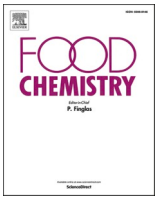
Food Chemistry 414 (2023) 135669

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Wild sweet cherry, strawberry and bilberry as underestimated sources of natural colorants and bioactive compounds with functional properties

Erika N. Vega [a,b], Patricia García-Herrera [a], María Ciudad-Mulero [a], Mª Ines Dias [b,c], Mª Cruz Matallana-González [a], Montaña Cámara [a], Javier Tardío [d], María Molina [d], José Pinela [b,c], Tânia C.S.P. Pires [b,c,e], Lillian Barros [b,c], Virginia Fernández-Ruiz [a], Patricia Morales [a,*]

[a] Dpto. Nutrición y Ciencia de los Alimentos, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, s/n, 28040 Madrid, Spain
[b] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[c] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[d] Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Finca "El Encín", Apdo. 127, 28800 Alcalá de Henares, Spain
[e] Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Science, E-32004 Ourense, Spain

ARTICLE INFO

Keywords:
Prunus avium L.
Fragaria vesca L.
Vaccinium myrtillus L.
Wild fruits
Anthocyanins food colorants
Antioxidant activity
Phenolic compounds
Natural functional ingredients

ABSTRACT

Wild edible fruits, neglected by the development of commercial agriculture, have recently aroused as a good source of natural colorants and bioactive compounds. These novel uses could cover the recent demand for healthier foods with functional properties. *Prunus avium, Fragaria vesca* and *Vaccinium myrtillus* wild fruits were characterized by individual anthocyanin profile and color CIELAB parameters, as well as phenolic fraction. In addition, some bioactivities were evaluated. In *P. avium* cyanidin-O-deoxyhexosyl-pentoside was the representative anthocyanin, in *F. vesca* pelargonidin-3-O-glucoside and in *V. myrtillus* delphinidin-O-hexoside. The three wild edible fruits showed interesting antioxidant activity especially in OxHLIA assays. *V. myrtillus* was the fruit with the best results for the bacterial growth inhibition, while *F. vesca* with better fungal growth inhibition. These results evidenced the richness of these wild fruits in bioactive compounds and pigments with antioxidant capacity, therefore, their potential use as natural colorants for healthier food products design.

1. Introduction

Wild fruits have played an important role in the survival of humanity, allowing it to obtain essential nutrients such as minerals and vitamins, as well as important bioactive compounds, among others. However, over the years, industrialization and commercial agriculture generated a decrease in their consumption leading to their depreciation (Sánchez-Mata & Tardío, 2016). In recent years, different studies have shown the great nutritional composition of wild fruits, which is associated with their beneficial health effects, such as reducing the risk of cardiovascular diseases, different types of cancers and neurological disorders, between others (Ferreira et al., 2017; Li et al., 2016; Ruiz-Rodríguez et al., 2014). These beneficial properties of wild fruits, along with their content of attractive pigments, are the reasons why there is currently a growing tendency to revalorize wild fruits as an interesting source of natural colorants. Wild sweet cherry (*Prunus avium* L.), strawberry (*Fragaria vesca* L.), and bilberry (*Vaccinium myrtillus* L.) are some fruits, which are currently studied for this purpose.

Sweet cherry (*Prunus avium*) is a vigorous tree from Rosaceae family, widely distributed around the world, and predominantly in areas with a temperate climate. Its fruits are commercially attractive due to their organoleptic characteristics (taste and color). Moreover, they have an interesting nutritional/bioactive composition, with a considerable content of phenolic compounds (anthocyanins, flavonoids, catechin and epicatechin, procyanidins, and hydroxycinnamic acids such as caffeic acid and *p*-coumaric acid derivatives), which contribute to the

* Corresponding author.
E-mail addresses: erinino@ucm.es (E.N. Vega), patrigar@ucm.es (P. García-Herrera), mariaciudad@ucm.es (M. Ciudad-Mulero), maria.ines@ipb.pt (M.I. Dias), mcmatall@ucm.es (M.C. Matallana-González), mcamara@ucm.es (M. Cámara), javier.tardio@madrid.org (J. Tardío), maria.molina.simon@madrid.org (M. Molina), jpinela@ipb.pt (J. Pinela), tania.pires@ipb.pt (T. C.S.P. Pires), lillian@ipb.pt (L. Barros), vfernand@ucm.es (V. Fernández-Ruiz), patricia.morales@farm.ucm.es (P. Morales).

https://doi.org/10.1016/j.foodchem.2023.135669
Received 21 October 2022; Received in revised form 2 January 2023; Accepted 8 February 2023
Available online 18 February 2023

antioxidant properties exhibited by this species (de Leo et al., 2021).

Wild strawberry (*Fragaria vesca*) is an herbaceous perennial plant from the Rosaceae family, whose fruits are small and sweet, and highly appreciated (Dias, Barros, Fernandes et al., 2015; Dias et al., 2017). Its fruits present vitamins (vitamin C and B_9, among others) and minerals (Mn, K, Mg, P and Ca), and they also show an interesting composition of bioactive compounds, including anthocyanins (mostly pelargonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside), flavonols (quercetin, kaemferol, fisetin), flavanols (catechin, proanthocyanidins) and phenolic acids (4-coumaric acid, ferulic acid, vanillic acid, sinapic acid) (Fierascu et al., 2020; Roy et al., 2018). The leaves and fruits of this species have been traditionally used in Europe for the treatment of external rashes and digestive diseases such as diarrhea, as well as kidney diseases (Dávila et al., 2018; Dias, Barros, Morales et al., 2015; Fierascu et al., 2020), and in the last years it has been reported that this fruit could exhibit beneficial effects in the prevention of different diseases, such as cancer, metabolic syndrome, cardiovascular diseases, obesity, diabetes, neurodegenerative processes, and microbial pathogenesis (Afrin et al., 2016; Miller et al., 2019).

Bilberry (*Vaccinium myrtillus*) is a spontaneous low-growing shrub from the Ericaceae family, native to the acid soils of temperate an artic area of Eurasia and North America (Villar, 1993). Its fruits have a great commercial importance, as they are consumed, mainly in processed products, as well incorporated in dietary supplements (Ancillotti et al., 2016). Regarding the composition of *V. myrtillus*, it can be highlighted its levels of sugars, principally glucose and fructose. These fruits also contain interesting amounts of bioactive compounds, including organic acids (mostly citric, malic and quinic), and phenolic compounds as anthocyanins (principally delphinidin-3-O-glucoside and delphinidin-3-O-galactoside), flavonoids (especially flavan-3-ols), flavonols (predominantly kaempferol, quercetin and myricetin), hydroxycinnamic acids (mostly chlorogenic acid) and derivates of stilbenes (Mikulic-Petkovsek et al., 2015; Pires, Caleja, Santos-Buelga, Barros, & Ferreira, 2020; Prencipe et al., 2014). Moreover, antioxidant, antibacterial, anti-obesity, anti-inflammatory, antiproliferative, cardioprotective and hypoglycaemic properties have been associated with *V. myrtillus* (Pires, Caleja et al., 2020).

Nowadays, consumers demand food products with added functional properties that provide health benefits. In this sense, wild edible fruits can play a key role as a healthy ingredient that can be consider a novel source of food additive (Albuquerque, Oliveira, Barros, & Ferreira, 2021; Carocho et al., 2014). It is known that fruits and their by-products are a good source of several pigments, which offer an attractive range of colors, and can be incorporated into different food products in order to improve their sensory properties. These pigments, including chlorophylls, carotenoids, anthocyanins, and betalains, are interesting not only for their potential use as natural colorants, but also due to their interesting bioactive properties (e.g., antioxidant, antimicrobial, etc.) (Albuquerque, Oliveira et al., 2021). Due to the current consumers demand targeted to consume food products with added functional properties and without synthetic additives, the main objective of this work has been to study the phenolic profile of three different wild fruits gathered in the Iberian Peninsula (*Prunus avium*, *Fragaria vesca*, and *Vaccinium myrtillus*) that could be used as novel sources of natural colorants with functional properties. To the best of the authors' knowledge there is information about chemical characterization and some data about bioactivity (mainly antioxidant activity carried out through methodologies other than QUENCHER) of similar commercial fruit, but scarce information about these specific wild species.

In order to highlight the potential use of these wild fruits for the development of new ingredients (as natural colorants) for healthier food products design. The studied wild fruits have been characterized in terms of CIELAB parameters, total and individual anthocyanins content as pigments responsible of its characteristic red-blue colors, as well as other bioactive compounds with antioxidant potential. In addition, the antioxidant, antibacterial and antifungal activities of the wild fruits under study have been evaluated.

2. Materials and methods

2.1. Samples

Mature fruits of *Fragaria vesca* L., *Prunus avium* L., and *Vaccinium myrtillus* L. were collected during 2021 from different locations from central Spain (Table 1) with harvesting permission Ref. PN_NC-032021 and Ref. ABSCH-IRCC-ES-257749–1 issued by the Spanish Ministry of Agriculture, Fisheries and Food (MAPA). The seeds of *P. avium* fruits were removed, then the peel of *P. avium* and the whole fruits of *F. vesca* and *V. myrtillus* were frozen, lyophilized and milled. Each sample was then passed through a 0.150 mm mesh to obtain a fine solid particle with a size about 0.037 mm as required for the QUENCHER methodology. The samples were stored in darkness at −4 °C for further analysis.

2.2. Physical-chemical characterization of the wild fruits

2.2.1. Moisture

Moisture determination was carried out according to AOAC loss of mass on drying method 984.25 (AOAC, 2005). Briefly, 2.5 g of the sample was weighted in triplicate in a capsule dried and weight previously and left in the oven at 100 °C for 24 h until total water elimination, then it was moved to a desiccator until room temperature and weighted again. The moisture was determined by weight difference.

2.2.2. pH

The pH determination was performed by potentiometric measure with a pH-meter Basic 20+, Crison, it was made according to the AOAC 981.12 method (AOAC, 2005). 25 mL of water were added to 2.5 g of the sample, mixed and put to rest, after 10 min the pH was measured. Each sample was determined in triplicated.

2.2.3. Titratable acidity

The titratable acidity was carried out according to the AOAC 942.15 method (AOAC, 2005) determining the acids presents in the sample through acid-base volumetric for that, the sample prepared for the pH measurement was neutralized with NaOH N/10 with known factor until a pH of 8.1. The titratable acidity was expressed as mL of NaOH 0.1 N needed for neutralized the acids contain in 100 g of sample.

2.2.4. Color Characterization: CIELAB parameters

The measurement of the color of the lyophilized samples was carried out by the tristilumus colorimetry method, based in the illumination of the sample with two rays, thus, the reflected ray pass through filters imitating the human eye (Loughrey, 2002). The measurements were made in cylindrical glass cuvettes of 5 cm of diameter and 1.3 cm of height with a colorimeter colorflex, HunterLab, under the follow specifications: CIE L*a*b* color space, through the illuminant C, 10° and 45/0° geometry. From the parameters a* and b* were determined the saturation index (C) and hue (h) applying the following equations:

$$C^*_{ab} = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$h_{ab} = \arctan(b^*/a^*) \quad (2)$$

2.3. Total polyphenols and phenolic families

The determination of the total polyphenols as well as the phenolic families (hydroxybenzoic acids, hydroxycinnamic acids, flavonols and total anthocyanin content), were made through QUENCHER (QUick, Easy, New, CHEap and Reproducible) methodology (del Pino-García et al., 2015; Fogliano et al., 1999) in which a small amount of the sample, previously homogenized until a particle size of 0.037 mm, is

Table 1
Analysed wild edible fruits: picture, collection sites and coordinates.

		
Prunus avium L. Horcajuelo de la Sierra,Madrid (Spain) 41° 03′ 47.1″ N 3° 32′ 57.2″ W	*Fragaria vesca* L. Montejo de la Sierra,Madrid (Spain) 41° 06′ 00.1″ N 3° 29′ 43.2″ W	*Vaccinium myrtillus* L. Hayedo de la Pedrosa, Segovia (Spain) 41° 12′ 58.8″ N 3° 24′ 33.8″ W

submitted to direct contact with the reagents of each determination. This methodology allows the measurement of the soluble molecules as well as the insoluble compounds, therefore, it is possible to obtain a more accurate and reliable result.

2.3.1. Determination of total polyphenols by QUENCHER methodology

The determination of total polyphenols was performed with Fast Blue BB method according to Medina (2011) and modified by Palombini et al. (2016) with some modifications. Briefly, 0.4 mL of 0.1 % fast blue BB was added to 1 ± 0.5 of the sample, it was homogenized by vortex, 0.4 mL of 5 % NaOH was added and mixed by vortex followed by the addition of 4 mL of distilled water, mixed by vortex again and incubated for 45 min in an orbital shaker, then it was centrifugated at 6500 rpm for 10 min and filtered, finally the absorbance was measured at 420 nm in an UV–vis spectrometer, synergy HTX, Biotek. All the determinations were carried out in triplicate. Calibration curve was obtained using different concentrations of gallic acid (0.625 – 160 μg/mL), hence, the results were expressed as milligrams of gallic acid equivalent per one hundred grams (mg GAE/100 g fw).

2.3.2. Determination of hydroxybenzoic acids by QUENCHER methodology

The determination of hydroxybenzoic acids (HBC) was carried out according to Bonoli, et al. (2004) with some modifications. Scarcely, 1 ± 0.5 mg of the sample was weighted in a falcon tube, 0.5 mL of distilled water and 4 mL of formic acid 3 % were added, the reaction was homogenized with a vortex and incubated for 15 min in an orbital shaker at room temperature, then it was centrifugated at 65,000 rpm for 5 min and filtered, the absorbance was measured at 280 nm in quartz cuvettes using an UV–vis spectrometer synergy HTX, Biotek. Each sample was made by triplicated. A gallic acid calibration curve was obtained through reaction of different concentration of the standard (3.125 – 400 μg/mL), hence, the results were expressed as milligrams of gallic acid equivalent per one hundred grams (mg GAE/100 g fw).

2.3.3. Determination of hydroxycinnamic acids by QUENCHER methodology

The determination of hydroxycinnamic acids (HCC) was performed according to Bonoli, et al. (2004). In brief, 1 ± 0.5 mg of the sample was weighted, and it was added 0.5 mL of distilled water and 4 mL of methanol, it was homogenized with vortex and incubated in an orbital shaker, after 15 min, the sample was centrifugated at 6500 rpm for 5 min and filtered. The absorbance was measure at 320 nm in an UV–vis spectrometer, synergy HTX, Biotek. All the determinations were carried out in triplicate. The results were expressed as milligram of ferulic acid equivalent per one hundred grams (mg FAE/100 g fw), as a calibration curve was obtained by reaction of different concentration (3.125 –200 μg/mL) of the standard.

2.3.4. Determination of flavonols by QUENCHER methodology

The determination of flavonols (FC) was adapted from the methodology proposed by Bonoli, et al. (2004). Briefly, 0.5 mL of distilled water and 4 mL of methanol were added to 1 ± 0.5 mg of the sample weighted in triplicate, then it was homogenized by vortex and incubated at room temperature an orbital shaker, after 15 min the sample was centrifugated for 15 min at 6500 rpm and filtered. The absorbance of the sample was measure at 370 nm with a UV–vis spectrometer, synergy HTX, Biotek. A calibration curve of quercetin was obtained with different concentrations of the standard (3.125 – 250 μg/mL). The results were expressed in milligrams of quercetin equivalent per one hundred grams (mg QE/100g fw).

2.3.5. Determination of pigments: Total anthocyanin content by QUENCHER methodology

The total anthocyanin content (TAC) was carried out through spectrophotometric methodology. Scarcely, 10 ± 0.1 mg were weight six times in different tubes, in three of them was add 10 mL of KCl buffer, pH 1.0 and in the other three was add 10 mL of CH_3CO_2Na, pH 4.5, then the six tubes were homogenized by vortex and left 15 min in orbital shaking, after which the tubes were centrifugated for 5 min to 7000 rpm and filtered. The absorbance was measure at 510 and 700 nm with a UV–vis spectrometer, synergy HTX, Biotek. For the calculations was used a calibration curve of cyanidin-3-O-glucoside, it was obtained with concentrations from 3.125 to 500 μg/mL of the standard. Hence, the results were expressed as milligrams of cyanidin-3-O-glucoside per one hundred grams of sample (mg cyd-3-glu/100 g fw).

2.4. Individual anthocyanin's identification

For the identification of the individual anthocyanins profile was previously made an extraction of 1 g of each sample with ethanol: water (80:20). The extract obtained was freeze dried and 5 mg of the extract were dissolved in 1 mL of water, then it was filtered. As previously described by Vega et al. (2021) the dissolution was injected in an HPLC equipment (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) coupled to a diode-array detector and an electrospray ionization mass spectrometer (Linear Ion Trap LTQ XL, Thermo Scientific) working in positive mode. An AQUA® reverse phase C18 column (5 μm, 150 mm × 4.6 mm, Phenomenex) was used at 35 °C for compound separation with gradients previously described (Vega et al., 2021). The anthocyanin identification was achieved by comparison of their retention time, UV–vis and mass spectra with standards and literature data. The quantification was performed through seven levels calibration curves obtained of different standards compounds and the results were expressed in mg/g of extract.

2.5. Bioactive properties of wild fruits

2.5.1. Antioxidant activity

The antioxidant activity was performed by using three different *in vitro* direct methods (QUENCHER procedure) applied to Folin-Ciocalteu, FRAP and DPPH methodology. Moreover, the *ex vivo* antioxidant activity was measured using the cell-based oxidative haemolysis inhibition assay (OxHLIA).

2.5.1.1. Folin-Ciocalteu assay. The capacity of the samples of reduce the Folin-Ciocalteu reagent was carried out according to del Pino-García et al. (2015). Concisely, 1 ± 0.5 mg of each sample was weight in triplicate, 0.2 mL of distiller water and 0.2 mL of Folin-Ciocalteu reagent were added, then it was homogenized by vortex and left in an orbital shaker for 5 min, after which it was added 4 mL of Na_2CO_3 and 5,6 mL of distiller water and homogenized by vortex, after 50 min of orbital shaken the sample was centrifugated for 5 min at 6500 rpm and filtered. The absorbance was measure at 750 nm in a UV–vis spectrometer, synergy HTX, Biotek. The results obtained were expressed in milligrams of gallic acid equivalent per one hundred grams of sample (mg GAE/100g), as a calibration curve was obtained by reaction of different concentrations (50 – 400 μg/mL) of the standard.

2.5.1.2. FRAP assay. The ferric reducing power by the samples were determined according to del Pino-García et al. (2015). Briefly, 10 mL of FRAP reagent was added to 1 ± 0.5 mg of the sample (weighed in triplicate), then it was homogenized by vortex and incubate at 37 °C by 30 min with continuous agitation, after which it was centrifugated at 7000 rpm for 5 min and filtrated. The absorbance was measure at 595 nm in an UV–vis spectrometer, synergy HTX, Biotek. A calibration curve was made with trolox (3.125 – 200 μg/mL), hence, the results were expressed as milligrams of trolox equivalent per one hundred grams of sample (mg TE/100g).

2.5.1.3. DPPH assay. The scavenging capacity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by the sample was carried out according to del Pino-García et al. (2015). 1 ± 0.5 mg was weighted by triplicated, then 10 mL of DPPH reagent were added, and it was homogenized by vortex, after 1 h of orbital shaking the sample were centrifugated for 5 min at 7000 rpm and filtered. The absorbance was measure at 517 nm in an UV–vis spectrometer, synergy HTX, Biotek. The results were expressed as milligrams of trolox equivalent per one hundred grams of sample (mg TE/100g) as a calibration curve was obtained by reaction of different concentration (6.25 – 400 μg/mL) of the standard.

2.5.1.4. OxHLIA assay. The antihaemolytic activity of the extracts was evaluated according to Lockowandt et al. (2019). An erythrocyte solution (2.8 %, v/v; 200 μL) prepared in phosphate-buffered saline (PBS, pH 7.4) was mixed with 400 μL of either: extract solution (4.69–600 μg/mL in PBS); PBS (negative control); distilled water (baseline); or the positive control trolox (7.81–250 μg/mL in PBS). After 10 min pre-incubation at 37 °C with shaking, 200 μL of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 160 mM in PBS) was added and the optical density was measured at 690 nm in a ELx800 microplate reader (Bio-Tek Instruments, Winooski, VT, USA) over time until complete haemolysis. The results were expressed as IC_{50} value in μg/mL, which is the extract concentration capable of promoting a Δt haemolysis delay of 60.

2.5.2. Antibacterial activity

The extracts were tested against eight foodborne bacteria, the Gram-negative *Enterobacter cloacae* (ATCC 49741), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica serotype Enteritidis* (ATCC 13076) and *Yersinia enterocolitica* (ATCC 8610) and the Gram-positive Bacillus cereus (ATCC 11778), *Listeria monocytogenes* (ATCC 19111) and *Staphylococcus aureus* (ATCC 25923). All these microorganisms are purchase at Frilabo, Porto, Portugal. The bacteria were incubated at 37 °C in an appropriate fresh medium, for 24 h before analysis to maintain the exponential growth phase.

The Minimum Inhibitory Concentration (MIC) determinations on all bacteria were conducted using colorimetric assay according to described by Pires et al. (2018). The samples were first dissolved in 5 % (v/v) Dimethyl sulfoxide (DMSO) and 95 % of autoclaved distilled water to give a final concentration of 20 mg/ mL for the stock solution. 90 μL of this concentration was added in the first well (96-well microplate) in duplicate with 100 μL of Tryptic Soy Broth (TSB). In the remaining wells 90 μL of TSB medium were added. Then the samples were serially diluted to obtain the concentration ranges (10 to 0.03125 mg/mL). To finish, 10 μL of inoculum (standardized at 1.5×10^6 Colony Forming Unit (CFU)/mL) was added at all the well assuring the presence of 1.5×10^5 CFU. Two negative controls were prepared, one with TSB and another one with the extract. Two positive controls were prepared with TSB and each inoculum, and another with medium, antibiotics, and bacteria. Ampicillin and Streptomycin were used for all bacteria tested and Methicillin was also used for *Staphylococcus aureus*. The microplates were incubated at 37 °C for 24 h. The MIC of samples was detected following addition (40 μL) of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT) and incubation at 37 °C for 30 min. MIC was defined as the lowest concentration (mg/mL) that inhibits the visible bacterial growth determinate by change the colouration from yellow to pink if the microorganisms are viable.

For the determination of Minimum Bactericidal Concentration (MBC), 10 μL of liquid from each well that showed no change in color was plated on solid medium, Blood agar (7 % sheep blood) and incubated at 37 °C for 24 h. The lowest concentration that yielded no growth determine the MBC. MBC was defined as the lowest concentration (mg/mL) required to kill bacteria.

2.5.3. Antifungal activity

Antifungal activity was performed according to described by Heleno et al. (2013) *Aspergillus fumigatus* (ATCC 204305) and *Aspergillus brasiliensis* (ATCC 16404) were used. The organisms were obtained from Frilabo, Porto, Portugal. The micromycetes were maintained on malt agar and the cultures stored at 4 °C and were further placed in new medium and incubated at 25 °C for 72 h. To investigate the antifungal activity, the fungal spores were washed from the surface of agar plates with sterile 0.85 % saline containing 0.1 % Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μL per well. The samples were first dissolved in 5 % (v/v) Dimethyl sulfoxide (DMSO) and 95 % of autoclaved distilled water to give a final concentration of 20 mg/mL for the stock solution. Afterwards, 90 μL of this concentration was added in the first well (96-well microplate) in duplicate with 100 μL of Malt Extract Broth (MEB).

In the remaining wells 90 μL of medium MEB were placed. Then the samples were serially diluted to obtain the concentration ranges (10 to 0.03125 mg/mL). Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microplate. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The fungicidal concentration (MFC) was determined by serial subcultivation of 2 μL of tested extracts dissolved in medium and inoculated for 72 h, into microplates containing 100 μL of MEB per well and further incubation 72 h at 26 °C. The lowest concentration (mg/mL) with no visible growth was defined as MFC indicating 99.5 % killing of the original inoculum. Commercial fungicide ketoconazole (Frilabo, Porto, Portugal), was use as positive control.

2.6. Statistical analysis

All assays carried out were made by triplicated and the results were

presented as mean values ± standards deviation. Duncan's multiple comparison test was applied to identify the differences among the sets of means. All the analysis were performed using the XLSTAT program (Addinsoft, 2022. New York, USA) using a significance level of 0.05.

3. Results and discussion

3.1. Physicochemical characteristics and color parameters of the analyzed wild fruits

Table 2 shows the results of the physicochemical characterization of the analyzed wild fruits. All the samples presented a high moisture content (80.52, 83.58 and 79.48 g of water per 100 g of fruit in *Prunus avium, Fragaria vesca*, and *Vaccinium myrtillus*, respectively). Regarding pH values, all the samples had an acid pH, with values between 3 and 4 and statistically significant differences ($p < 0.05$) were observed among them. The obtained results are in accordance with those found by other authors who have previously studied the physicochemical characterization of *P. avium* (Bastos et al., 2015; Hayaloglu & Demir, 2015; Kalyoncu et al., 2009; Karlidag et al., 2009), *F. vesca* (Aslantas et al., 2007; Dias et al., 2016; Yildiz et al., 2014) and *V. myrtillus* (Aliman et al., 2020).

Regarding color parameters, all the fruits presented values towards the reds (a^*), especially *F. vesca*. In the same way, the three wild fruits were in positives values in parameter b^*, thus, they were in the yellow range, especially *P. avium*, however, *V. myrtillus* was in the lowest values of yellow, being very close to the blue range, this may be the reason of its dark purple color. *P. avium* also presented the highest values of saturation index and hue, followed by *F. vesca* and with the lowest values *V. myrtillus* in both cases. The lightest fruits were those of *P. avium* with the highest L^*, followed by those of *F. vesca*, whereas the fruits of *V. myrtillus* were those with the darkest color.

The color parameters of *P. avium* peel agreed with the surface color measured previously reported by Hayaloglu & Demir (2015) (L^*: 18.95 – 63.74; a^*: 1.86 – 29.46; b^*: 6.00 – 27.00) in 12 different wild sweet cherry cultivars from Turkey. In the case of whole *F. vesca* fruit, the luminosity achieved (56.66) was higher than reported previously by de Bubba et al. (2016) (34 –44) for commercial *F. vesca* fruits recollected in different years and different soils from Italy. The parameters a^* (23.59) and b^* (13.33) were under the ranges reported by the same author (a^*: 29 – 39; b^*: 22 – 40). Lastly, the values of a^* and b^* obtained in this work for *V. myrtillus* (17.02; 7.01, respectively) were higher than those shown by Celik et al. (2018) for wild and commercial bilberries (*Vaccinium corimbosum* L.) cultivated in different years in Turkey (a^*: 0.50 – 0.68; b^*: 0.89 – 4.71). Though, L^* value obtained (25.66) was similar to the commercial variety (L^*: 32.4).

Table 2
Physicochemical characterization of the analyzed wild fruits.

	Prunus avium L.	*Fragaria vesca* L.	*Vaccinium myrtillus* L.
Moisture (g/100 g fw)	80.52 ± 0.07^{b}	83.58 ± 1.35 ^{a}	79.48 ± 1.55^{b}
pH	4.01 ± 0.02 ^{a}	3.62 ± 0.01^{b}	3.35 ± 0.02^{c}
Titratable acidity (mL NaOH/100 g fw)	173.13 ± 6.71^{b}	240.87 ± 12.76 ^{a}	157.79 ± 3.81^{b}
	Color parameters		
L^*	62.88 ^{a}	56.66^{b}	25.66^{c}
a^*	12.68^{c}	23.59 ^{a}	17.02^{b}
b^*	24.11 ^{a}	13.33^{b}	7.01^{c}
C^*	27.24 ^{a}	27.09^{b}	18.41^{c}
h	62.26 ^{a}	29.47^{b}	22.39^{c}

L^*: luminosity, a^*: red – green, b^*: yellow – blue. Different small superscript letters in each line mean statistically significant differences ($p < 0.05$) compared by Duncan's test.

3.2. Total polyphenols and phenolic families in the analyzed wild fruits

In the present study, the total polyphenols as well as the content of different families of phenolic compounds were analyzed, and the results are shown in Table 3. Statistically significant differences ($p < 0.05$) were observed among the studied wild fruits. It was noticed that *P. avium* was the species which contained the lowest ($p < 0.05$) values of total polyphenols (TP), total anthocyanin content (TAC), hydroxybenzoic acids content (HBC) and flavonols content (FC). On the contrary, the highest values ($p < 0.05$) of TP, HBC and FC were found in *F. vesca*, while *V. myrtillus* was the species richest ($p < 0.05$) in TAC and hydroxycinnamic acids content (HCC).

The TP in *P. avium* (361.90 mg GAE/100 g, fw) was slightly above the ranges previously reported by other authors in cultivated sweet cherries (35.6 – 278 mg/100 g, fw) (Ballistreri et al., 2013; Gonçalves et al., 2004; Hu et al., 2021; Schmitz-Eiberger & Blanke, 2012). Regarding the FC, the obtained results showed values of 21.95 mg QE/100 g (fw) in *P. avium*. These concentrations were slightly lower that those found by Hu et al. (2021), who studied the phenolic content and antioxidant potential of four different sweet cherry Australian cultivars and reported values of total flavonoid content between 31 and 51 mg QE/100 g (fw). It is known that the content of phenolic acids differs between cultivars, year, ripeness stage, and storage conditions (Gonçalves et al., 2004). In the present study, the total amount of phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids) in *P. avium* was 206.73 mg GAE/100 g (fw). Other authors have reported values of total phenolic acids, determined by Folin-Ciocalteu procedure, between 46.9 and 222 mg GAE/100 g (fw) in different cultivars of sweet cherries from Portugal (Gonçalves et al., 2004). The results obtained in the present work showed that HBC was higher compared to HCC. On the other hand, regarding anthocyanin content, as can be seen in Table 2, the TAC of *P. avium* was 4.67 mg cya-3-glu/100 g (fw), which was in accordance with values previously reported by Usenik et al. (2008) (1.15 – 16.2 mg cyanidin 3-glucoside equivalents (CGE) per 100 g of fresh cultivated sweet cherry). However, other authors have reported higher content of total anthocyanins in cultivated sweet cherries (6.21 – 94.20 mg CGE/100 g, fw) (Ballistreri et al. 2013). These differences could be attributed to the employed methodology, since according to the scientific literature, it is possible to conclude that the amounts of TAC found by QUENCHER methodology, were lower than those obtained by HPLC technique (Bastos et al., 2015; Schmitz-Eiberger & Blanke, 2012).

Regarding the achieved results for wild *Fragaria vesca* and comparing them with previous studies, it was observed that the analyzed fruits of these species presented a highest amount of TP (1315.10 mg GAE/100 g, fw) than those previously reported by Peñarrieta et al. (2009) who reported TP values from 165.01 to 364.02 mg GAE/100 g (fw) for wild

Table 3
Profile of phenolic compounds present in the analyzed wild fruits, determined by QUENCHER methodology.

	Prunus avium L.	*Fragaria vesca* L.	*Vaccinium myrtillus* L.
TP (mg GAE/100 g fw)	361.90 ± 21.55^{c}	1315.10 ± 94.22 ^{a}	1151.96 ± 113.21^{b}
HBC (mg GAE/100 g fw)	132.16 ± 2.44^{c}	293.32 ± 6.94 ^{a}	281.47 ± 1.99^{b}
HCC (mg FAE/100 g fw)	74.57 ± 1.78^{b}	60.34 ± 3.16^{c}	102.12 ± 2.18 ^{a}
FC (mg QE/100 g fw)	21.95 ± 1.68^{c}	106.79 ± 5.52 ^{a}	89.74 ± 4.32^{b}
TAC (mg cya-3-glu/100 g fw)	4.67 ± 0.30^{c}	25.56 ± 2.04^{b}	210.06 ± 10.23 ^{a}

TP: Total polyphenols, HBC: Hydroxybenzoic acids content, HCC: Hydroxycinnamic acids content, FC: Flavonols content, TAC: Total anthocyanin content, cya-3-glu: cyanidin-3-*O*-glucoside; GAE: gallic acid equivalent; FAE: ferulic acid equivalent; QE: quercetin equivalent. Values were expressed in fresh weight. Different small superscript letters in each line mean statistically significant differences ($p < 0.05$) compared by Duncan's test.

strawberries from Bolivia, or Yildiz et al. (2014), who found values from 138 to 228 mg GAE/100 g (fw) in wild strawberries from 15 locations from Turkey. In the present study, FC content was measured using QUENCHER methodology (spectrophotometric method) obtaining higher values (106.79 mg/100 g, fw) comparing with those reported by other authors as Häkkinen & Törrönen (2000), in 14 different cultivars from Poland and Finland (36.1 – 61.1 mg/100 g, fw) through HPLC. The wide difference can be due to the use of different methods of determination, QUENCHER method allows the quantification of extractable and non-extractable phenolic compounds, thus obtaining more reliable results. In regard to the phenolic acids, in the present study, a total amount of 353.66 mg GAE/100 g, (fw) was found, where the HBC (293.32 mg GAE/100 g, fw) was significantly higher (almost five times) than hydroxycinnamic acids content (HCC, 60.34 mg FAE/100 g, fw). Other authors have reported values of total phenolic acids in wild strawberry fruit from 164.8 to 284.0 mg of caffeic acid/100 g (fw) (Dyduch-Siemińska et al., 2015; Najda et al., 2014) or 0.04 mg/100 g determined by HPLC (Roy et al., 2018). The total anthocyanins content (TAC) obtained (25.56 mg cya-3-glu/100 g, fw) was slightly under the ranges reported by Yildiz et al. (2014) in wild strawberries (31.64 – 51.78 mg pelargonidin-3-glucoside/100 g, fw), however, it was above the values reported by Roy et al. (2018) in cultivated *F. vesca* (8.36 mg/100 g, fw) by HPLC.

Regarding *Vaccinium myrtillus* wild fruits, a higher amount of total polyphenols was obtained (TP, 1151.96 mg GAE/100 g, fw) comparing with the previously reported by other authors in wild (147.5 – 536.0 mg/100 g, fw) bilberries (Mikulic-Petkovsek et al., 2015), being hydroxybenzoic acids the majoritarian phenolic fraction (HBC, 281.47 mg/100 g, fw). On the other hand, the total anthocyanins content (TAC, 210.06 mg cya-3-glu/100 g, fw), hydroxycinnamic acids content (HCC, 102.12 mg/100 g, fw) and flavonols content (FC, 89.74 mg QE/100 g, fw) were in accordance with the amounts reported by different authors (Laaksonen et al., 2010; Mikulic-Petkovsek et al., 2015; Miller et al., 2019; Neamtu et al., 2020; Pires, Dias et al., 2020).

In general, *V. myrtillus* showed the best polyphenol profile, followed by *F. vesca* and *P. avium*. It was also showed that in most of the assays carried out in this study, a higher amount was observed comparing with other authors studies, which could be due to the use of QUENCHER methodology.

3.3. Anthocyanins profile in the analyzed wild fruits

Table 4 shows the peak characteristics, tentative identifications, and quantification of individual anthocyanins in hydroethanolic extracts prepared from the analyzed wild fruits (*Prunus avium*, *Fragaria vesca*, and *Vaccinium myrtillus*). In addition, the chromatographic profiles of anthocyanins found in the studied samples are shown in Fig. 1.

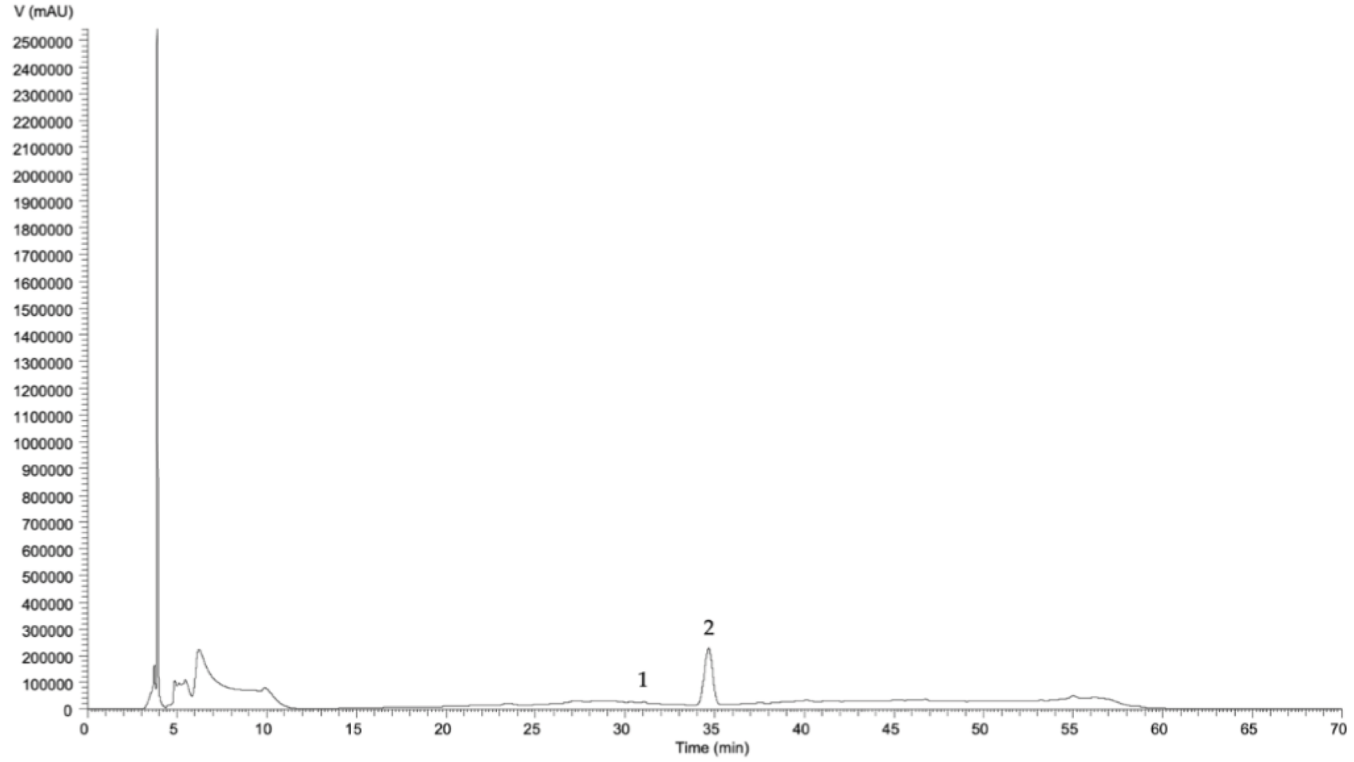
Only two different anthocyanins were identified in *P. avium*, and consequently, it was the wild fruit that presented the lowest amount of these compounds. Nevertheless, the total anthocyanin quantified was higher than the previously reported (Bastos et al., 2015). Both anthocyanins found were cyanidin derivatives, being peak 1 ($[M]^+$ at *m/z* 449) identified by comparing the mass spectral data and UV-spectra with the available standard compound. The same compound was found by Bastos et al. (2015) in *P. avium*. Peak 2 presented a protonated ion ($[M]^+$ at *m/z* 449) and a unique MS^2 fragment at *m/z* 287 (cyanidin aglycone) corresponding to the loss of a subsequent loss of a deoxyhexosyl ($[H\text{-}146]^+$) and pentosyl moieties ($[H\text{-}132]^+$) leading to a possible identification as cyanidin-*O*-deoxyhexosyl-pentoside, being the anthocyanin found in higher amount (3.006 ± 0.001 mg/g extract).

Regarding to *F. vesca*, five anthocyanins were found, derivatives of cyanidin (peaks 1′ and 2′), pelargonidin (peaks 3′ and 4′) and peonidin (peak 5). Peaks 2′ ($[M]^+$ at *m/z* 449) and 3′ ($[M]^+$ at *m/z* 433) were identified as cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside, respectively, by comparison with authentic standards. Both compounds are commonly found in strawberries (Dias et al., 2016; Sun et al., 2014), the latter being the major one. Peak 1′ ($[M]^+$ at *m/z* 611), a second cyanidin derivative with a MS^2 product ion at *m/z* 287 ($[M-162-162]^+$) was tentatively identified as cyanidin-*O*-hexosyl-*O*-hexoside. A second pelargonidin derivative (peak 4′, $[M]^+$ at *m/z* 463) was also found, with some representativeness in the sample, 6.194 ± 0.003 mg/g extract, but unfortunately was not possible to reach a tentative identification of the compound due to the inability to identify the moiety lost between the protonated ion and aglycone fragment. Finally, peak 5′, the only petunidin derivative found (*m/z* at 317),

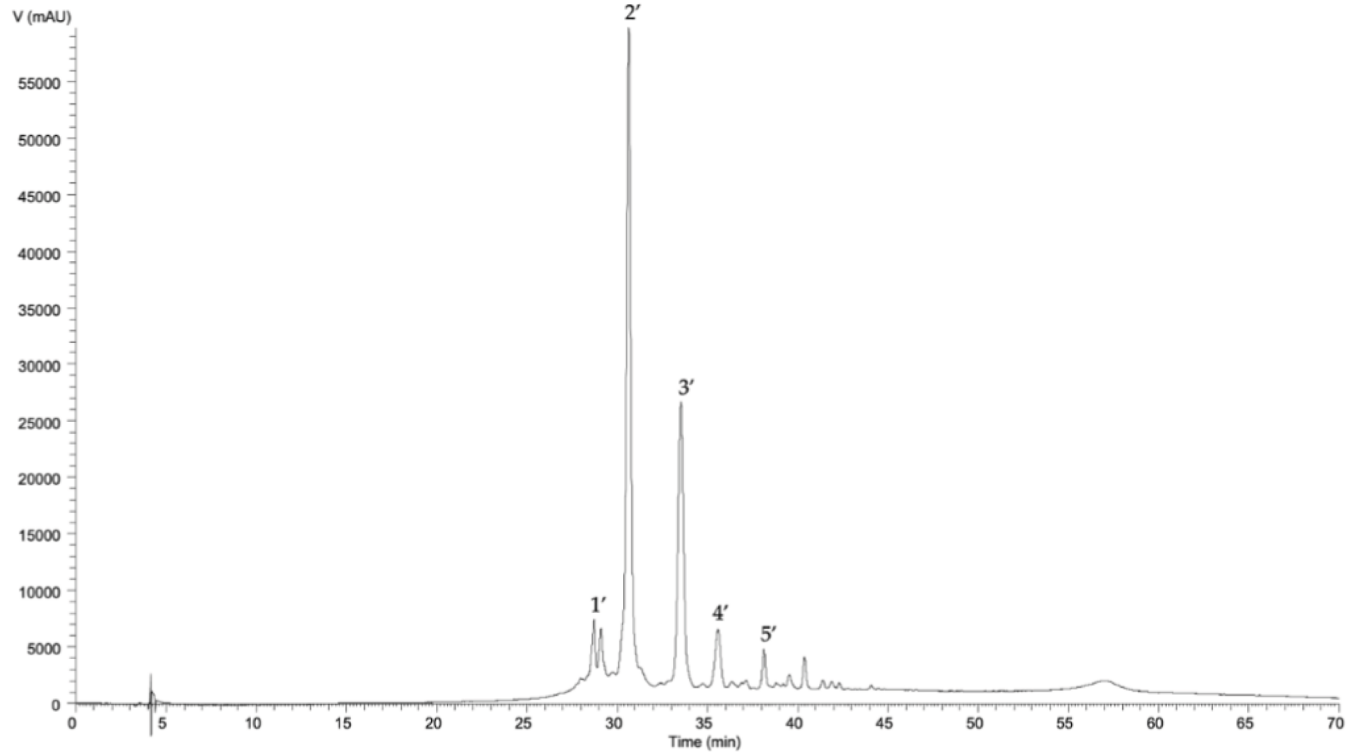
Table 4
Individual anthocyanin identification of *Prunus avium*, *Fragaria vesca* and *Vaccinium myrtillus* extract.

Peak	Rt	UV	$[M]^+$	MS^2	Tentative identification	mg/g extract, dw
Prunus avium L.						
1 [A]	30,43	516	449	287 (100)	Cyanidin-3-*O*-glucoside	2.964 ± 0.0002
2 [A]	31,11	516	565	287 (100)	Cyanidin-*O*-deoxyhexosyl-pentoside	3.006 ± 0.001
					Total anthocyanins	5.970 ± 0.001
Fragaria vesca L.						
1′ [A]	28,61	517	611	449 (12), 287 (100)	Cyanidin-*O*-hexosyl-*O*-hexoside	2.9799 ± 0.0002
2′ [A]	30,67	515	449	287 (100)	Cyanidin-3-*O*-glucoside	3.400 ± 0.001
3′ [B]	33,54	502	433	271 (100)	Pelargonidin-3-*O*-glucoside	6.800 ± 0.004
4′ [B]	35,6	517	463	271 (100)	Pelargonidin derivative	6.194 ± 0.003
5′ [C]	38,115	517	449	317 (100)	Petunidin-*O*-pentoside	0.059 ± 0.001
					Total anthocyanins	19.43 ± 0.01
Vaccinium myrtillus L.						
1″ [A]	28,77	523	465	303 (100)	Delphinidin-*O*-hexoside	3.87 ± 0.02
2″ [A]	28,82	523	465	303 (100)	Delphinidin-*O*-hexoside	4.47 ± 0.01
3″ [A]	29,75	520	449	287 (100)	Cyanidin-*O*-hexoside	3.71 ± 0.02
4″ [A]	30	523	435	303 (100)	Delphinidin-*O*-pentoside	3.43 ± 0.01
5″ [A]	30,64	516	449	287 (100)	Cyanidin-3-*O*-glucoside	4.02 ± 0.01
6″ [C]	31,02	522	479	317 (100)	Petunidin-*O*-hexoside	0.417 ± 0.001
7″ [C]	32,15	522	479	317 (100)	Petunidin-*O*-hexoside	1.99 ± 0.01
8″ [C]	33,91	522	463	301 (100)	Peonidin-*O*-hexoside	0.187 ± 0.004
9″ [C]	34,48	525	449	317 (100)	Petunidin-*O*-pentoside	0.304 ± 0.004
10″ [C]	35,6	519	463	301 (100)	Peonidin-*O*-hexoside	0.705 ± 0.002
11″ [C]	36,87	525	493	331 (100)	Malvidin-3-*O*-glucoside	1.02 ± 0.02
12″ [C]	38,51	525	463	331 (100)	Malvidin-*O*-pentoside	0.197 ± 0.004
13″ [A]	39,66	525	477	287 (100)	Cyanidin derivative	2.986 ± 0.004
14″ [C]	45,22	522	301		Peonidin	0.061 ± 0.001
15″ [C]	45,77	525	331		Malvidin	0.043 ± 0.001
					Total anthocyanins	27.370 ± 0.003

In the Peak Column, each capital letter means the calibration curve used for quantification. A- Cyanidin-3-*O*-glucoside ($y = 103505x + 3E + 06$; r^2: 0.9914; LOD: 0.15 g/mL; LOQ: 0.45 g/mL); B- Pelargonidin-3-*O*-glucoside ($y = 33872x + 2E + 06$; r^2: 0.9996; LOD: 0.28 g/mL; LOQ: 0.84 g/mL); and C- Peonidin-3-*O*-glucoside ($y = 123036x - 9227.9$; r^2: 0,9992; LOD: 0.30 g/mL; LOQ: 0,91 g/mL).

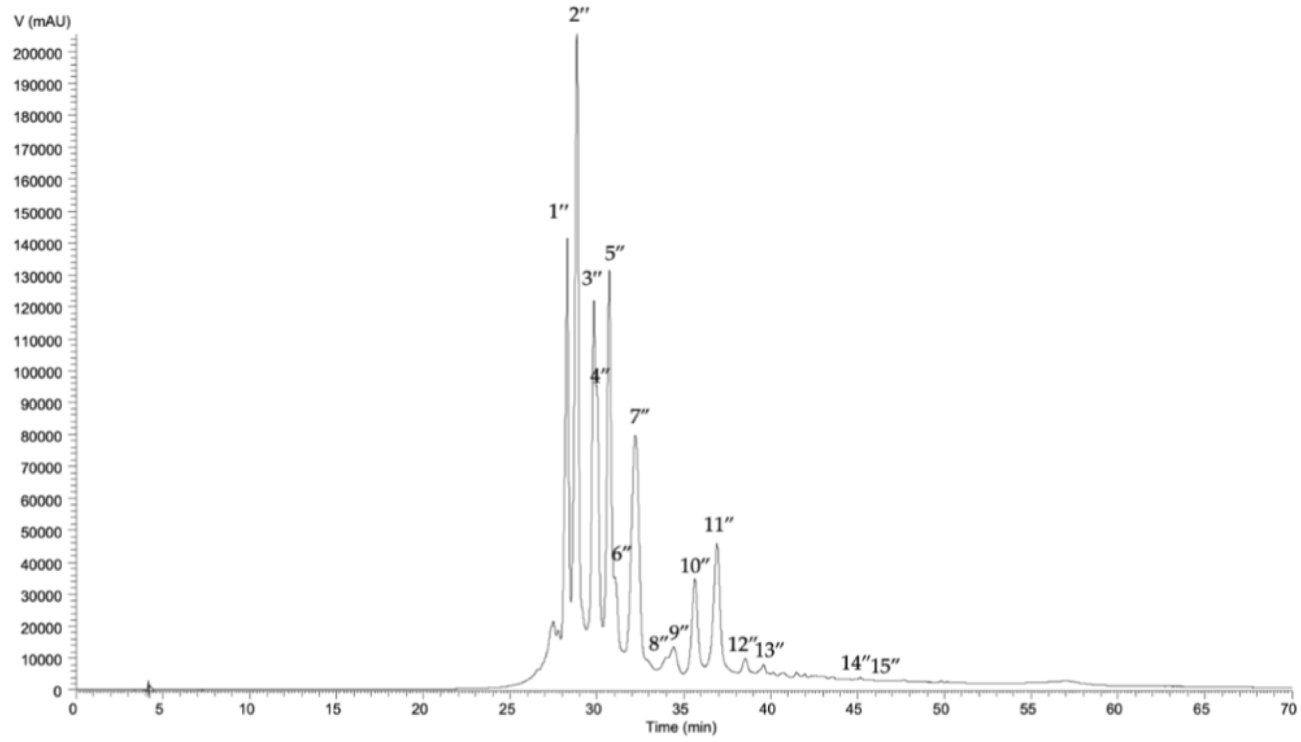


(a)



(b)

Fig. 1. Chromatographic profiles at 520 nm of the anthocyanin compounds in *Prunus avium* (a), *Fragaria vesca* (b) and *Vaccinium myrtillus* (c). (1: Cyanidin-3-*O*-glucoside; 2: Cyanidin-*O*-deoxyhexosyl-pentoside; 1′: Cyanidin-*O*-hexosyl-*O*-hexoside; 2′: Cyanidin-3-*O*-glucoside; 3′: Pelargonidin-3-*O*-glucoside; 4′: Pelargonidin-derivative; 5′: Petunidin-*O*-pentoside; 1″: Delphinidin-*O*-hexoside; 2″: Delphinidin-*O*-hexoside; 3″: Cyanidin-*O*-hexoside; 4″: Delphinidin-*O*-pentoside; 5″: Cyanidin-3-*O*-glucoside; 6″: Petunidin-*O*-hexoside; 7″: Petunidin-*O*-hexoside; 8″: Peonidin-*O*-hexoside; 9″: Petunidin-*O*-pentoside; 10″: Peonidin-*O*-hexoside; 11″: Malvidin-3-*O*-glucoside; 12″: Malvidin-*O*-pentoside; 13″: Cyanidin derivative; 14″: Peonidin; 15″: Malvidin).



(c)

Fig. 1. (*continued*).

presented a protonated ion $[M]^+$ at *m/z* 449, corresponding to the loss of 132 u, hence, tentatively identified as petunidin-*O*-pentoside.

The fruits of *V. myrtillus* L. showed the widest range of anthocyanins, 13*O*-glycosylated derivatives of delphinidin (peaks 1″, 2″ and 4″), cyanidin (peaks 3″, 5″ and 13″), petunidin (peaks 6″, 7″, and 9″), peonidin (peaks 8″ and 10″), and malvidin (peaks 11″ and 12″), also 2 aglycones, peonidin and malvidin (peaks 14′’ and 15′’, respectively). Peaks 5″ ($[M]^+$ at *m/z* 449) and 11″ ($[M]^+$ at *m/z* 493) were identified by comparison with available authentic standards. Peaks 1″, 2″, 3″, 6″, 7″, 8″ and 10″ presented a protonated ion $[M]^+$ at *m/z* 465, 449, 479, and 463, respectively, and a unique MS2 fragment that corresponded to the loss of a unique hexosyl unit (162 u), and, therefore these compounds were tentatively identified as their respective anthocyanidin linked to a hexose. In the case of the peaks 4″ ($[M]^+$ at *m/z* 435), 9″ ($[M]^+$ at *m/z* 449) and 12″ ($[M]^+$ at *m/z* 463), there was a loss of 132 u indicating the presence of a pentose, hence, these compounds were tentatively identified as their corresponding anthocyanidin linked to a pentose. Peak 13′’ was only possible to identify as a cyanidin derivative, again, the moiety lost did not correspond to any bibliographic data available on the matter. This anthocyanins profile was similar than those reported by Pires, Dias et al. (2020b) and Mikulic-Petkovsek et al. (2015) in bilberry fruits extracts. In the analyzed wild fruits from *V. myrtillus*, the anthocyanins derived from delphinidin were the most abundant, representing 40.59 % of the total anthocyanins content. Colak et al. (2017) reported a similar profile of anthocyanins in different colored berries of bilberry (*V. myrtillus*) samples. These authors also identified the presence of 15 anthocyanins, being delphinidin the major anthocyanidin glycoside found in blue, blue/black, and purple berry phenotypes (Colak et al., 2017). In addition, the total anthocyanin content (27.370 mg/g, dw) obtained in this work was slightly higher than that reported by the latter author (12.46 mg/g, dw).

3.4. Bioactive properties: Antioxidant activity of the analyzed wild edible fruits

As explained in Materials and Methods, the antioxidant activity of the wild fruits was evaluated using three different *in vitro* assays (DPPH, FRAP and Folin-Ciocalteu) and tested in a cellular model (OxHLIA). Regardless of the employed method of analysis, *P. avium* was the species that exhibited the lowest ($p < 0.05$) antioxidant properties (Table 5), in accordance with the phenolic composition described in section 2.2. The results obtained by direct *in vitro* methods, such as Folin-Ciocalteu and FRAP assays, as well as OxHLIA method showed that *F. vesca* had better antioxidant activity ($p < 0.05$) than *V. myrtillus*, however, the values achieved through DPPH indicated that *V. myrtillus* had the highest ($p < 0.05$) antioxidant activity for this assay. This may be due to the facts that *V. myrtillus* presented the highest amount of total anthocyanins content of the evaluated fruits, together with the fact that DPPH assay is measured at a wavelength of 517 nm, where anthocyanins present a high absorbance, thus, these molecules could generate interferences and therefore an overestimation of the antioxidant activity by this method. Even though FRAP and Folin-Ciocalteu were measured with different reference standards, it was expected a correlation since in both assays the reaction mechanism is based on an electron transfer reaction (Magalhães et al., 2008).

Despite of the fact that *P. avium* was the wild fruit that presented the lowest antioxidant activity, in this study the values obtained by DPPH (186.83 mg TE/100 g, fw) and FRAP (902.98 mg TE/100 g, fw) assays were higher than those previously reported by other authors (136 mg TE/100 g, and 124 – 1200.39 mg TE/100 g, fw, by DPPH and FRAP, respectively) (Blando & Oomah, 2019; Chaovanalikit & Wrolstad, 2004). Furthermore, it can be noticed that FRAP stood out as the highest value. The results obtained by Folin-Ciocalteu were in accordance with the values (54 –558 GAE mg/100 g, fw) reported by Chaovanalikit & Wrolstad (2004) for cultivated sweet and sour cherries.

In the case of *F. vesca*, it is interesting to stand out that the results of

Table 5
Bioactive properties of *P. avium, F. vesca* and *V. myrtillus*: *in vitro* antioxidant activity (mg/100 g fw fruit), *in vivo* antioxidant activity, antibacterial and antifungal activity of hydroethanolic extract (mg/mL).

Antioxidant activity			
Antioxidant activity *in vitro*	*P. avium*	*F. vesca*	*V. myrtillus*
Folin-Ciocalteu (mg GAE/100 g fw)	138.48 ± 7.46^{c}	937.53 ± 15.22^{a}	341.69 ± 30.94^{b}
DPPH (mg TE/100 g fw)	186.83 ± 1.89^{c}	201.79 ± 2.89^{b}	255.38 ± 6.26^{a}
FRAP (mg TE/100 g fw)	902.98 ± 49.97^{c}	1877.58 ± 113.74^{a}	1601.42 ± 86.96^{b}
Antioxidant activity *ex vivo*			
OxHLIA (IC_{50} Values, μg/mL)	114.02 ± 3.66^{c}	24.69 ± 1.02 ^{a}	47.03 ± 2.43^{b}

Antibacterial activity (mg/mL hydroethanolic extract)

	P. avium		*F. vesca*		*V. myrtillus*		Streptomycin		Methicillin		Ampicillin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria												
Enterobacter cloacae	>10	>10	>10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Escherichia coli	5	>10	5	>10	5	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	>10	>10	10	>10	10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterocolitica	2.5	>10	5	>10	2.5	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	0.3	>10	0.6	>10	0.6	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria												
Bacillus cereus	>10	>10	>10	>10	10	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	1.25	>10	1.25	>10	0.3	10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	0.6	>10	0.6	>10	0.3	10	0.007	0.007	0.007	0.007	0.15	0.15

Antifungal activity (mg/mL hydroethanolic extract)

	P. avium		*F. vesca*		*V. myrtillus*		Ketoconazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus brasiliensis	5	>10	2.5	>10	>10	>10	0.06	0.125
Aspergillus fumigatus	10	>10	5	>10	10	>10	0.5	1.0

GAE: gallic acid equivalent; TE: trolox equivalent; IC_{50}: extract concentration providing 50 % of antioxidant activity. Different small superscript letters in each line mean statistically significant differences ($p < 0.05$) compared by Duncan's test. MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MBC: minimum fungicidal concentration; n.t.: not tested. IC_{50} for Trolox OxHLIA assay = 21.72 ± 0.80 μg/mL.

antioxidant activity obtained in this work were higher in comparison with those previously reported by other authors. This fact is especially remarkable in the case of FRAP assay (1877.58 mg TE/100 g, fw), as the obtained results were almost double the highest value reported by Peñarrieta et al. (2009) (425 – 978.63 mg TE/100 g, fw). Moreover, the values obtained by Folin-Ciocalteu were two and four times higher than the values reported by the same author (425 – 978.63 mg GAE/100 g, fw) (Peñarrieta et al., 2009) and Najda et al. (234.8 mg/100 g, fw) (2014), respectively.

Regarding the antioxidant properties of *V. myrtillus*, Table 5 shows that the values obtained through Folin-Ciocalteu method were in accordance with those previously reported by Ciulca et al. (2021) and Urbonaviciene et al. (2022). However, the activity by FRAP (1601.42 mg TE/100 g, fw) was higher than previously reported for wild bilberries fruits collected in different years in Norway, Finland, Latvia, and Lithuania (901.04 –1444.17 mg TE/100 g, fw) (Urbonaviciene et al., 2022). Likewise, the activity by DPPH (255.38 mg TE/100 g, fw) stood out with respect to the values reported by Mikulic-Petkovsek et al. (2015) for wild bilberries (160–180 mg TE/100 g, fw).

Respecting to OxHLIA assay, as shown in Table 5, *F. vesca* was the most effective extract in protecting the erythrocyte population from the hemolytic action caused by the AAPH-derived free radicals, with values close to Trolox (the positive control), which represented a great activity for our natural fruit extracts (24.69 *vs* 21.72 μg/mL, respectively). *V. myrtillus* and *F. vesca* presented a higher antioxidant activity (values lower than 50 μg/mL), comparing to the antioxidant activity of other red fruits such as *Lonicera caerulea* L. (145–394 μg/mL) [54]. However, *P. avium* presented results closed to those reported by other authors (Molina et al., 2019). *F. vesca* showed similar activity to other fruits such as commercial *Sicana odorifera* (Vell.) Naudin epicarp (EC_{50} value of 27 μg/mL) (Albuquerque, Dias et al., 2021) and higher than commercial passion fruit (*Passiflora edulis* Sims) extract (EC_{50} value of 78 μg/mL) (Ghada et al., 2020).

3.5. Bioactive properties: Antibacterial activity of the analyzed wild edible fruits

The growth inhibition of eight common Gram-negative and Gram-positive bacteria responsible of food transmission diseases with the selected wild edible fruits extracts were also tested. Results of the antibacterial activity are shown in Table 5. The growth of *Yersinia enterocolitica* and *Staphylococcus aureus* were highly inhibited by the studied wild fruit extracts, being *P. avium* and *V. myrtillus* those that presented the most effective concentration for each one respectively (0.3 mg/mL). *Enterobacter cloacae, Pseudomonas aeruginosa* and *Bacillus cereus* were the more resistant strains against the tested extracts, and only *V. myrtillus* showed scarce activity for these three bacteria, while *P. avium* did not inhibit the growth of none of them. As can be seen in the Table 5, *V. myrtillus* was the wild fruit with better antibacterial activity. Other authors reported similar bilberry bioactivity results for *E. coli* inhibition (Pires et al., 2021), but higher concentrations (2.5 mg/mL) were required (lower antibacterial activity) for *Listeria monocytogenes* and *Staphylococcus aureus*.

3.6. Bioactive properties: Antifungal activity of the analyzed wild edible fruits

Regarding the antifungal activity against *Aspergillus brasiliensis* and *Aspergillus fumigatus*, Table 5 shows that *A. fumigatus* growth was inhibited by all wild fruit extracts (in different concentrations). However, the growth of *A. brasiliensis* was inhibited only by *F. vesca* and *P. avium* fruit extracts, with *F. vesca* bring the extract with better fungal growth inhibition (MIC values). On the other hand, none extract showed fungicidal activity (>10 mg/mL). To the best of the authors' knowledge,

there is not literature about antifungal activity in *F. vesca* and *V. myrtillus* fruits, however studies on antifungal properties were found for *P. avium*. In this case, other authors have reported antifungal activity against other fungal strains, such as *Botrytis cinerea* and *Penicillium expansum* (Liu et al., 2020; Wang et al., 2017). Furthermore, other fruits extract such as *Sicana odorifera* showed better results (MIC: 2.2 mg/mL), against *A. fumigatus* than those obtained in the present study (Albuquerque, Dias et al., 2021).

4. Conclusions

The wild fruits of *Prunus avium*, *Fragaria vesca* and *Vaccinium myrtillus* were analyzed as underestimated sources of natural colorants and bioactive compounds with functional properties. The highest values of total polyphenols, hydroxybenzoic acids content, and flavonols were shown by *F. vesca*. The total anthocyanin content and hydroxycinnamic acids content stood out in *V. myrtillus*. However, most of the results obtained in the three fruits were much higher to those previously reported. The predominant anthocyanins of each wild fruit were petunidin-*O*-hexoside, pelargonidin-3-*O*-glucoside and cyanidin-*O*-deoxyhexosyl-pentoside, in *V. myrtillus, F. vesca* and *P. avium,* respectively.

The three wild fruits presented a great *in vitro* antioxidant activity, especially *F. vesca* through Folin-Ciocalteu and FRAP assays and *V. myrtillus* through DPPH assay, and as evidenced in the determination of phenolic compounds. These results were also confirmed *ex vivo* in erythrocytes by the OxHLIA bioassay. Moreover, the antibacterial and antifungal activities was evaluated. *V. myrtillus* was the fruit with better results for the bacterial growth inhibition, while *F. vesca* showed the best antifungal activity.

In view of the above results, *Prunus avium*, *Fragaria vesca* and *Vaccinium myrtillus* wild fruits are a great source of phenolic compounds, mostly anthocyanins, with great antioxidant and antimicrobial activity, which make them a great possible source of natural food ingredients for the development of healthier food products.

Funding

This research was funded by Spanish Ministry of Science and Innovation, grant PID2019-109365RA-I00 (Ref. AEI/10.13039/501100011033).

CRediT authorship contribution statement

Erika N. Vega: Data curation, Writing – original draft. **Patricia García-Herrera:** Investigation, Writing – original draft. **María Ciudad-Mulero:** Writing – original draft, Writing – review & editing. **Ma Inés Dias:** Data curation, Writing – review & editing. **Ma Cruz Matallana-González:** . **Montaña Cámara:** Methodology, Validation. **Javier Tardío:** . **María Molina:** . **José Pinela:** Methodology, Writing – review & editing. **Tânia C.S.P. Pires:** Data curation. **Lillian Barros:** Methodology, Supervision, Validation, Writing – review & editing. **Virginia Fernández-Ruiz:** Conceptualization, Methodology, Supervision, Validation, Validation, Writing – review & editing. **Patricia Morales:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgments

The authors are grateful to ALIMNOVA Research Group (UCM Ref, 951505). E.N.V. to her FPI contract (PRE2020-092030). To the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021); to FCT for the contracts of M.I.D. (CEEC Institutional), J.P. (CEECIND/01011/2018) and L.B. (CEEC Institutional).

References

Afrin, S., Gasparrini, M., Forbes-Hernandez, T. Y., Reboredo-Rodriguez, P., Mezzetti, B., Varela-López, A., … Battino, M. (2016). Promising Health Benefits of the Strawberry: A Focus on Clinical Studies. *Journal of Agricultural and Food Chemistry, 64*(22), 4435–4449. https://doi.org/10.1021/acs.jafc.6b00857

Albuquerque, B. R., Dias, M. I., Pereira, C., Petrović, J., Soković, M., Calhelha, R. C., … Barros, L. (2021). Valorization of *Sicana odorifera* (Vell.) Naudin Epicarp as a Source of Bioactive Compounds: Chemical Characterization and Evaluation of Its Bioactive Properties. *Foods, 10*(4), 700. https://doi.org/10.3390/foods10040700

Albuquerque, B. R., Oliveira, M. B. P. P., Barros, L., & Ferreira, I. C. F. R. (2021). Could fruits be a reliable source of food colorants? Pros and cons of these natural additives. *Critical Reviews in Food Science and Nutrition, 61*(5), 805–835. https://doi.org/10.1080/10408398.2020.1746904

Aliman, J., Michalak, I., Bušatlić, E., Aliman, L., Kulina, M., Radović, M., & Hasanbegović, J. (2020). Study of the physicochemical properties of highbush blueberry and wild bilberry fruit in central Bosnia. *Turkish Journal of Agriculture and Forestry, 44*(2), 156–168. https://doi.org/10.3906/tar-1902-36

Ancillotti, C., Ciofi, L., Pucci, D., Sagona, E., Giordani, E., Biricolti, S., … del Bubba, M. (2016). Polyphenolic profiles and antioxidant and antiradical activity of Italian berries from *Vaccinium myrtillus* L. and *Vaccinium uliginosum* L. subsp. *gaultherioides* (Bigelow) S.B. Young. *Food Chemistry, 204*, 176–184. https://doi.org/10.1016/j.foodchem.2016.02.106

AOAC INTERNATIONAL. (2005). *Official Methods of Analysis* (18th ed.). AOAC International.

Aslantas, R., Pirlak, L., & Güleryüz, M. (2007). The Nutritional Value of Wild Fruits from the North Eastern Anatolia Region of Turkey. *Asian Journal of Chemistry, 19*(4), 3072–3078.

Ballistreri, G., Continella, A., Gentile, A., Amenta, M., Fabroni, S., & Rapisarda, P. (2013). Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *Food Chemistry, 140*(4), 630–638. https://doi.org/10.1016/j.foodchem.2012.11.024

Bastos, C., Barros, L., Dueñas, M., Calhelha, R. C., Queiroz, M. J. R. P., Santos-Buelga, C., & Ferreira, I. C. F. R. (2015). Chemical characterisation and bioactive properties of *Prunus avium* L.: The widely studied fruits and the unexplored stems. *Food Chemistry, 173*, 1045–1053. https://doi.org/10.1016/j.foodchem.2014.10.145

Blando, F., & Oomah, B. D. (2019). Sweet and sour cherries: Origin, distribution, nutritional composition and health benefits. *Trends in Food Science & Technology, 86*, 517–529. https://doi.org/10.1016/j.tifs.2019.02.052

Bonoli, M., Verardo, V., Marconi, E., & Caboni, M. F. (2004). Antioxidant Phenols in Barley (*Hordeum vulgare* L.) Flour: Comparative Spectrophotometric Study among Extraction Methods of Free and Bound Phenolic Compounds. *Journal of Agricultural and Food Chemistry, 52*(16), 5195–5200. https://doi.org/10.1021/jf040075c

Carocho, M., Barreiro, M. F., Morales, P., & Ferreira, I. C. F. R. (2014). Adding Molecules to Food, Pros and Cons: A Review on Synthetic and Natural Food Additives. *Comprehensive Reviews in Food Science and Food Safety, 13*(4), 377–399. https://doi.org/10.1111/1541-4337.12065

Celik, F., Bozhuyuk, M. R., Ercisli, S., & Gundogdu, M. (2018). Physicochemical and bioactive characteristics of wild grown bilberry (*Vaccinium myrtillus* L.) genotypes from Northeastern Turkey. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46*(1), 128–133. https://doi.org/10.15835/nbha461210842

Chaovanalikit, A., & Wrolstad, R. E. (2004). Total Anthocyanins and Total Phenolics of Fresh and Processed Cherries and Their Antioxidant Properties. FCT67–FCT72 *Journal of Food Science, 69*(1). https://doi.org/10.1111/j.1365-2621.2004.tb17858.x.

Ciulca, S., Roma, G., Alexa, E., Radulov, I., Cocan, I., Madosa, E., & Ciulca, A. (2021). Variation of Polyphenol Content and Antioxidant Activity in Some Bilberry (Vaccinium myrtillus L.) Populations from Romania. *Agronomy, 11*(12), 2557. https://doi.org/10.3390/agronomy11122557

Colak, N., Primetta, A. K., Riihinen, K. R., Jaakola, L., Grúz, J., Strnad, M., … Ayaz, F. A. (2017). Phenolic compounds and antioxidant capacity in different-colored and non-pigmented berries of bilberry (*Vaccinium myrtillus* L.). *Food Bioscience, 20*, 67–78. https://doi.org/10.1016/j.fbio.2017.06.004

Dávila, P., Pardo de Santayana, M., & Tardío, J. (2018). *Fragaria vesca* L. In M. Pardo de Santayana, R. Morales, J. Tardío, L. Aceituno-Mata, & M. Molina (Eds.), *Inventario Español de los Conocimientos Tradicionales relativos a la Biodiversidad. Fase II (3)* (pp. 333–336). Ministerio para la Transición Ecológica.

de Leo, M., Iannuzzi, A. M., Germanò, M. P., D'Angelo, V., Camangi, F., Sevi, F., … Braca, A. (2021). Comparative chemical analysis of six ancient italian sweet cherry (*Prunus avium* L.) varieties showing antiangiogenic activity. *Food Chemistry, 360*, Article 129999. https://doi.org/10.1016/j.foodchem.2021.129999

del Bubba, M., Giordani, E., Ancillotti, C., Petrucci, W. A., Ciofi, L., Morelli, D., … Furlanetto, S. (2016). Morphological, nutraceutical and sensorial properties of cultivated *Fragaria vesca* L. berries: Influence of genotype, plant age, fertilization treatment on the overall fruit quality. *Agricultural and Food Science, 25*(3). https://doi.org/10.23986/afsci.56867

del Pino-García, R., García-Lomillo, J., Rivero-Pérez, M. D., González-SanJosé, M. L., & Muñiz, P. (2015). Adaptation and Validation of QUick, Easy, New, CHEap, and Reproducible (QUENCHER) Antioxidant Capacity Assays in Model Products Obtained from Residual Wine Pomace. *Journal of Agricultural and Food Chemistry, 63*(31), 6922–6931. https://doi.org/10.1021/acs.jafc.5b01644

Dias, M. I., Barros, L., Fernandes, I. P., Ruphuy, G., Oliveira, M. B. P. P., Santos-Buelga, C., … Ferreira, I. C. F. R. (2015). A bioactive formulation based on *Fragaria vesca* L. vegetative parts: Chemical characterisation and application in κ-carrageenan gelatin. *Journal of Functional Foods, 16*, 243–255. https://doi.org/10.1016/j.jff.2015.04.044

Dias, M. I., Barros, L., Morales, P., Cámara, M., Alves, M. J., Oliveira, M. B. P. P., … Ferreira, I. C. F. R. (2016). Wild *Fragaria vesca* L. fruits: A rich source of bioactive phytochemicals. *Food & Function, 7*(11), 4523–4532. https://doi.org/10.1039/C6FO01042C

Dias, M. I., Barros, L., Morales, P., Sánchez-Mata, M. C., Oliveira, M. B. P. P., & Ferreira, I. C. F. R. (2015). Nutritional parameters of infusions and decoctions obtained from *Fragaria vesca* L. roots and vegetative parts. *LWT - Food Science and Technology, 62*(1), 32–38. https://doi.org/10.1016/j.lwt.2015.01.034

Dias, M. I., Barros, L., Sousa, M. J., Oliveira, M. B. P. P., Santos-Buelga, C., & Ferreira, I. C. F. R. (2017). Enhancement of nutritional and bioactive compounds by *in vitro* culture of wild *Fragaria vesca* L. vegetative parts. *Food Chemistry, 235*, 212–219. https://doi.org/10.1016/j.foodchem.2017.05.060

Dyduch-Siemińska, M., Najda, A., Dyduch, J., Gantner, M., & Klimek, K. (2015). The Content of Secondary Metabolites and Antioxidant Activity of Wild Strawberry Fruit (*Fragaria vesca* L.). *Journal of Analytical Methods in Chemistry, 2015*, 1–8. https://doi.org/10.1155/2015/831238

Ferreira, I. C. F. R., Morales, P., & Barros, L. (2017). *Wild Plants, Mushrooms and Nuts: Functional Food Properties and Applications* (I. C. F. R. Ferreira, P. Morales, & L. Barros, Eds.; First). Wiley Blackwell.

Fierascu, R. C., Temocico, G., Fierascu, I., Ortan, A., & Babeanu, N. E. (2020). *Fragaria* genus: Chemical composition and biological activities. *Molecules, 25*(3), 498. https://doi.org/10.3390/molecules25030498

Fogliano, V., Maria Monti, S., Musella, T., Randazzo, G., & Ritieni, A. (1999). Formation of coloured Maillard reaction products in a gluten-glucose model system. *Food Chemistry, 66*(3), 293–299. https://doi.org/10.1016/S0308-8146(99)00058-8

Ghada, B., Pereira, E., Pinela, J., Prieto, M. A., Pereira, C., Calhelha, R. C., … Ferreira, I. C. F. R. (2020). Recovery of Anthocyanins from Passion Fruit Epicarp for Food Colorants: Extraction Process Optimization and Evaluation of Bioactive Properties. *Molecules, 25*(14), 3203. https://doi.org/10.3390/molecules25143203

Gonçalves, B., Landbo, A.-K., Knudsen, D., Silva, A. P., Moutinho-Pereira, J., Rosa, E., & Meyer, A. S. (2004). Effect of Ripeness and Postharvest Storage on the Phenolic Profiles of Cherries (*Prunus avium* L.). *Journal of Agricultural and Food Chemistry, 52*(3), 523–530. https://doi.org/10.1021/jf030595s

Häkkinen, S. H., & Törrönen, A. R. (2000). Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: Influence of cultivar, cultivation site and technique. *Food Research International, 33*(6), 517–524. https://doi.org/10.1016/S0963-9969(00)00086-7

Hayaloglu, A. A., & Demir, N. (2015). Physicochemical Characteristics, Antioxidant Activity, Organic Acid and Sugar Contents of 12 Sweet Cherry (*Prunus Avium* L.) Cultivars Grown in Turkey. *Journal of Food Science, 80*(3), C564–C570. https://doi.org/10.1111/1750-3841.12781

Heleno, S. A., Ferreira, I. C. F. R., Esteves, A. P., Ćirić, A., Glamočlija, J., Martins, A., … Queiroz, M. J. R. P. (2013). Antimicrobial and demelanizing activity of *Ganoderma lucidum* extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food and Chemical Toxicology, 58*, 95–100. https://doi.org/10.1016/j.fct.2013.04.025

Hu, T., Subbiah, V., Wu, H., BK, A., Rauf, A., Alhumaydhi, F. A., & Suleria, H. A. R. (2021). Determination and Characterization of Phenolic Compounds from Australia-Grown Sweet Cherries (*Prunus avium* L.) and Their Potential Antioxidant Properties. *ACS Omega, 6*(50), 34687–34699. doi:10.1021/acsomega.1c05112.

Kalyoncu, I. H., Ersoy, N., & Yılmaz, M. (2009). Some physico-chemical properties and mineral contents of sweet cherry (*Prunus avium* L.) type grown in Konya. *African Journal of Biotechnology, 8*(12), 2744–2749. https://doi.org/10.4314/ajb.v8i12.60849

Karlidag, H., Ercisli, S., Sengul, M., & Tosun, M. (2009). Physico-Chemical Diversity in Fruits of Wild-Growing Sweet Cherries (*Prunus Avium* L.). *Biotechnology & Biotechnological Equipment, 23*(3), 1325–1329. https://doi.org/10.1080/13102818.2009.10817663

Laaksonen, O., Sandell, M., & Kallio, H. (2010). Chemical factors contributing to orosensory profiles of bilberry (*Vaccinium myrtillus*) fractions. *European Food Research and Technology, 231*(2), 271–285. https://doi.org/10.1007/s00217-010-1278-7

Li, Y., Zhang, J.-J., Xu, D.-P., Zhou, T., Zhou, Y., Li, S., & Li, H.-B. (2016). Bioactivities and Health Benefits of Wild Fruits. *International Journal of Molecular Sciences, 17*(8), 1258. https://doi.org/10.3390/ijms17081258

Liu, X., Ji, D., Cui, X., Zhang, Z., Li, B., Xu, Y., … Tian, S. (2020). p-Coumaric acid induces antioxidant capacity and defense responses of sweet cherry fruit to fungal pathogens. *Postharvest Biology and Technology, 169*, Article 111297. https://doi.org/10.1016/j.postharvbio.2020.111297

Lockowandt, L., Pinela, J., Roriz, C. L., Pereira, C., Abreu, R. M. V., Calhelha, R. C., … Ferreira, I. C. F. R. (2019). Chemical features and bioactivities of cornflower (*Centaurea cyanus* L.) capitula: The blue flowers and the unexplored non-edible part. *Industrial Crops and Products, 128*, 496–503. https://doi.org/10.1016/j.indcrop.2018.11.059

Loughrey, K. (2002). Overview of color analysis. *Current protocols in food analytical chemistry*. John Wiley & Sons Inc. (p. F51.1-F51.13).

Magalhães, L. M., Segundo, M. A., Reis, S., & Lima, J. L. F. C. (2008). Methodological aspects about in vitro evaluation of antioxidant properties. *Analytica Chimica Acta, 613*(1), 1–19. https://doi.org/10.1016/j.aca.2008.02.047

Medina, M. B. (2011). Determination of the total phenolics in juices and superfruits by a novel chemical method. *Journal of Functional Foods, 3*(2), 79–87. https://doi.org/10.1016/j.jff.2011.02.007

Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Stampar, F., & Veberic, R. (2015). A comparison of fruit quality parameters of wild bilberry (*Vaccinium myrtillus* L.) growing at different locations. *Journal of the Science of Food and Agriculture, 95*(4), 776–785. https://doi.org/10.1002/jsfa.6897

Miller, K., Feucht, W., & Schmid, M. (2019). Bioactive Compounds of Strawberry and Blueberry and Their Potential Health Effects Based on Human Intervention Studies: A Brief Overview. *Nutrients, 11*(7), 1510. https://doi.org/10.3390/nu11071510

Molina, A. K., Vega, E. N., Pereira, C., Dias, M. I., Heleno, S. A., Rodrigues, P., … Ferreira, I. C. F. R. (2019). Promising Antioxidant and Antimicrobial Food Colourants from Lonicera caerulea L. var. *Kamtschatica. Antioxidants, 8*(9), 394. https://doi.org/10.3390/antiox8090394

Najda, A., Dyduch-Siemińska, M., Dyduch, J., & Gantner, M. (2014). Comparative analysis of secondary metabolites contents in *Fragaria vesca* L. fruits. *Annals of Agricultural and Environmental Medicine, 21*(2), 339–343. https://doi.org/10.5604/1232-1966.1108601

Neamtu, A.-A., Szoke-Kovacs, R., Mihok, E., Georgescu, C., Turcus, V., Olah, N. K., Frum, A., Tita, O., Neamtu, C., Szoke-Kovacs, Z., Cziaky, Z., & Mathe, E. (2020). Bilberry (*Vaccinium myrtillus* L.) Extracts Comparative Analysis Regarding Their Phytonutrient Profiles, Antioxidant Capacity along with the *In Vivo* Rescue Effects Tested on a *Drosophila melanogaster* High-Sugar Diet Model. *Antioxidants, 9*(11), 1067. doi:10.3390/antiox9111067.

Palombini, S. v., Claus, T., Maruyama, S. A., Carbonera, F., Montanher, P. F., Visentainer, J. v., Gomes, S. T. M., & Matsushita, M. (2016). Optimization of a New Methodology for Determination of Total Phenolic Content in Rice Employing Fast Blue BB and QUENCHER Procedure. *Journal of the Brazilian Chemical Society, 27*(7), 1188–1194. doi:10.5935/0103-5053.20160013.

Peñarrieta, J. M., Alvarado, J. A., Bergenståhl, B., & Ákesson, B. (2009). Total Antioxidant Capacity and Content of Phenolic Compounds in Wild Strawberries (*Fragaria vesca*) Collected in Bolivia. *International Journal of Fruit Science, 9*(4), 344–359. https://doi.org/10.1080/15538360903378526

Pires, T. C. S. P., Caleja, C., Santos-Buelga, C., Barros, L., & Ferreira, I. C. F. R. (2020). *Vaccinium myrtillus* L. Fruits as a Novel Source of Phenolic Compounds with Health Benefits and Industrial Applications - A Review. *Current Pharmaceutical Design, 26*(16), 1917–1928. https://doi.org/10.2174/1381612826666200317132507

Pires, T. C. S. P., Dias, M. I., Barros, L., Alves, M. J., Oliveira, M. B. P. P., Santos-Buelga, C., & Ferreira, I. C. F. R. (2018). Antioxidant and antimicrobial properties of dried Portuguese apple variety (*Malus domestica* Borkh. cv Bravo de Esmolfe). *Food Chemistry, 240*, 701–706. https://doi.org/10.1016/j.foodchem.2017.08.010

Pires, T. C. S. P., Dias, M. I., Calhelha, R. C., Alves, M. J., Santos-Buelga, C., Ferreira, I. C. F. R., & Barros, L. (2021). Development of new bilberry (*Vaccinium myrtillus* L.) based snacks: Nutritional, chemical and bioactive features. *Food Chemistry, 334*, Article 127511. https://doi.org/10.1016/j.foodchem.2020.127511

Pires, T. C. S. P., Dias, M. I., Carocho, M., Barreira, J. C. M., Santos-Buelga, C., Barros, L., & Ferreira, I. C. F. R. (2020). Extracts from *Vaccinium myrtillus* L. fruits as a source of natural colorants: Chemical characterization and incorporation in yogurts. *Food & Function, 11*(4), 3227–3234. https://doi.org/10.1039/C9FO02890K

Prencipe, F. P., Bruni, R., Guerrini, A., Rossi, D., Benvenuti, S., & Pellati, F. (2014). Metabolite profiling of polyphenols in *Vaccinium* berries and determination of their chemopreventive properties. *Journal of Pharmaceutical and Biomedical Analysis, 89*, 257–267. https://doi.org/10.1016/j.jpba.2013.11.016

Roy, S., Wu, B., Liu, W., & Archbold, D. D. (2018). Comparative analyses of polyphenolic composition of *Fragaria* spp. color mutants. *Plant Physiology and Biochemistry, 125*, 255–261. https://doi.org/10.1016/j.plaphy.2018.02.003

Ruiz-Rodríguez, B. M., Sánchez-Moreno, C., de Ancos, B., de Cortes Sánchez-Mata, M., Fernández-Ruiz, V., Cámara, M., & Tardío, J. (2014). Wild *Arbutus unedo* L. and *Rubus ulmifolius* Schott fruits are underutilized sources of valuable bioactive compounds with antioxidant capacity. *Fruits, 69*(6), 435–448. https://doi.org/10.1051/fruits/2014035

Sánchez-Mata, M. C., & Tardío, J. (2016). *Mediterranean wild edible plants. Ethnobotany and food composition tables* (M. C. Sánchez-Mata & J. Tardío, Eds.; 1st ed.). Springer New York, NY. doi:10.1007/978-1-4939-3329-7.

Schmitz-Eiberger, M. A., & Blanke, M. M. (2012). Bioactive components in forced sweet cherry fruit (*Prunus avium* L.), antioxidative capacity and allergenic potential as dependent on cultivation under cover. *LWT - Food Science and Technology, 46*(2), 388–392. https://doi.org/10.1016/j.lwt.2011.12.015

Sun, J., Liu, X., Yang, T., Slovin, J., & Chen, P. (2014). Profiling polyphenols of two diploid strawberry (*Fragaria vesca*) inbred lines using UHPLC-HRMSn. *Food Chemistry, 146*, 289–298. https://doi.org/10.1016/j.foodchem.2013.08.089

Urbonaviciene, D., Bobinaite, R., Viskelis, P., Bobinas, C., Petruskevicius, A., Klavins, L., & Viskelis, J. (2022). Geographic Variability of Biologically Active Compounds, Antioxidant Activity and Physico-Chemical Properties in Wild Bilberries (*Vaccinium myrtillus* L.). *Antioxidants, 11*(3), 588. https://doi.org/10.3390/antiox11030588

Usenik, V., Fabčič, J., & Štampar, F. (2008). Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). *Food Chemistry, 107*(1), 185–192. https://doi.org/10.1016/j.foodchem.2007.08.004

Vega, E. N., Molina, A. K., Pereira, C., Dias, M. I., Heleno, S. A., Rodrigues, P., Fernandes, I. P., Barreiro, M. F., Stojković, D., Soković, M., Carocho, M., Barreira, J. C. M., Ferreira, I. C. F. R., & Barros, L. (2021). Anthocyanins from *Rubus fruticosus* L. and *Morus nigra* L. Applied as Food Colorants: A Natural Alternative. *Plants*, *10*(6), 1181. doi:10.3390/plants10061181.

Villar, L. (1993). *Vaccinium* L. In S. Castroviejo, C. Aedo, C. Gómez Campo, M. Laínz, P. Montserrat, R. Morales, F. Muñoz Garmendia, G. Nieto Feliner, E. Rico, S. Talavera, & L. Vilar (Eds.), *Flora ibérica. Plantas vasculares de la Península Ibérica e Islas Baleares: Vol. IV* (pp. 519–523). Real Jardín Botánico, CSIC.

Wang, M., Jiang, N., Wang, Y., Jiang, D., & Feng, X. (2017). Characterization of Phenolic Compounds from Early and Late Ripening Sweet Cherries and Their Antioxidant and Antifungal Activities. *Journal of Agricultural and Food Chemistry, 65*(26), 5413–5420. https://doi.org/10.1021/acs.jafc.7b01409

Yildiz, H., Ercisli, S., Hegedus, A., Akbulut, M., Topdas, E. F., & Aliman, J. (2014). Bioactive content and antioxidant characteristics of wild (*Fragaria vesca* L.) and cultivated strawberry (*Fragaria* × *ananassa* Duch.) fruits from Turkey. *Journal of Applied Botany and Food Quality, 87*, 274–278. https://doi.org/10.5073/JABFQ.2014.087.038