

Effect of green extraction techniques on phenolic recovery and bioactivities of onion peel extracts

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Abstract

Onion peels are rich in bioactive compounds that can be recovered using green extraction methods. In this study, red (RO) and yellow onion (YO) peel extracts were prepared using ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). The extracts were characterized in terms of phenolic composition by high-performance liquid chromatography (HPLC) and total phenolic content (TPC), antioxidant activity using ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging and oxygen radical absorbance capacity (ORAC) assays, and antimicrobial activity against common foodborne and spoilage bacteria by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The highest TPC, FRAP and ORAC values were observed in YO peel extracts obtained by UAE: 369.72 ± 7.52 mg gallic acid equivalents (GAE)/g dry extract, 10.17 ± 0.45 mM Trolox equivalents (TE)/L and 134.18 ± 2.59 mM TE/L, respectively. Quercetin was the most abundant phenolic compound in all extracts tested. For YO peel extracts, UAE yielded higher antioxidant activity, while the extraction method had no effect on the antioxidant activity of RO peel extracts. Gram-positive bacteria were most susceptible to MAE extracts from YO peels and UAE extracts from RO peels. RO peel extracts showed stronger antimicrobial activity against Gram-negative bacteria than YO peel extracts. The lowest MIC and MBC values (0.31 mg/mL) were found against *Listeria monocytogenes*. These results indicate that the effects of UAE and MAE depend on onion type and the targeted functional property, with UAE being particularly effective for yellow onion peels, as reflected by the higher total phenolic content and antioxidant activity.

1. Introduction

The global annual production of onions is around 47.6 million tonnes, with yellow onions accounting for 75% of global cultivation and the average person consuming almost 6.35 kg per year [1]. The increasing global demand for onions has led to a considerable accumulation of onion skin waste, which is both ecologically and economically questionable. In the European Union alone, around 450,000 tonnes of onion skin waste is generated annually [2]. Onion processing generates substantial quantities of by-products, primarily consisting of outer dry scales, peels, and trimmed tissues, which are commonly discarded despite their high content of biologically active compounds. Studies have demonstrated that onion peels contain considerably higher concentrations of phenolic compounds, particularly flavonoids, than the edible bulb tissue [3–5]. Quercetin and its glycosylated derivatives represent the predominant flavonoids in onion peels, while phenolic acids like protocatechuic, ferulic, vanillic, and *p*-coumaric acids contribute to the overall phenolic profile [6, 7]. In addition to their antioxidant potential, onion peel extracts have been associated with anti-inflammatory [8, 9], cardioprotective [10], gastroprotective [11], anti-diabetic [12, 13], and anti-cancer effects [14, 15]. Quercetin, in particular, has been extensively studied for its role in reducing oxidative stress and regulating inflammation [5]. Furthermore, onion peels have demonstrated wound-healing potential by suppressing inflammatory cytokines and promoting tissue regeneration [8, 16]. Their antimicrobial activity also makes them suitable for use in cosmetics and health products aimed at treating infections and oxidative skin damage [7, 8]. Onion peels therefore represent a valuable and underutilised source of phenolic compounds with considerable potential for food, nutraceutical and pharmaceutical applications [5, 7, 17].

Besides extraction conditions, the chemical composition of onion peels is also strongly influenced by genetic and environmental factors. Onion is characterized by considerable genetic diversity, and the accumulation of flavonoids is strongly influenced by cultivar, geographical origin, climatic conditions, agricultural practices and environmental stress. Previous studies have demonstrated pronounced differences in the concentration of quercetin, flavonol glycosides and phenolic acids among onion cultivars grown in different regions, indicating that conclusions obtained for one cultivar cannot be directly extrapolated to another [18, 19]. Despite this well-recognized variability, onion cultivars originating from the eastern Adriatic and Mediterranean production area have not yet been comprehensively characterized. Among different species of the *Allium* genus, yellow and red onion peels are particularly rich in quercetin and its glycosides, as well as other phenolic compounds such as kaempferol and protocatechuic acid [20]. These phenolics are largely responsible for the strong antioxidant and antimicrobial properties of onion peel extracts [7, 20, 21]. Their composition

and biomedical potential have recently been reviewed [5]. Onion peel extracts have also shown potential as natural preservatives capable of reducing lipid oxidation and microbial spoilage in foods [17, 22].

The efficient recovery of bioactive compounds and the biological potential of onion peels are largely influenced by the choice of extraction method. In recent years, green extraction technologies have increasingly replaced conventional solvent extraction because they can improve extraction efficiency while reducing solvent consumption, extraction time and environmental impact. Several techniques have been investigated, including ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), ultrasound–enzyme-assisted extraction (UEAE), microwave-assisted extraction (MAE) and enzyme–ultrasound-assisted extraction (EUAE) using a choline chloride/lactic acid-based natural deep eutectic solvent (ChCl–La NADES) [20, 23–27]. The extraction method affects yield, composition and antioxidant properties because these techniques rely on fundamentally different extraction mechanisms. UAE promotes mass transfer through acoustic cavitation, whereas MAE relies on rapid dielectric heating and matrix disruption; consequently, their effects can vary depending on the plant matrix and the individual compounds being extracted. For example, supercritical fluid extraction, a promising green technique, uses carbon dioxide (CO₂), which is suitable for the extraction of temperature-sensitive and non-polar compounds [28]. UAE is often described as reducing extraction time and improving yield [24, 27, 29]. Consequently, individual phenolic compounds, particularly thermolabile flavonoids and glycosides, may respond differently to these extraction mechanisms depending on plant matrix characteristics. Direct comparisons of UAE and MAE performed under harmonized experimental conditions are therefore necessary to determine whether extraction efficiency is governed primarily by extraction technology or by cultivar-specific matrix properties [7]. Solvent selection also influences polyphenol recovery, with aqueous ethanol being among the most effective and sustainable solvent systems for onion peel extraction [30–33]. On the other hand, subcritical water extraction can increase extraction yield and selectively improve the recovery of specific compounds such as quercetin. Consequently, optimization of both extraction technology and solvent remains essential for maximizing biological activity.

Although numerous studies have investigated onion peel extracts and their antioxidant properties, comparative information regarding the influence of different green extraction technologies on phenolic composition, antioxidant capacity and antimicrobial activity of different onion varieties remains limited. Furthermore, studies integrating phenolic profiling with multivariate statistical approaches to define the relationship between composition and bioactivity are still scarce. In addition, Croatian cultivars have not been studied.

The aim and novelty of the present work lies not simply in evaluating onion peel extracts, but in the integrated comparison of two industrially relevant green extraction technologies (UAE and MAE) applied to two genetically distinct Croatian onion cultivars under identical extraction conditions. By combining quantitative phenolic profiling, multiple antioxidant assays, antibacterial activity against representative foodborne microorganisms and multivariate statistical analysis, this study provides new insight into the relative contributions of cultivar and extraction technology to the functional properties of onion peel extracts. Such information provides new insight into the relative contributions of cultivar and extraction technology to the functional properties of onion peel extracts.

2. Materials and Methods

2.1. Collection, preparation and extraction of material

Two varieties of onions (*Allium cepa* L.) were obtained as agro-food by-products in Kladnjice, Croatia (43°40'34.3"N, 16°17'25.0"E) in August 2023. The outer layers (peels) of the yellow onion (YO, Stuttgarter Riesen) and red onion (RO, domestic variety of the Trogir area) were separated and shade-dried (Fig. 1). After drying, the peels were milled into powder (MultiDrive control, IKA-Werke GmbH & Co. KG, Staufen, Germany) and extracted with a 50% (v/v) ethanol-water mixture using two different extraction methods: UAE for 1 h at 40 kHz and 60°C in an ultrasonic bath (Argolab, Carpi, Italy),

and MAE for 5 min at 600 W (EthosX, Milestone S.r.l., Sorisole, Italy). Each raw material was extracted three times, and each extract was freeze-dried and stored in a cool, dark place until analysis.

2.2. Chemical analysis

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method [34]. A standard calibration curve (0–500 mg/L) was prepared using gallic acid. The results are expressed as gallic acid equivalents (GAE) in mg/g of dried extract.

The dominant phenolic compounds were quantified using high-performance liquid chromatography (HPLC) equipped with a UV/VIS detector (Shimadzu Nexera LC-40, Shimadzu, Kyoto, Japan) and processed using LabSolutions software (Shimadzu, Kyoto, Japan), following the method described by Sharma et al. [35], with slight modifications. Chromatographic separation was carried out on a Phenomenex C18 column (250 × 4.6 mm, 5 µm particle size; Phenomenex, California, USA). The mobile phase consisted of solvent A (0.1% acetic acid in water) and solvent B (methanol). A 20 µL sample was injected into the column, which was maintained at 27°C. Detection was performed at 280 nm and 360 nm, with each sample injected in duplicate. The total run time was 25 min, using the following gradient: 20% B for the first 5 min, increasing linearly to 80% B by 10 min, held at 80% B until 15 min, then returning to 20% B from 15 to 20 min and maintained until the end of the run. Concentrations of identified phenolic compounds were expressed in mg per liter of extract (mg/L). The selection of protocatechuic acid, epicatechin, rutin, quercetin, myricetin and kaempferol was based on their prominent presence and key role in the phenolic profile of onion peel extracts [7, 35, 36].

2.3. Antioxidant assays

Antioxidant assays, diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC), were used to measure the antioxidant activity of onion by-product extracts. The methods are based on two distinct mechanisms of action, hydrogen atom transfer (DPPH and ORAC) and electron transfer (FRAP). Analyses were performed in triplicate and reported as mean ± standard deviation.

The FRAP assay was performed to quantify the reducing activity of the extracts following the method of Benzie and Strain [37], with slight modifications [38]. In brief, 300 µL of FRAP reagent was added to each microplate well, and the absorbance was recorded at 592 nm. Then, 10 µL of extract was added to the reagent, and the change in absorbance was measured after 4 min. The results were compared with a Trolox standard curve and expressed as millimoles of Trolox equivalents per liter of extract (mM TE/L).

The DPPH radical scavenging activity of the extracts was measured using the method described by Von Gadow et al. [39], with modifications [38]. A 290 µL solution of DPPH radical was pipetted into the microplate wells, and the initial absorbance at 517 nm was recorded. Then, 10 µL of the extract was added to the wells, and the decrease in absorbance was measured after 60 min using a plate reader. The antioxidant activity was expressed as mM TE/L.

The ORAC assay was carried out based on the modified method described in [40] to assess the ability of extracts to inhibit free peroxy radicals. The degradation of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) was monitored in the presence of fluorescein. Briefly, 150 µL of fluorescein was pipetted into the microplate wells together with 25 µL of sample, Trolox standard or blank. The plate was incubated at 37°C for 30 min, after which 25 µL of AAPH was added. Fluorescence intensity was measured every minute for 80 min at excitation and emission wavelengths of 485 nm and 520 nm, respectively. Results were expressed as mM TE/L.

2.4. Antimicrobial assays

The obtained extracts (10 mg/mL in 50% ethanol) were evaluated for antibacterial activity using the broth microdilution method, with 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT) serving as a growth indicator. Following

the protocols of Elez Garofulić et al. [41] and Klančnik et al. [42], the minimum inhibitory concentration (MIC) was defined as the lowest extract concentration at which no visible bacterial growth was observed, as indicated by the absence of a color change from colorless INT to red formazan.

Briefly, the extracts were diluted in Mueller–Hinton broth (MHB), and 100 µL of each dilution was added to the first well of a 96-well microtiter plate. Serial two-fold dilutions were performed across the remaining wells. Subsequently, 50 µL of a prepared bacterial inoculum was added to each well. The plates were mixed using a microtiter plate shaker (Plate Shaker-Thermostat PST-60 HL, Biosan, Riga, Latvia) at 600 rpm for 1 min, followed by incubation for 24 h at 37°C.

After incubation, 20 µL of INT solution (2 mg/mL) was added to each well. The plates were gently mixed and incubated in the dark for an additional hour. MIC values were determined visually as the lowest concentration of extract that prevented the formation of red formazan, indicating inhibition of bacterial growth. The minimum bactericidal concentration (MBC) was defined as the lowest concentration at which no bacterial growth was observed after recultivation on Mueller–Hinton Agar plates [41].

The antibacterial assays were performed against common foodborne and spoilage bacteria at concentrations of 1×10^6 to 10^7 colony-forming units (CFU)/mL. The tested strains included *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 14579, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Enteritidis ATCC 13076 and *Shigella sonnei* ATCC 9290.

2.5. Statistical analyses

Analyses of variance (one-way ANOVA followed by Fisher's least significant difference test) were used to express the statistical difference for the results of the TPC, FRAP, DPPH and ORAC assays, and chemical profile between the different onion types and extracts [43]. The analyses were performed with Statgraphics Centurion-Ver. 16.1.11 (StatPoint Technologies, Inc., Warrenton, VA, USA). The principal component analyses (PCA) was used to determine the relationship between the antioxidant activity, dominant phenolics and activity against Gram+ pathogens using the software STATISTICA® (version 13, StatSoft Inc., Tulsa, OK, USA). Before analyses, the data were log-transformed [44].

3. Results and discussion

3.1. Phenolic profiling

The TPC results for YO and RO peel extracts (Fig. 2) show the influence of the different extraction methods on phenolic concentration. For YO peel, the UAE extract had the highest TPC (369.72 ± 7.52 mg GAE/g), while the MAE extract yielded an approximately 2-fold lower value of 176.83 ± 9.07 mg GAE/g. In RO peel extracts, TPC values did not vary significantly between extraction methods. The extract produced using UAE yielded a TPC of 266.06 ± 7.49 mg GAE/g, while MAE slightly increased the TPC to 274.13 ± 6.51 mg GAE/g. Overall, the results show that UAE was more effective for maximizing TPC in YO peel, whereas MAE yielded the slightly higher TPC value in RO peel extracts. Khalili et al. [45] determined the phenolic content of red onion peels macerated for 72 h in water and pure ethanol. The TPC of the water macerate was 204.56 ± 10.22 mg GAE/g of extract, while that of the ethanol macerate was 330.51 ± 7.88 mg GAE/g, which is within the range of our results and demonstrates the direct influence of the solvent on TPC. Bozinou et al. [30] extracted red onion peels using water, ethanol and their mixtures for 2 h under stirring at 40 °C, with two extraction cycles. The TPC of the 50% ethanol extract was 505.57 ± 28.63 mg GAE/g of extract, which is approximately twice the values obtained in this study and confirms the suitability of this solvent for extracting higher amounts of phenolic compounds. Gorrepati et al. [21] investigated TPC in the peels of eight onion cultivars (dark red, red, light red and white). TPC ranged from 78.77 ± 0.25 to 1738.21 ± 2.74 mg/100 g dry weight (DW), with the highest values recorded for dark red varieties. Mounir et al. [8] reported the chemical profile and biological activity of red and yellow onion peels extracted using a Soxhlet apparatus (80% methanol, 65 °C, 1 h). ultra-performance liquid chromatography–quadrupole time-of-flight tandem

mass spectrometry (UPLC-QTOF–MS/MS) analysis confirmed flavonols as the most frequently detected compounds in both red and yellow onion peels, mainly quercetin, kaempferol, taxifolin, isorhamnetin and myricetin derivatives. Constantin et al. [46] investigated the influence of conventional solvent extraction and novel extraction methods (UAE, enzyme-assisted extraction (EAE), and supercritical fluid extraction (SFE)) on TPC, chemical composition and antioxidant activity of yellow onion peel extracts. The solvent used was a 70% ethanol solution and glacial acetic acid in an 8:1 ratio. The highest TPC and antioxidant activity were recorded for SFE, and the most dominant compound identified was quercetin 3,4-diglycoside.

HPLC analysis of the most abundant phenolic compounds in yellow (YO) and red onion (RO) peel extracts obtained using UAE and MAE is summarized in Table 1. Significant differences ($p < 0.05$) were observed between extraction methods and onion types for several compounds.

The selected phenolic compounds, protocatechuic acid, epicatechin, rutin, quercetin-3-glucoside, myricetin, quercetin and kaempferol, represent structurally diverse bioactive molecules, including phenolic acids, flavanols and flavonoids. These compounds are associated with a variety of health-promoting effects, such as cardioprotective, neuroprotective, anti-obesity, antidiabetic, anti-cholesterol, anticancer and antimicrobial activities [47, 48]. Their presence not only contributes to the antioxidant capacity of onion peel extracts but also serves as a basis for quality control and the potential standardization of functional products [49]. Analysing these specific phenolics allows comparative studies between different onion cultivars and various onion parts, such as onion peel, offering valuable insights for research and potential industrial applications in functional foods and dietary supplements.

Quercetin, often the predominant flavonoid in onion peels, along with its derivatives, contributes significantly to the antioxidant capacity of these matrices [7, 21]. Quercetin was the most abundant compound in all extracts, although its recovery varied with both onion variety and extraction method. In YO peels, MAE yielded a slightly higher quercetin concentration (117.11 ± 8.14 mg/L) than UAE (110.35 ± 2.01 mg/L), whereas the opposite trend was observed in RO peels, where UAE resulted in a higher quercetin content (63.14 ± 2.58 mg/L) than MAE (57.68 ± 0.97 mg/L). This reflects the influence of both onion variety and extraction technique on compound recovery. Protocatechuic acid, a degradation product of quercetin, showed the highest concentration in the YO UAE extract (30.54 ± 1.61 mg/L), followed by the RO UAE extract (18.50 ± 1.10 mg/L), with significantly lower concentrations in both MAE extracts, suggesting that UAE better preserves or facilitates the extraction of this phenolic acid. These findings are consistent with the study conducted by Hajiguliyeva et al. [50], who reported higher levels of protocatechuic acid in red onion peels than in yellow onion peels, although in our study yellow peels yielded higher values under UAE conditions. Epicatechin concentrations were highest in the YO UAE extract (46.84 ± 7.01 mg/L), while RO peels yielded comparable concentrations regardless of extraction method (approximately 26 mg/L for both UAE and MAE), suggesting that YO peels may represent a richer source of flavanols and that UAE is a more efficient extraction method for this compound in YO matrices. Rutin concentrations were consistently higher in UAE extracts for both onion varieties, with the highest levels recorded in the RO UAE extract (5.98 ± 1.17 mg/L), followed by the YO UAE extract (5.01 ± 0.92 mg/L). Both MAE samples exhibited significantly lower rutin levels, highlighting the importance of solvent dynamics and extraction conditions in preserving or enhancing specific phenolics. A similar trend was observed for quercetin-3-glucoside, which reached the highest concentration in the RO UAE extract (6.03 ± 2.34 mg/L) and the lowest concentration in the RO MAE extract (2.14 ± 0.20 mg/L), further supporting the superior efficiency of UAE in the extraction of glycosylated flavonoids from onion peel.

A similar trend was observed for myricetin, with the highest concentration measured in RO UAE (8.25 ± 1.16 mg/L), while in YO peel extracts UAE was more effective than MAE (6.10 ± 0.38 mg/L vs. 4.78 ± 0.37 mg/L, respectively). Kaempferol showed a matrix-dependent response to the extraction method. In RO peels, UAE yielded the highest concentration (12.80 ± 0.19 mg/L) compared with MAE (10.61 ± 0.82 mg/L). In contrast, in YO peels, MAE yielded more kaempferol (5.65 ± 0.15 mg/L) than UAE (4.03 ± 0.58 mg/L), indicating that the extraction method plays a compound-specific role depending on the matrix.

The total concentration of quantified phenolics was highest in the YO UAE extract (205.72 ± 12.94 mg/L), followed by YO MAE (181.84 ± 10.88 mg/L), RO MAE (141.09 ± 20.26 mg/L) and RO UAE (119.95 ± 4.32 mg/L). These results suggest that YO peels generally yielded higher total concentrations of the quantified phenolics than RO peels and that UAE was particularly effective at extracting a broader range of compounds from yellow onions. The observed variability also supports the idea that phenolic content and composition can serve as markers for onion peel extract standardization and quality profiling, with potential applications in functional food and nutraceutical product development.

Table 1. HPLC concentrations (mg/L) of the most abundant compounds in yellow (YO) and red (RO) onion peels extracted by ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). Different letters a–d denote statistically significant differences ($p < 0.05$) among extracts.

Sample	YO		RO	
Extraction method	UAE	MAE	UAE	MAE
Protocatechuic acid	30.54 ± 1.61^a	16.97 ± 1.04^b	18.50 ± 1.10^c	14.04 ± 0.21^c
Epicatechin	46.84 ± 7.01^a	32.31 ± 0.42^{ab}	26.38 ± 11.70^b	26.44 ± 1.18^b
Rutin	5.01 ± 0.92^b	2.77 ± 0.10^b	5.98 ± 1.17^a	3.57 ± 0.36^b
Quercetin-3-glucoside	2.87 ± 0.42^a	2.24 ± 0.65^a	6.03 ± 2.34^b	2.14 ± 0.20^a
Myricetin	6.10 ± 0.38^a	4.78 ± 0.37^a	8.25 ± 1.16^b	5.49 ± 0.59^a
Quercetin	110.35 ± 2.01^a	117.11 ± 8.14^b	63.14 ± 2.58^c	57.68 ± 0.97^c
Kaempferol	4.03 ± 0.58^a	5.65 ± 0.15^b	12.80 ± 0.19^c	10.61 ± 0.82^d
Total	205.72 ± 12.94^a	181.84 ± 10.88^b	119.95 ± 4.32^c	141.09 ± 20.26^d

The results of our study are consistent with previous reports confirming quercetin as the dominant flavonoid in onion peel extracts, although absolute concentrations vary considerably depending on solvent, extraction technique and onion variety. Bozinou et al. [30] reported a quercetin concentration of 259.27 ± 9.59 mg/L using 50% ethanol, while Joković et al. [7] identified quercetin as the most abundant compound among 23 detected phenolics in yellow onion peel extracts (48.53 ± 7.95 mg/g DW in the methanol extract). Celano et al. [2] similarly reported quercetin derivatives as the predominant phenolic compounds in UHPLC-HRMS/MS analysis of UAE extracts obtained from yellow and red onion peels. Their findings also agree with the higher kaempferol content observed in RO UAE extracts. Moreover, the generally higher concentrations of several phenolic compounds obtained by UAE support previous evidence that UAE can enhance the release of bound phenolics more effectively than conventional extraction methods. Notably, RO peels yielded higher concentrations of several phenolic compounds than YO peels, whereas quercetin was present at a higher concentration in YO peels.

3.2. Antioxidant activity

The antioxidant activity of YO and RO peel extracts obtained by UAE and MAE was evaluated using FRAP, DPPH and ORAC methods. The results show significant differences between extraction methods and between the two samples (Fig. 3). In the FRAP assay, the highest value was observed for YO extracts obtained by UAE (10.17 ± 0.45 mM TE/L), while the lowest value was observed for RO extracts obtained by MAE (6.27 ± 0.19 mM TE/L). For the FRAP assay, UAE was the more effective extraction method for both samples; however, the overall antioxidant response differed according to assay and onion type. For DPPH radical scavenging activity, the results did not vary significantly between samples, with values ranging from 35.13 ± 0.97 to 38.10 ± 0.09 mM TE/L. The highest DPPH antioxidant capacity was observed for YO

extracted by MAE, while the lowest was observed for RO extracted by UAE. The ORAC assay showed differences between extraction methods and the analysed onion samples. The highest ORAC value (134.18 ± 2.59 mM TE/L) was observed for YO extract prepared using UAE, compared with the YO extract prepared using MAE, which had the lowest value (82.86 ± 5.95 mM TE/L). For RO peels, the MAE extract had a higher ORAC value of 113.27 ± 9.84 mM TE/L compared with 110.70 ± 8.33 mM TE/L for UAE. Celano et al. [2] extracted yellow and red onion peels using UAE (25 °C, 20 kHz, 70% ethanol). In contrast to our results, the authors found almost 2-fold higher antioxidant activity for red onion peel extract than for yellow onion peel extracts using ORAC and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) methods. Gorrepati et al. [21] investigated the antioxidant activity (FRAP, DPPH and ABTS) of onion peel powder from eight onion cultivars (dark red, red, light red and white). The highest antioxidant activity was recorded for dark red varieties. Bozinou et al. [30] measured the antioxidant activity of red onion peel extracts obtained with water-ethanol mixtures using DPPH, FRAP and H2O2 scavenging methods and found higher values compared with water-only extracts. Their results cannot be directly compared with those of the present study because different measurement units were used; however, they support the choice of 50% ethanol as one of the most suitable solvents for extracting phenolic compounds with high antioxidant activity. Overall, UAE was the better extraction method for preserving the antioxidant activity of YO peel extracts in our study, except for DPPH inhibition, for which MAE yielded a higher value, although the difference was not statistically significant. In contrast, the choice of extraction method did not substantially affect the antioxidant activity of RO peels.

3.3. Antimicrobial activity

The antimicrobial activity of yellow onion (YO) and red onion (RO) peel extracts against four Gram-positive bacterial strains, *Enterococcus faecalis*, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*, is presented in Table 2. The lowest MIC and MBC values were observed for *L. monocytogenes*, demonstrating the strong antimicrobial potential of MAE extracts from both YO and RO. Differences in antimicrobial activity against *S. aureus* were observed between extraction methods; while most extracts exhibited similar inhibitory and bactericidal effects, the YO extract obtained by MAE demonstrated reduced activity compared with the other treatments.

Notable variations were also found between the two onion types and the applied extraction techniques. YO peel extracts obtained using MAE exhibited strong activity against *E. faecalis*, with both MIC and MBC values of 0.62 mg/mL, but showed lower efficacy against *S. aureus* (MIC = 2.5 mg/mL and MBC = 5 mg/mL). In contrast, RO peel extracts obtained by UAE demonstrated good antimicrobial activity, particularly against *E. faecalis* and *B. cereus*, with MIC and MBC values of 0.62 mg/mL and 1.25 mg/mL, respectively. These results show the influence of extraction method on the antimicrobial activity of onion peel extracts, with MAE yielding extracts with enhanced efficacy against specific pathogens such as *L. monocytogenes*.

The high susceptibility of *L. monocytogenes* observed in this study may be explained by its pronounced sensitivity to plant polyphenols [51]. Previous studies indicate a negative impact of phenolic compounds on the growth of *L. monocytogenes* through multiple mechanisms, including disruption of bacterial membrane integrity and inhibition of adhesion, biofilm formation and motility, thereby reducing its ability to survive and proliferate [51, 52]. Consistent with these findings, the exploratory PCA associated *L. monocytogenes* with kaempferol, myricetin, quercetin-3-glucoside and rutin rather than with total phenolic content or phenolic acids, suggesting an association between specific flavonols and their glycosides and the antimicrobial activity observed against this pathogen.

The antimicrobial activity of YO and RO peel extracts was also assessed against four Gram-negative bacterial strains: *E. coli*, *P. aeruginosa*, *S. Enteritidis* and *S. sonnei* (Table 3). Both YO and RO extracts exhibited identical MIC and MBC values (5 mg/mL) against *E. coli* and *S. enteritidis*, irrespective of the extraction method used. The YO peel extract obtained by UAE did not demonstrate inhibitory or bactericidal activity against *P. aeruginosa* at the tested concentrations, whereas the MAE extract exhibited inhibitory effects but no bactericidal effect (MBC >5 mg/mL). In contrast, RO extracts, especially those obtained by MAE, showed antimicrobial activity against *P. aeruginosa* at the highest tested concentration.

Differences in activity were also observed for *S. sonnei*. YO extracts showed weak inhibitory effects without bactericidal activity, while RO extracts, particularly the MAE extract, exhibited superior efficacy, with the lowest MIC and MBC values among the samples tested. Overall, RO peel extracts showed slightly greater antimicrobial activity against Gram-negative bacteria than YO peel extracts, particularly against *P. aeruginosa* and *S. sonnei*.

The more pronounced antimicrobial activity of onion peel extracts against Gram-positive bacteria can be attributed to differences in cell-envelope structure. Because Gram-positive bacteria do not have an outer membrane, phenolic compounds, including flavonoids and phenolic acids present in onion peel extracts, can interact more readily with the cytoplasmic membrane, disrupting its integrity and function [53]. This can result in leakage of intracellular contents and ultimately lead to cell death. In contrast, Gram-negative bacteria possess an additional outer membrane rich in lipopolysaccharides, which acts as a barrier to the penetration of phenolic compounds and consequently reduces their susceptibility to these bioactive compounds.

Compared with our results, Khalili et al. [45] reported significantly lower MIC and MBC values for red onion peel extracts prepared by ethanol and water maceration. For *S. aureus*, the MIC and MBC were 400 and 800 µg/mL for the water extract and 200 and 400 µg/mL for the ethanol extract, respectively; for *E. coli*, both extracts exhibited MIC and MBC values of 400 and 800 µg/mL. Similarly, Bozinou et al. [30] evaluated the antimicrobial activity of a 50% ethanol extract of red onion peel using the agar disk diffusion method and confirmed activity against *L. monocytogenes*, *E. coli*, *E. faecalis*, *P. aeruginosa* and *S. aureus*, with *E. faecalis* being the most susceptible.

Joković et al. [7] tested onion peel extracts against eight microbial strains, including *S. aureus* ATCC 6538, *E. faecalis* ATCC 19433, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 12453, *Klebsiella aerogenes* ATCC 13048, *S. Enteritidis* ATCC 13076 and *S. sonnei* ATCC 25931. The extracts showed inhibitory effects, with the best MIC results recorded for the pure ethanolic extract (0.78–12.50 mg/mL). MIC values for selected strains included *S. aureus* (1.56 mg/mL), *E. faecalis* (3.13 mg/mL), *E. coli* (12.50 mg/mL), *S. Enteritidis* (6.25 mg/mL) and *P. aeruginosa* (6.25 mg/mL). In comparison, our study found lower MIC values for YO peel extracts against the same strains. Contrary to our findings, Mounir et al. [8] reported poor activity of yellow onion peel extract against *P. aeruginosa*, with no observed inhibition, while the red onion peel extract showed limited activity (MIC = 31.25 mg/mL, MBC = 62.5 mg/mL). However, their findings for *S. aureus* were consistent with ours, reporting an MIC and MBC of 1.9 mg/mL for red onion peel extract. In contrast, yellow onion peel extract showed lower efficacy in their study (MIC and MBC = 15.6 mg/mL) than observed in ours. Several factors can explain differences in antimicrobial activity between onion peel extracts. These include growing conditions (soil type, climate, fertilization, harvest time), post-harvest handling and storage conditions (temperature, light exposure), varietal differences in phytochemical composition and different extraction methods.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of yellow (YO) and red (RO) onion peel extracts against Gram-positive bacterial strains, expressed in mg/mL (MAE – microwave-assisted extraction; UAE – ultrasound-assisted extraction)

Onion type	Method	<i>Enterococcus faecalis</i>		<i>Bacillus cereus</i>		<i>Listeria monocytogenes</i>		<i>Staphylococcus aureus</i>	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
YO	UAE	1.25	1.25	2.5	2.5	1.25	1.25	1.25	2.5
YO	MAE	0.62	0.62	1.25	1.25	0.31	0.31	2.5	5
RO	UAE	0.62	0.62	1.25	1.25	1.25	1.25	1.25	2.5
RO	MAE	1.25	2.5	1.25	2.5	0.31	0.31	1.25	2.5

Table 3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of yellow (YO) and red (RO) onion peel extracts against Gram-negative bacterial strains, expressed in mg/mL (MAE – microwave-assisted

extraction; UAE – ultrasound-assisted extraction)

Onion type	Method	<i>Escherichia coli</i>		<i>Pseudomonas aeruginosa</i>		<i>Salmonella Enteritidis</i>		<i>Shigella sonnei</i>	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
YO	UAE	5	5	n.d.	n.d.	5	5	5	n.d.
YO	MAE	5	5	5	n.d.	5	5	5	n.d.
RO	UAE	5	5	5	5	5	5	5	5
RO	MAE	5	5	5	5	5	5	2.5	5

n.d., not determined; >5 mg/mL.

The intermediate position of *E. faecalis* and *S. aureus* between the antioxidant and flavonoid-associated clusters suggests that their susceptibility may be associated with the combined action of multiple phenolic compounds. This is consistent with previous reports highlighting additive and synergistic interactions among phenolic constituents as an important determinant of the antibacterial activity of plant extracts [61]. Such behaviour supports the concept that the antimicrobial activity of plant extracts can arise from multiple mechanisms, including membrane disruption, alteration of membrane permeability, enzyme inhibition, metal chelation and interference with cellular redox balance. Similar to antioxidant activity, synergistic interactions among phenolic constituents may therefore contribute substantially to the antibacterial efficacy of onion peel extracts. The importance of such interactions has been highlighted for phenolic-rich systems, where the biological activity of the whole extract frequently exceeds the activity predicted from individual compounds alone [57, 62].

Principal component analysis (PCA) was applied as an exploratory multivariate approach to investigate the relationships among phenolic composition, antioxidant properties and antimicrobial activity against Gram-positive bacteria in onion peel extracts (Fig. 4a,b). Although the PCA was performed using four extract types, the analysis provided a useful exploratory overview of the relationships among the measured variables. Therefore, the observed associations should be interpreted as indicative trends that may guide future studies involving larger sample sets. The first two principal components explained 85.80% of the total variance, indicating that the two-dimensional model adequately represented the variability of the dataset. PC1 was primarily associated with the overall antioxidant potential of the extracts. Strong negative loadings were observed for TPC (−0.944), FRAP (−0.908), ORAC (−0.911), protocatechuic acid (−0.915), epicatechin (−0.795) and *B. cereus* MIC values (−0.908). This clustering suggests that extracts characterized by higher concentrations of phenolic compounds, particularly protocatechuic acid and epicatechin, also tended to exhibit strong reducing power and peroxy radical scavenging activity. The close positioning of these variables indicates a strong association with the antioxidant potential of the extracts. The observed clustering of TPC, FRAP, ORAC, protocatechuic acid and epicatechin may be explained by the contribution of phenolic acids to both electron transfer (ET) and hydrogen atom transfer (HAT) reactions [54].

Although FRAP and ORAC are based on different reaction mechanisms, previous studies have shown that phenolic compounds exhibit high activity in both assays. This activity is determined not only by the number of hydroxyl groups but also by their position on the aromatic ring and the presence of methoxy or other substituent groups. These structural features influence their ability to donate electrons and hydrogen atoms as well as to stabilize the resulting phenoxyl radicals, thereby contributing to antioxidant activity in both electron transfer- and hydrogen atom transfer-based assays [55, 56]. Skroza et al. [57] reported that phenolic acids with ortho-dihydroxyl substitution patterns, such as protocatechuic acid, contribute substantially to antioxidant capacity measured by both FRAP and ORAC assays, highlighting their multifunctional antioxidant behaviour. Furthermore, the cooperative action of multiple phenolics should also be considered, as the overall antioxidant potential of complex extracts is often influenced by synergistic interactions among individual compounds rather than by a single dominant constituent.

In contrast, DPPH activity was strongly negatively associated with PC2 (-0.980), together with quercetin (-0.815), suggesting that radical scavenging activity measured by DPPH reflects a different mechanism of action compared with the FRAP and ORAC assays. The positioning of quercetin close to DPPH in the correlation plot supports an association between quercetin and hydrogen atom transfer reactions, consistent with its well-documented radical scavenging properties. The strong association between quercetin and DPPH activity is consistent with the structural characteristics of quercetin, which contains a catechol moiety in the B-ring and multiple hydroxyl groups capable of efficient radical quenching. Onion peel is particularly rich in quercetin and its glycosides, which are recognized as primary contributors to the antioxidant potential of this by-product [5]. These structural features facilitate hydrogen atom donation and stabilization of the resulting semiquinone radical through resonance, making quercetin one of the most effective naturally occurring radical scavengers. Previous studies have shown that quercetin exhibits particularly high activity in radical-based antioxidant assays, including DPPH, because its favourable hydroxylation pattern and conjugated molecular structure promote efficient radical scavenging and stabilization of the resulting phenoxyl radical [58, 59].

This is consistent with the exploratory PCA results obtained in the present study, in which quercetin was closely associated with DPPH activity. Positive PC2 loadings were dominated by kaempferol (0.917), myricetin (0.891), quercetin-3-glucoside (0.871), rutin (0.714) and *L. monocytogenes* MIC values (0.430). Unlike phenolic acids, flavonols and their glycosides vary significantly in polarity and affinity for bacterial cell surfaces, which may affect their antimicrobial activity [60]. The clustering of kaempferol, myricetin, rutin and quercetin-3-glucoside with *L. monocytogenes* suggests an association between the qualitative composition of flavonoids and the antimicrobial response rather than with total phenolic content alone. In addition, a strong positive correlation was observed between rutin (quercetin-3-O-rutinoside) and *L. monocytogenes* MIC values ($r = 0.934$), suggesting a potential association of quercetin glycosides with the antimicrobial profile of the extracts. As rutin is the glycosylated form of quercetin, its biological activity differs from that of the aglycone due to changes in polarity and molecular interactions. Glycosylation can affect the accessibility of flavonoids to bacterial cells, suggesting that quercetin derivatives may be associated differently with the antimicrobial profile of onion peel extracts compared with free quercetin [5, 60].

The exploratory PCA results demonstrated that extraction conditions were associated with differences in the functional properties of the resulting extracts. The multivariate approach highlighted two distinct bioactivity patterns: one associated with high antioxidant capacity and elevated levels of protocatechuic acid and epicatechin, and another linked to specific flavonoids and differential antimicrobial responses. These findings support the concept that onion peel extracts represent multifunctional matrices whose biological activity is associated with both phenolic composition and interactions among individual compounds.

Overall, the exploratory PCA suggests that the biological activity of onion peel extracts is associated not only with total phenolic content but also with the qualitative composition of individual phenolic compounds. The distinct clustering of antioxidant parameters and antimicrobial variables indicates that different phenolic groups may be associated differently with the biological properties of the extracts.

4. Conclusions

Onion peels represent a valuable agro-food by-product rich in phenolic compounds with significant antioxidant and antimicrobial potential. This study demonstrated that both onion variety and extraction methods influence the phenolic composition and biological activity of the obtained extracts. UAE proved particularly effective for recovering phenolic compounds from yellow onion peels, resulting in higher total phenolic content and enhanced antioxidant activity, while MAE showed advantages for specific antimicrobial responses. Quercetin was the predominant phenolic compound in all extracts, whereas protocatechuic acid, epicatechin and rutin were associated with differences in bioactivity among samples. Multivariate analysis revealed that the biological properties of onion peel extracts are associated not only with total phenolic content but also with the composition and interactions of individual phenolic compounds. Extracts enriched

in protocatechuic acid and epicatechin were associated with stronger antioxidant activity and greater efficacy against *B. cereus*, while flavonoid-rich profiles were associated with distinct antimicrobial responses. These results support the valorisation of onion peels as sustainable sources of natural antioxidant and antimicrobial ingredients for use in food, nutraceutical and related applications, while contributing to agro-food by-product waste reduction and sustainable development.

Declarations

Author Contributions: “Conceptualization, Vida Šimat and Danijela Skroza.; methodology, Danijela Skroza and Maja Veršić Bratinčević; validation, Danijela Skroza and Maja Veršić Bratinčević; formal analysis, Vida Šimat, Martina Čagalj, Roberta Frleta Matas, Petra Pavić, Danijela Skroza and Maja Veršić Bratinčević; investigation, Vida Šimat, Martina Čagalj, Danijela Skroza and Maja Veršić Bratinčević; resources, Vida Šimat and Danijela Skroza; data curation, Martina Čagalj, Roberta Frleta Matas, Petra Pavić, Danijela Skroza and Maja Veršić Bratinčević; writing—original draft preparation, Martina Čagalj, Danijela Skroza, Petra Pavić, and Roberta Frleta Matas; writing—review and editing, Vida Šimat; supervision, Vida Šimat and Danijela Skroza; project administration, Vida Šimat, Martina Čagalj and Danijela Skroza; funding acquisition, Vida Šimat and Danijela Skroza. All authors have read and agreed to the published version of the manuscript.”

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Ethics approval and consent to participate: Not applicable. This study did not involve human participants or animals.

Plant guidelines: The plant material originated from cultivated yellow and red onion (*Allium cepa* L.) intended for food production. No wild or protected plant species were used in this study. The use of the plant material was conducted in accordance with applicable local and national guidelines.

Permissions to collect plant material: The onion peels used in this study were obtained as agro-food waste/by-products from cultivated onions grown on privately owned land in Kladnjice, Croatia. Permission to use this material for scientific research was obtained from the landowner. Since no wild plant material or plant specimens were collected from the natural environment, no specific collection permits or licences were required.

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Competing interests: The authors declare no competing interests.

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Figures



Figure 1

Yellow (Stuttgarter Riesen, left) and red (domestic variety of the Trogir area, right) onion peels

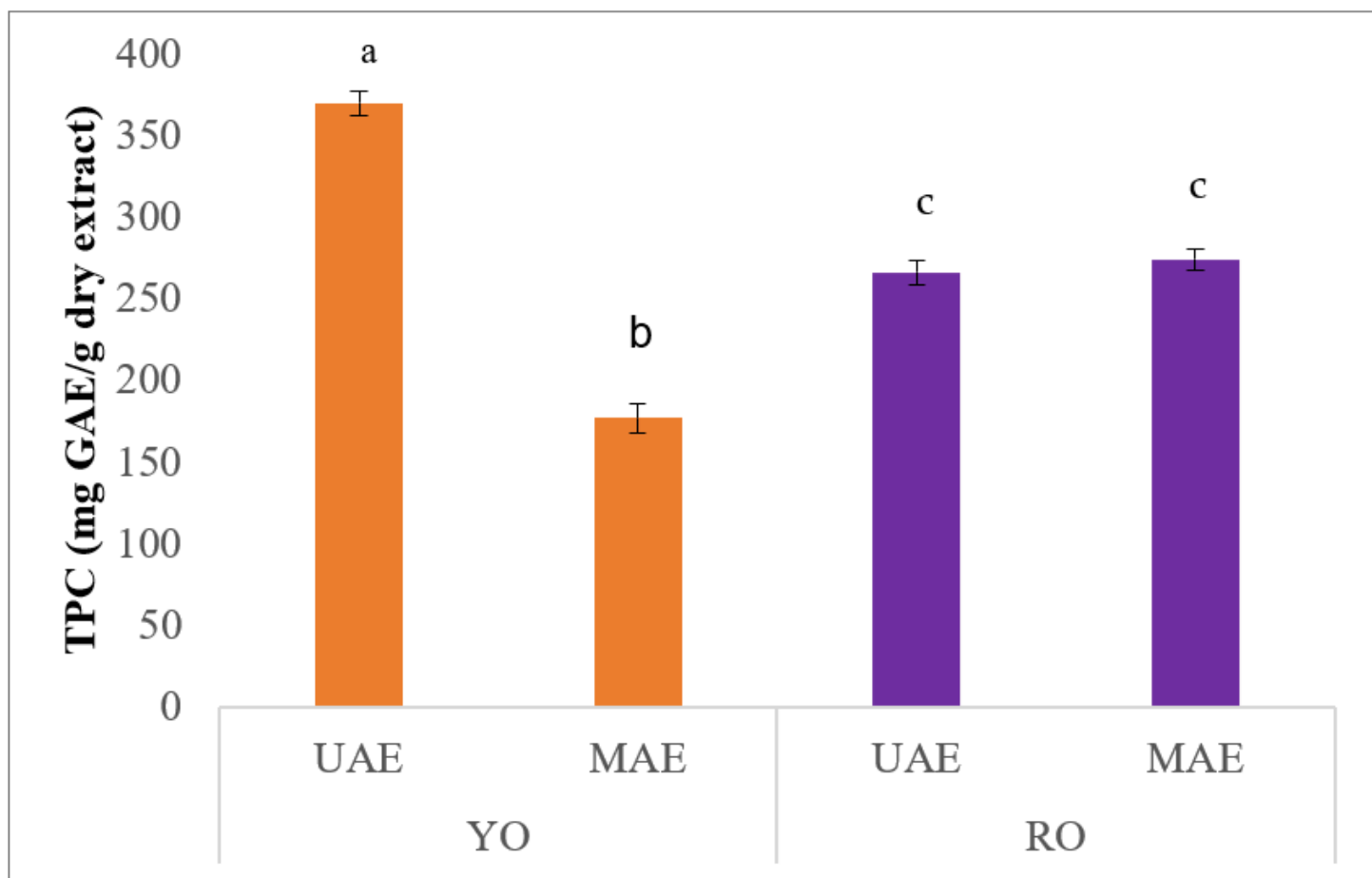


Figure 2

Total phenolic content (TPC) in yellow (YO) and red (RO) onion peel extracts (MAE – microwave-assisted extraction; UAE – ultrasound-assisted extraction; GAE – gallic acid equivalents); different letters a–c denote statistically significant differences ($p < 0.05$) among extracts



Figure 3

Antioxidant activity measured by ferric reducing antioxidant power (FRAP) (a), diphenyl-1-picrylhydrazyl (DPPH) radical scavenging (b), and oxygen radical absorbance capacity (ORAC) (c) in yellow (YO) and red (RO) onion peel extracts (MAE – microwave-assisted extraction; UAE – ultrasound-assisted extraction; TE – Trolox equivalents); different letters a–c denote statistically significant differences ($p < 0.05$) among extracts

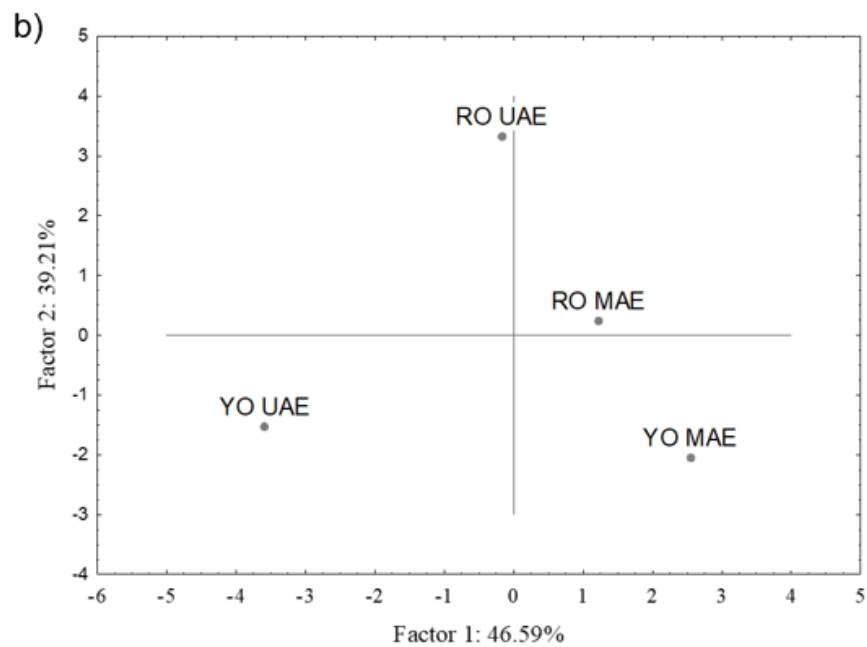
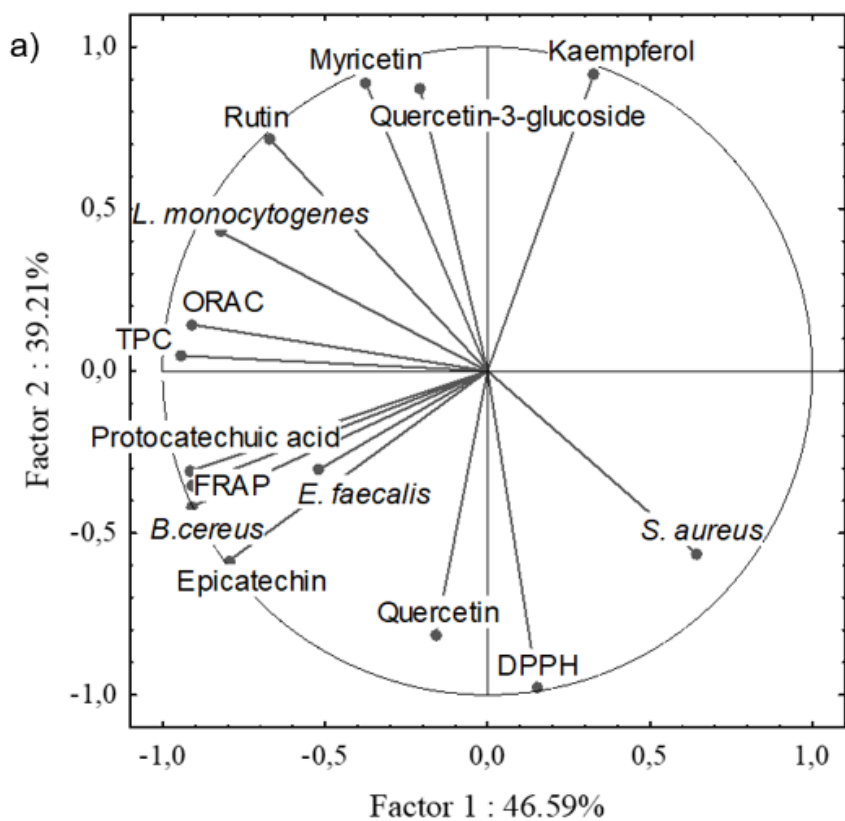


Figure 4

Correlation loadings (a) and score plot (b) of antioxidant activity, dominant phenolics and activity against Gram-positive pathogens