al. [51] used *Delonix regia* flower extract with different solvents against *S. aureus* (among others), and found MIC values between 12.5 and 56.3 mg/mL. *Asphodelus fistulosus* aqueous and ethanolic crude extracts of different parts of the plant were subjected to antibacterial tests against Gram-positive and Gram-negative bacteria [52]. As to the flower extract, both aqueous and ethanolic extracts achieved a similar MIC, 2.83 mg/mL and 2.49 mg/

mL, respectively. No microdilution assay of these flowers has been reported in the literature, as to the authors' knowledge; however, Pereira et al. [46] found that these methanolic extracts of these flowers have a higher antibacterial activity against *S. aureus* compared to other Gram-positive and Gram-negative bacteria in an antibiogram assay.

LC-MS

The sample which showed the highest total phenolic content (200 °C) was selected for further analysis to profile these chemicals. The total ion chromatogram (Fig. 10) obtained was characterized by an initial region (0–3 min) of well-resolved sharp peaks, followed by a broad hump eluting between approximately 8 and 18 min. Early eluting compounds were mainly organic acids, particularly quinic acid and citric acid ($[M-H]^-$ at m/z 191 and 133, respectively), as well as a hydroxybenzoyl hexoside ($[M-H]^-$ at m/z 299), a hydroxybenzoic acid derivative [53, 54].

The later “hump” region is well known to arise from the co-elution of structurally related polyphenols and exhibited a UV-Vis maximum at 272–280 nm, consistent with tannins.

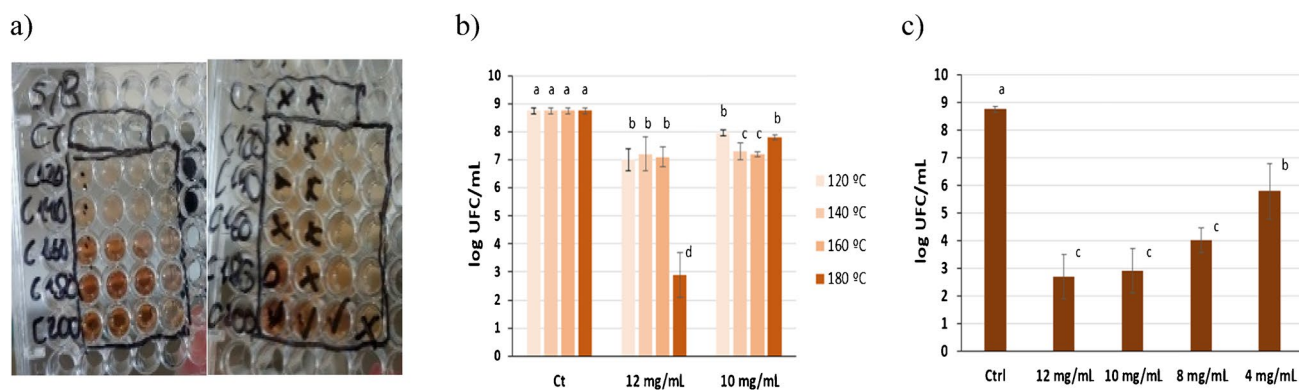
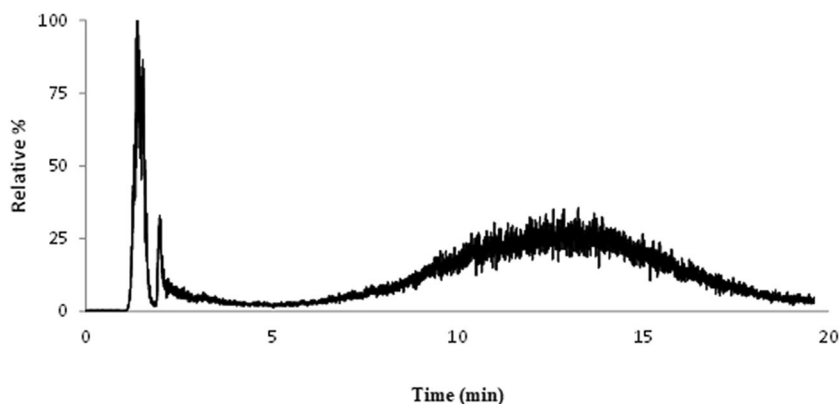


Fig. 9 Antimicrobial activity against *Staphylococcus aureus* of the pressurized hot water extracts from *Camellia japonica* var. X petals, expressed in log CFU/mL. **a** 96-well microplates. X symbols indi-

cate inhibition has not been found. **b** 120–180 °C samples. **c** 200 °C sample. Statistical analysis was performed by extract content ($n=3$, $p<95\%$)

Fig. 10 Total ion chromatogram (TIC) of the product obtained after hydrothermal treatment at 200 °C of *Camellia japonica* unidentified variety X petals previously dried by MHG and extracted by 5% ethanol modified supercritical CO₂



These included the proanthocyanidins (epi)catechin gallate ($[M-H]^-$ at m/z 441), B-type (epi)catechin dimers, trimers, and tetramers ($[M-H]^-$ at m/z 577, 865, and 1153, respectively), and a (epi)catechin-type derivative ($[M-H]^-$ at m/z 433), all characterized by a characteristic fragment ion at m/z 289 in their MS/MS spectra [55, 57]. In addition, prodelphinidins were observed, namely (epi)gallocatechin-*O*-hexoside ($[M-H]^-$ at m/z 467) and (epi)catechin-(epi)gallocatechin gallate ($[M-H]^-$ at m/z 745), both of which displayed the diagnostic fragment ion at m/z 305. An unknown hydrolyzable tannin ($[M-H]^-$ at m/z 489), suggested by a base peak at m/z 301 in its MS² spectrum, was also detected. Furthermore, a cinchonain-type tetramer i.e., a structure composed of three catechin-type flavan-3-ols linked to a phenylpropanoid unit via single carbon-carbon (B-type) bonds, was tentatively identified in this sample [55, 56]. However, its definitive characterization was hindered by the lack of fragmentation under the established MS/MS conditions.

Incorporation of anthocyanin-rich extract in starch films

The extracted potato starch used to develop the proposed films showed a yield of 35% which is consistent with the results previously found for the selected potato variety used

as raw material employing other green extraction procedures [28, 29, 58]. The extracted biopolymer exhibited high values of total starch content (92.4%) and amylose percentage (31.3%) when compared with their commercial counterparts or potato starches extracted from other potato varieties [58]. The amylose content was higher than those identified for corn (around 25%), rice (around 20%), and chestnut starches (around 22%) [28, 60].

The relative crystallinity of the extracted starch was 27.7% (Fig. 11a). This value is slightly lower than those previously reported for other varieties varying between 34.2 and 37.3% [61]. Latter authors indicated that the starch crystallinity is highly influenced by the amylose content (relatively high in this study) and amylopectin chain structure. They stated that the amylopectin is considered responsible for the starch crystallinity while amylose can interrupt the crystalline packing.

Figure 11b shows representative flow curves of the starch-based film-forming solutions. In all cases, the apparent viscosity decreased (about 2 decades) with increasing shear rate, exhibiting a characteristic shear-thinning behavior. At fixed shear rate, the apparent viscosity was lower for those forming solutions made incorporating the anthocyanin extracts. This is consistent with the literature, since the presence of hydrophilic extracts can compete for the water

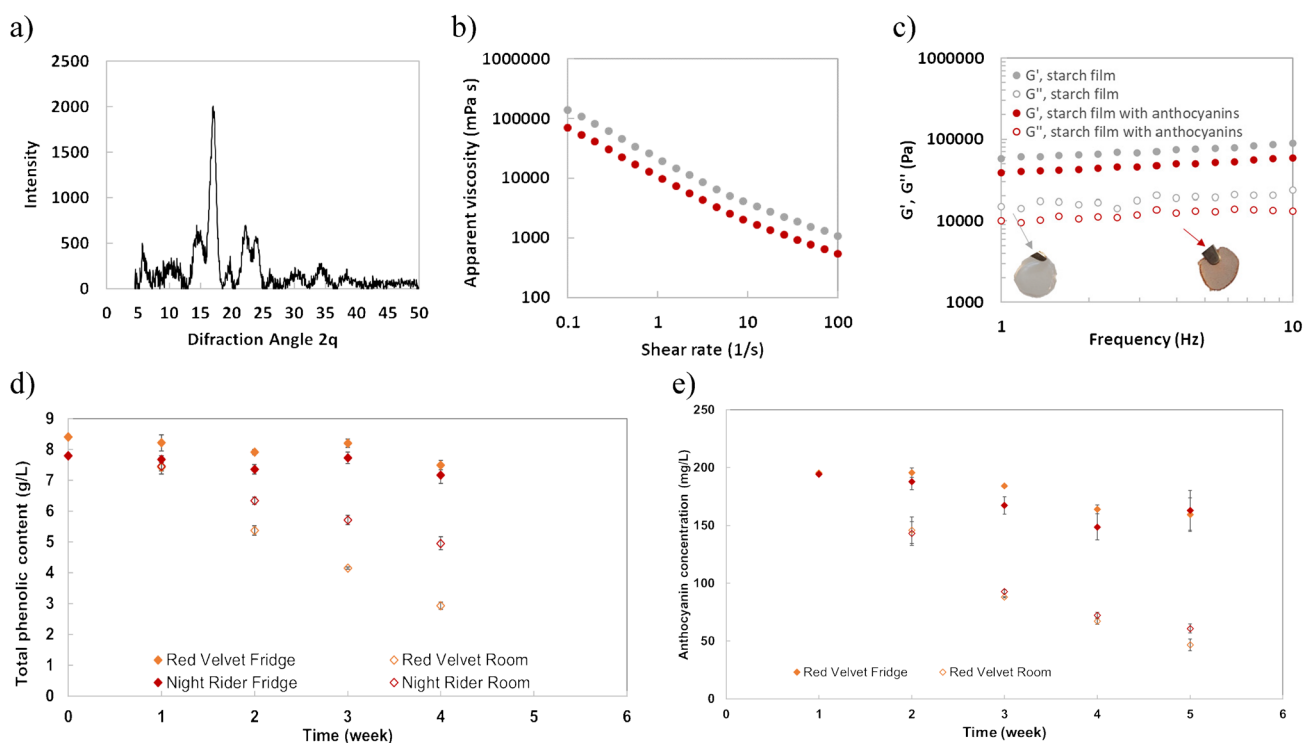


Fig. 11 Properties of the starch and the corresponding films prepared with ethanolic extracts from conventional and from MHG drying of *C. japonica* Royal Velvet variety: **a** crystallinity, **b** steady-shear measurements of the film-forming solutions, **c** small amplitude oscillatory

measurements of the developed films, stability of **d** anthocyanins and **e** phenolics during storage under room and refrigerated conditions. Note here that error bars were not included in those plots where they were lower than the symbol size

during the solubilization of the biopolymer [62]. The viscoelastic properties of the corresponding films are presented in Fig. 11c. The formulated matrices presented a typical gel behavior ($G' > G''$, both moduli almost invariant with the frequency) with intermediate strength. The magnitude of the viscous and the elastic moduli agrees with those of other biopolymer-based films enriched with bioactive fractions [63]. The color parameters varied from (L^* , 80.2 ± 2.1 ; a^* , 18.2 ± 1.3 ; b^* , 7.3 ± 0.5) for the starch-based films to [L^* , 69.3 ± 1.3 ; a^* , 49.5 ± 2.2 ; b^* , 19.4 ± 0.4] for those enriched with anthocyanins, with AE^* values higher than 3.5 indicating significant differences of color according to the classification of Adekunle et al. [34].

The stability of the films containing bioactives obtained by ethanolic extraction from the dried petal was evaluated during one month at room temperature (Fig. 11d, e). The storage temperature was highly influencing the stability of both phenolics and anthocyanins. Under refrigerated conditions, 75% of the anthocyanins and 80% of the phenolics were maintained.

Conclusions

The results confirmed the potential of green technologies for the extraction of bioactives with in vitro antioxidant properties from *Camellia japonica* flowers. An initial microwave hydrodiffusion and gravity allowed a rapid drying of the material with higher efficiency in terms of time and energy than oven drying. The separated hydrolate contained phenolic compounds with antioxidant properties. The dried solids showed enhanced yields during ethanolic extraction of anthocyanins and phenolics. Pressurized techniques were also proposed. Supercritical carbon dioxide with ethanol as modifier provided low performance, but pressurized hot water extraction allowed the solubilization of almost half of the raw material and the soluble product exhibited up to 20% phenolic purity. The antimicrobial activity obtained was similar to what was found in the literature, and tannins like epicatechin gallate and B-type epicatechin oligomers, and prodelphinidins like epigallocatechin-*O*-hexoside and epicatechin–epigallocatechin gallate, were found. The anthocyanin-rich extracts obtained in ethanol extraction could be incorporated in starch-based films for refrigerated storage.

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Julie Queffelec: investigation, visualization. Noa González-Martínez: writing—original draft, investigation, visualization, methodology. Kelly V. Kurman: writing—original draft, investigation, methodology. Adelaide Almeida: investigation, visualization, methodology, software. Rita Reis: investigation, visualization, methodology, software. Catia Vieira: investigation, visualization, methodology, software. Beatriz Díaz-Reinoso: writing—original draft, investigation, visualization, methodology. Noelia Flórez-Fernández: writing—original draft, investigation, visualization, methodology, software. M. Dolores Torres: writing—review and editing, investigation, visualization, methodology, software. Beatriz Piñeiro-Lago: writing—review and editing, visualization, conceptualization, funding acquisition. Susana M. Cardoso: writing—review and editing, visualization, conceptualization, funding acquisition. Herminia Domínguez: writing—review and editing, visualization, conceptualization, funding acquisition.

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Data availability The data supporting this study are available within the paper. Should any raw data files be needed in another format, they are available from the corresponding author upon request.

Declarations

Ethics approval This research did not involve human or animal samples.

Conflict of interest The authors declare no competing interests.

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