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Identification of the bioactive compounds and antioxidant, antimutagenic and antimicrobial activities of thermally processed agro-industrial waste

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ABSTRACT

The purpose of the research was to identify the bioactive compounds and to evaluate the antioxidant, antimutagenic and antimicrobial activities of the major Romanian agro-industrial wastes (apple peels, carrot pulp, white- and red-grape peels and red-beet peels and pulp) for the purpose of increasing the wastes' value. Each type of waste material was analyzed without (fresh) and with thermal processing (10 min, 80 °C). Based on the obtained results, the thermal process enhanced the total phenolic content. The highest antioxidant activity was exhibited by thermally processed red-grape waste followed by thermally processed red-beet waste. Linoleic acid was the major fatty acid in all analyzed samples, but its content decreased significantly during thermal processing. The carrot extracts have no antimicrobial effects, while the thermally processed red-grape waste has the highest antimicrobial effect against the studied strains. The thermally processed red-grape sample has the highest antimutagenic activity toward *S. typhimurium* TA98 and TA100.

1. Introduction

In recent years, there has been a worldwide concern regarding the use of industrial waste. The industrial waste is generated during processing of raw materials in the food industry. The insufficient collection and improper disposal of agro-industrial wastes can cause pollution problems and the loss of biomass, which can serve as a source of bioactive compounds (Shyamala & Jamuna, 2010). By-products from the fruit and vegetable industry are particularly of interest because they are inexpensive and available in large quantities. Some of the agricultural by-products such as apples and citrus fruits have indeed already been used in the production of dietary fiber (Figuerola, Hurtado, Estevez, Choffelle, & Asenjo, 2005). The compositions and physicochemical properties of dietary fibers depend on both the characteristics of the raw materials and the processing steps (Chau, Chen, & Lee, 2004).

The presence of bioactive molecules, such as fatty acids and phenolic compounds, in agro-industrial waste makes fruit and vegetable leftovers more valuable for the food industry. Fruits and vegetables contain bioactive compounds that impart health benefits beyond basic nutrition (Oomah & Mazza, 2000). It has been reported that the antioxidant capacity of fruits correlates to their total phenolic content and composition (Corral-Aguayo, Yahia, Carrilo-Lopez, & Gonzalez-Aguilar, 2008). Recent studies show that

Abbreviations: AF, apple waste fresh; CF, carrot waste fresh; WGF, white-grape waste fresh; FAMEs, fatty acid methyl esters; RGF, red-grape waste fresh; BF, red-beet waste fresh; AT, apple waste thermally processed; CT, carrot waste thermally processed; WGT, white-grape waste thermally processed; RGT, red-grape waste thermally processed; BT, red-beet waste thermally processed; HPLC-DAD-ESI-MS, high-performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry; I%, percentage inhibition of DPPH radical; RIC, radical inhibition capacity; TLs, total lipids; GC–MS, gas chromatograph mass spectrometry; wt%, weight percentages; MIC, minimum inhibitory concentration; MBC, minimum bacterial concentration; SD, standard deviation; GAE, gallic acid equivalents; TF, total flavonoids; DW, dry weight; QE, quercetin equivalents; CFU, colony forming unit; TP, total phenolic/polyphenols.

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the antimicrobial potential of natural extracts is higher than that shown by synthetic antibiotics (Katalinić et al., 2010).

The Romanian fruit and vegetable juice and pulp industries stand out. Much of the waste, including apple peels, carrot pulp, white and red grape peels and red beet peels and pulp, is discarded; however, this waste is rich in bioactive compounds and can thus be improved and incorporated into food supplements (European Commission Final Report, 2010).

One of the five major Romanian agro-industrial wastes is apple peel. Studies report that apple peel, in addition to its ability to inhibit lipid oxidation, has phytochemicals that exhibit cardioprotective and anticancer properties (Knekt et al., 2002). These health advantages of apple peels are correlated with the presence of flavonols, anthocyanins, flavan-3-ols, phenolic acids and dihydrochalcones (Boyer & Liu, 2004). Other previous results have indicated that approximately 80% of polyphenols are concentrated in apple peel (Leccese, Bartolini, & Viti, 2009), whose total antioxidant capacity is five-to-six-fold higher than that of apple flesh; the peel also possesses unique flavonoids, such as quercetin glycosides, that are not found in the flesh (Rupasinghe & Kean, 2008).

Another major Romanian agro-industrial waste is grape peel (white and red). Approximately 80% of the grape harvest is used in the winemaking industry, resulting in huge amounts of waste and a serious disposal problem. A high proportion of phenolic compounds remains in the winemaking waste (Lafka, Sinanoglou, & Lazos, 2007), and these compounds are effective as inhibitors of human low-density lipoprotein oxidation, in addition to other positive impacts that they have on human health (Folts, 2002). It is known that there is a strong correlation between antioxidant capacity and the total phenolic compounds present (Corral-Aguayo et al., 2008).

Carrot pulp represents another important Romanian agro-food waste. This particular waste has been shown to have high amounts of phenolic compounds and dietary fiber, which give some physical characteristics to the carrot. For example, anthocyanins and carotenoids are responsible for the color, aroma and bitterness of carrots (Gonçalves, Pinheiro, Abreu, Brandao, & Silva, 2010). Moreover, its phenolic acids have a strong antioxidant potential, and anthocyanins have been proven to reduce cardiovascular heart disease by decreasing the inflammation and lipid oxidation (Arscott & Tanumihardjo, 2010).

Red beet by-products are also found among major Romanian agro-industrial wastes. This type of waste, namely, the pomace/pulp from the juice industry, accounts for 15–30% of the raw material and is usually discarded as feed or manure, even though it has a high content of betalains. Betalains are water-soluble nitrogenous pigments, which consist of two main groups, the red betacyanins and the yellow betaxanthins (Pedreno & Escribano, 2001). They actually give the color of the beet, and the phenolic portion of the peel has l-tryptophan, p-coumaric and ferulic acids and cyclodopa glucoside derivatives. Red beets are considered among the 10 most effective vegetables, in terms of antioxidant capacity, with the largest amount of total phenolics being found in the peel (50%) (Kujala, Loponen, Kika, & Pihlaja, 2000). Thus, it is crucial to explore red beet pulp and peel, as little is known about their *in vivo* absorption.

The utilization of Romanian agro-industrial waste could provide an extra source of income and, at the same time, help to reduce the solid-waste disposal problem of the country. However, there is limited information on the bioactive potential of these specific agro-food wastes after being treated by a thermal process. In this context, the present study is aimed at evaluating the phenol, flavonoid, and lipid (fatty acids) content, as well as the antioxidant, antimicrobial, and antimutagenic activities of these five major Romanian agro-industrial wastes, as both fresh and thermally processed matrices. The thermal treatment was used to test a future practical application of these wastes in the food industry. Through the results reported in this study, it is expected that food-processing industries may better direct their waste, thus avoiding a growing environmental problem.

2. Materials and methods

2.1. Materials and chemicals

The apple peels (Ionatan of Voineşti) (AW), carrot peels and pulp (Nabuco variety) (CW), white (Fetească Regală) (WGW) and red (Isabella variety) grape peels (RGW) and red beet (Beta vulgaris) peels and pulp (RBW) were collected from a commercial juice producer and transported in plastic containers at −20 °C to the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The waste materials were immediately crumbled and bioactive compounds were extracted as fresh samples (F). Each crumbled material was mixed with water (10 mL of distilled water for every 1 g of waste material) and thermally processed (10 min at 80 °C), to make thermal samples (T). The results were calculated based on the dry matter.

Folin-Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), sodium nitrate ($NaNO_2$), ammonium nitrate (NH_4NO_3), hydrochloric acid (HCl), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), sea salts, glucose, acetic acid, acetonitrile, methanol, gallic acid, quercetin, chlorogenic acid, rutin, cyanidin chloride, DPPH (1,1-diphenyl-2-picrylhydrazyl), lipid standards and chemicals (used for oil extraction, fractionation and preparation of fatty acid methyl esters (FAMEs)) were purchased from Sigma-Aldrich (Steinheim, Germany). For antimicrobial assays, Mueller-Hinton agar, thioglycollate broth with resazurin, and Mueller-Hinton broth were purchased from BioMerieux (France) and Tween 80 and Broth Malt medium were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Extraction and analysis of phenolic compounds

The fresh (AF, CF, WGF, RGF, BF) and thermally processed samples (AT, CT, WGT, RGT, BT) were individually extracted three times with 20 mL of extraction mixture (hydrochloric acid/methanol/water ratio of 1:80:19) at 40 °C for 30 min in an ultrasonic bath (Dulf, Vodnar, Dulf, & Tosa, 2015). After centrifugation (4000*g* for 10 min), the supernatants were filtered; the filtrates were evaporated to dryness under vacuum, dissolved in methanol and stored (4 °C) until analysis (total and individual phenolic compounds, total flavonoids, antioxidant, antimutagenic and antimicrobial activities).

2.2.1. Total phenolic content

Determination of total phenolic content (TP) was performed by using the Folin-Ciocalteu method (Dulf, Vodnar, & Socaciu, 2016; Dulf et al., 2015). 25 μl of each extract was mixed with 125 μl of Folin-Ciocalteu regent (0.2 N) and 100 μl of 7.5% (w/v) Na_2CO_3 solution. The mixture was incubated for 2 h in the dark at room temperature (25 °C). The absorbance against a methanol blank was recorded at 760 nm. A standard curve was prepared using gallic acid (0.01–1 mg/mL), and the TP content in the extract was expressed as gallic acid equivalents (GAE) in mg/100 g dry weight (DW) of waste.

2.2.2. Total flavonoid content

The total flavonoid content (TF) of the extracts was determined using methods described previously (Barakat & Rohn, 2014). A 100 μl aliquot of 2% aluminum chloride ethanol solution was added to 100 μl of the extracts and mixed. After incubating for 1 h at room

temperature, the absorbance at 510 nm was measured against a prepared regent blank. Total flavonoid content was expressed as quercetin equivalents (QE) in mg/g dry weight (DW).

2.2.3. Analysis of phenolic compounds by HPLC-DAD-ESI-MS

The characterization of the phenolic compounds in the samples was carried out on an HPLC-DAD-ESI-MS system consisting of an Agilent 1200 HPLC with DAD detector, coupled to an MS-detector single-quadrupole Agilent 6110. Separations of phenolic compounds were performed at 25 °C on an Eclipse column, XDB C18 (4.6 × 150 mm, 5 μm) (Agilent Technologies, USA). The binary gradient consisted of 0.1% acetic acid/acetonitrile (99:1) in distilled water (v/v) (solvent A) and 0.1% acetic acid in acetonitrile (v/v) (solvent B) at a flow rate of 0.5 mL/min, following the elution program used by Dulf et al. (2015).

Various phenolic compounds were identified by comparing the retention times, UV–visible and mass spectra of unknown peaks with reference standards. For MS fragmentation, the ESI (+) module was applied, with a scanning range between 100 and 1000 *m*/*z*, capillary voltage 3000 V, at 350 °C and with a nitrogen flow of 8 L/min. The eluent was monitored by DAD, and the absorbance spectra (200–600 nm) were collected continuously during the course of each run. The flavonols were detected at 340 nm and the anthocyanins at 520 nm (Dulf et al., 2015). Data analysis was performed using Agilent ChemStation Software (Rev B.04.02 SP1, Palo Alto, California, U.S.A). The anthocyanin levels were determined using cyanidin chloride as the external standard and expressed as equivalents of cyanidin (mg cyanidin/100 g DW of substrate) (r^2 = 0.9935). The chlorogenic and neochlorogenic acids were expressed in mg chlorogenic acid/100 g DW of substrate (r^2 = 0.9935) and flavonol glycosides were calculated as equivalents of rutin (mg rutin/100 g DW of substrate) (r^2 = 0.9987).

2.2.4. DPPH free-radical-scavenging assay

Phenolic extracts of fresh and thermally processed samples were subject to DPPH radical-scavenging activity assessment, using the method described by Toma, Olah, Vlase, Mogosan, and Mocan (2015) and Dezsi et al. (2015). The percentage inhibition (I%) was calculated as [1 − (test sample absorbance/blank sample absorbance)] × 100.

2.3. Lipid extraction and GC–MS analysis of FAMEs

Total lipids (TLs) of fresh (apple peels, carrot peels and pulp, and white and red grape peels (AF, CF, WGF, RGF)) and thermally processed samples (AT, CT, WGT, RGT) were extracted using the method described by Dulf et al. (2015). The red beet waste material registered low content in lipids, and the results are not shown. Five grams of each material was homogenized in 50 mL of methanol for 1 min using a high-power homogenizer (MICCRA D-9, ART Prozess- und Labortechnik, Mullheim, Germany). One hundred mL of chloroform was then added, and homogenization continued for 2 min. The mixture was filtered, and the solid residue was resuspended in chloroform/methanol (2:1, v/v, 150 mL) and homogenized again for 3 min. After filtration of the mixture, the residue was washed with 150 mL chloroform/methanol (2:1, v/v). The filtrates and washes were combined and washed with 0.88% aqueous potassium chloride, followed by methanol/water (1:1, v/v) solution. The purified lipid (bottom) layer was filtered and dried over anhydrous sodium sulfate, and the solvent was removed in a rotary evaporator. The amount of lipid was determined gravimetrically. The recovered oils were transferred to vials with 2 mL of chloroform (stock solution) and stored at −18 °C until further analysis.

FAMEs were prepared by acid-catalyzed transesterification of TLs (Dulf, Oroian, Vodnar, Socaciu, and Pintea, 2013; Dulf, Pamfil, Baciu, and Pintea, 2013). The samples were analyzed with a gas chromatograph (GC) coupled to a mass spectrometer (MS); PerkinElmer Clarus 600T GC–MS (PerkinElmer, Inc., Shelton, CT, U.S.A) (Dulf et al., 2015). The GC column was a Supelcowax 10 (60 m × 0.25 mm i.d., 0.25 μm film thickness; Supelco INC., Bellefonte, PA, U.S.A). The oven temperature was set at 140 °C and then increased to 220 °C. Helium was used as the carrier gas with a constant flow rate of 0.8 mL/min. Mass spectra (E.I. positive-ion electron-impact mode) were recorded at 70 eV using a trap current of 100 μA with a source temperature of 150 °C. The MS was scanned from *m*/*z* 22 to 395 for all GC–MS experiments. The FAME peak identification was based on comparing both the retention time and MS of the unknown peak to those of known standards and compounds listed in an MS database (NIST MS Search 2.0). The amount of each fatty acid was calculated as peak area percentage of total fatty acids.

2.4. Antimicrobial and antifungal activity

2.4.1. Bacteria and culture conditions

For this bioassay, six bacterial strains were used: four Gram-positive bacteria (*Staphylococcus aureus* (ATCC 49444), *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19114), *Enterococcus faecalis* (ATCC 29212)), three Gram-negative bacteria (*Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 14028) and *Escherichia coli* (ATCC 25922)) and two anaerobic strains: *Fusobacterium nucleatum* (Gram-negative, ATCC 25586) and *Peptostreptococcus anaerobius* (Gram-positive, ATCC 27337). All tested microorganisms were obtained from Food Biotechnology Laboratory, UASVM CN, Romania. The aerobic strains were cultured on Mueller-Hinton agar and cultures were stored at 4 °C and subcultured once a month. Anaerobic bacteria were cultured overnight at 37 °C on thioglycollate broth with resazurin, stored at 4 °C and subcultured once a month.

2.4.2. Microdilution method

The modified microdilution technique was used to evaluate antimicrobial activity. Briefly, fresh overnight cell suspensions were adjusted with sterile saline solution to a concentration of approximately 2×10^5 CFU/mL in a final volume of 100 μL per well. The inoculum was stored at 4 °C for further use. Determinations of minimum inhibitory concentrations (MICs) were performed by a serial dilution technique using 96-well plates. Different dilutions of the extracts were carried out with wells containing 100 μL of Mueller-Hinton broth and, afterwards, 10 μL of inoculum was added to all the wells. The microplates were incubated for 24–48 h at 37 °C. The MIC of the samples was detected after the addition of 20 μL (0.2 mg/mL) of resazurin solution to each well, and the plates were incubated for 2 h at 37 °C. A change from blue to pink indicates reduction of resazurin and, therefore, bacterial growth. The MIC was defined as the lowest concentration that prevented this color change. The minimum bactericidal concentrations (MBCs) were determined by serial subcultivation of 2 μL into 96-well plates containing 100 μL of broth per well and further incubation for 48 h at 37 °C. The lowest concentration with no visible growth was defined as the MBC, indicating death of 99.5% of the original inoculum. Streptomycin (Sigma P 7794, Santa Clara, CA, USA) (0.05–3 mg/mL) was used as positive control for bacterial growth. Water was used as negative control.

2.4.3. Antifungal activity

To investigate the antifungal activities, the following fungi were used: *Aspergillus flavus* (ATCC 9643), *Aspergillus niger* (ATCC 6275), *Candida albicans* (ATCC 10231), *Candida parapsilosis* (ATCC 22019), and *Penicillium funiculosum* (ATCC 56755), all bought from the same source stated above. Cultures were maintained on malt agar at 4 °C and subcultured every month. Spore suspensions

(1.5×10^5) were obtained by washing agar plates with sterile solution that contained 0.85% saline and 0.1% Tween 80 (v/v), which was then added to each well to a final volume of 100 μL. The minimum inhibitory (MIC) and minimum fungicidal (MFC) concentration assays were performed using the microdilution method by preparing a serial of dilutions in 96-well plates. The extracts were diluted in 0.85% saline (10 mg/mL), then added to microplates containing Broth Malt medium with inoculum and incubated for 72 h at 28 °C on a rotary shaker. The lowest concentrations without visible growth (observed through a binocular microscope) were defined as minimal inhibitory concentrations (MICs). The fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μL of tested extracts that were dissolved in medium and inoculated for 72 h into microtiter plates containing 100 μL of broth per well and followed by further incubation 72 h at 28 °C. The lowest concentration with no visible growth was defined as the MFC, indicating death of 99.5% of the original inoculum. The fungicide fluconazole (Sigma F 8929, Santa Clara, CA, USA) was used as positive control (1–3500 μg/mL). All the experiments were performed in duplicate and repeated thrice. Water was used as negative control.

2.5. Mutagenic and antimutagenic activity

2.5.1. Mutagenic and antimutagenicity test

Mutagenic and antimutagenicity of fresh (AF, CF, WGF, RGF, BF) and thermally processed samples (AT, CT, WGT, RGT, BT) were examined using the plate incorporation method (Maron & Ames, 1983) described in detailed by Sarac and Sen (2014). 4-nitro-o-phenylenediamine (4-NPD, 3 μg/plate) and sodium azide (NaN_3, 8 μg/plate) were used as positive controls for *S. typhimurium* TA98 and *S. typhimurium* TA100. Ethanol: water (1:1, v/v) was used as negative control. The extracts were prepared at a concentration of 5 mg/plate. The antimutagenicity of the reference mutagens in the absence of the extract was defined as 0% inhibition, and the antimutagenicity was calculated according to the formula given by Ong, Whong, Stewart, and Brockman (1986): %Inhibition = $[1 - T/M] \times 100$, where T is the number of revertants per plate in the presence of mutagen and the extract, and M is the number of revertants per plate in the positive control (without extract). The tests were performed in duplicate with three subsamples each, and the data is presented as the mean ± standard deviation (SD). Antimutagenicity was recorded as follows: strong: 40% or more inhibition; moderate: 25–40% inhibition; low/none: 25% or less inhibition (Evandri et al., 2005).

2.6. Statistical analysis

All tests were conducted in triplicate and the results were expressed as the mean ± standard deviation (SD). Correlations between the antioxidant capacity and phenolic content were determined using Person's correlation. Statistical differences among samples were estimated using Student's *t*-test (GraphPad Prism Version 5.0, Graph Pad Software Inc., San Diego, CA). Differences between means at the 5% level were considered statistically significant.

3. Results and discussion

3.1. Total phenolics and total flavonoids

The total polyphenol content (TP) of extracts measured by the Folin-Ciocalteu method is shown in Fig. 1 (A). There were significant differences ($p < 0.05$) between the TPs content in the fresh and thermally processed waste materials. As shown in Fig. 1 (A), the thermally processed samples of apple and white grapes had higher total phenolic content, red grape waste had the highest (1990 ± 52.9 mg GAE/100 g dry weight), and carrot waste had the lowest (5.1 ± 0.25 mg GAE/100 g dry weight). Polyphenol content in the extracts of carrot and red beet waste showed significantly ($p < 0.05$) higher values (37.5 mg GAE/100 g DW for CF and 14.1 mg GAE/100 g DW for BF) in fresh samples than in thermally processed samples (5.1 mg GAE/100 g dry weight for CT and 5.5 mg GAE/100 g dry weight for BT). As an overall result, it can be stated that fresh samples of carrot waste and red beet waste have the lowest phenolic content and, during thermal processing, the phenolic content decreases significantly; in contrast, thermal processing increases the phenolic content in the rest of the samples. This can be attributed to the fact that extraction of intracellular contents is enhanced by thermal treatment. Additionally, Wang, He, and Chen (2014) reported that high temperature increased the content of phenolic compounds due to the hydrolysis of polysaccharides. The TP content in fresh apple peels was (564 ± 39.5 mg GAE/100 g DW); this value is 3.3-fold lower than those reported by Henríquez, Córdova, Almonacid, and Saavedra (2014) but agree with those reported by Drogoudi, Michailidis, and Pantelidis (2008). After thermal treatment (10 min at 80 °C), the polyphenolics increased in AT to 20.56 ± 1.9 (mg GAE/100 g DW). The TP decrease (in CT and BT) can be explained by three possible mechanisms: (1) the release of bound phenolic compounds; (2) the partial degradation of lignin, which can release phenolic acids; and (3) the thermal degradation of the phenolic acids or catechins (Larrauri, Ruperez, & Saura-Calixto, 1997). Additionally, there is a possibility that thermal treatment inactivates the enzymes responsible for the loss of phenolic compounds (like polyphenol oxidase) or could also accelerate the activity of enzymes that participate in the biosynthesis of phenols (like phenylalanine ammonia-lyase) (Morales-de la Peña, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2011).

The total flavonoids content was calculated and is displayed in Fig. 1 (B); the obtained values range from 8.5 ± 1.1 (fresh carrot waste) to 1050 ± 62.1 mg (thermally processed red grape waste). As observed for flavonoids, no significant differences were registered between fresh and thermally processed samples for apple and white grape waste. The significant properties of flavonoids have produced a growing interest in their isolation and separation from natural products and in the development of analytical databases of the flavonoid content of foods (Bajalan, Mohammadi, Alaei, & Pirbalouti, 2016). In the Medina-Meza and Barbosa-Cánovas (2015) study on grape peels processed by electric fields, the total flavonoid contents were 98 (mg/mL) quercetin eq in fresh samples, which increased by 96% when the sample was exposed to pulsed electric fields.

3.2. Antioxidant activity

The changes in antioxidant activity of the extracts were assessed by measuring the DPPH radical-inhibition capacity (RIC), and the results are presented in Fig. 1(C). After thermal processing, statistically significant increases (58.37%) ($p < 0.05$) in RIC of red grape waste was registered. Red-grape waste extract exhibits the highest scavenging activity, followed by red-beet waste extract. The increase in antioxidant activities of thermally processed beet waste shows that the antioxidant activity depends not only on the presence of betacyanins but also on other polyphenols that could have increased during the treatments. These observations agree with the results of Dewanto, Wu, Adom, and Liu (2002), who found that thermal processing enhances the nutritional value of tomatoes and corn by increasing the total antioxidant activity.

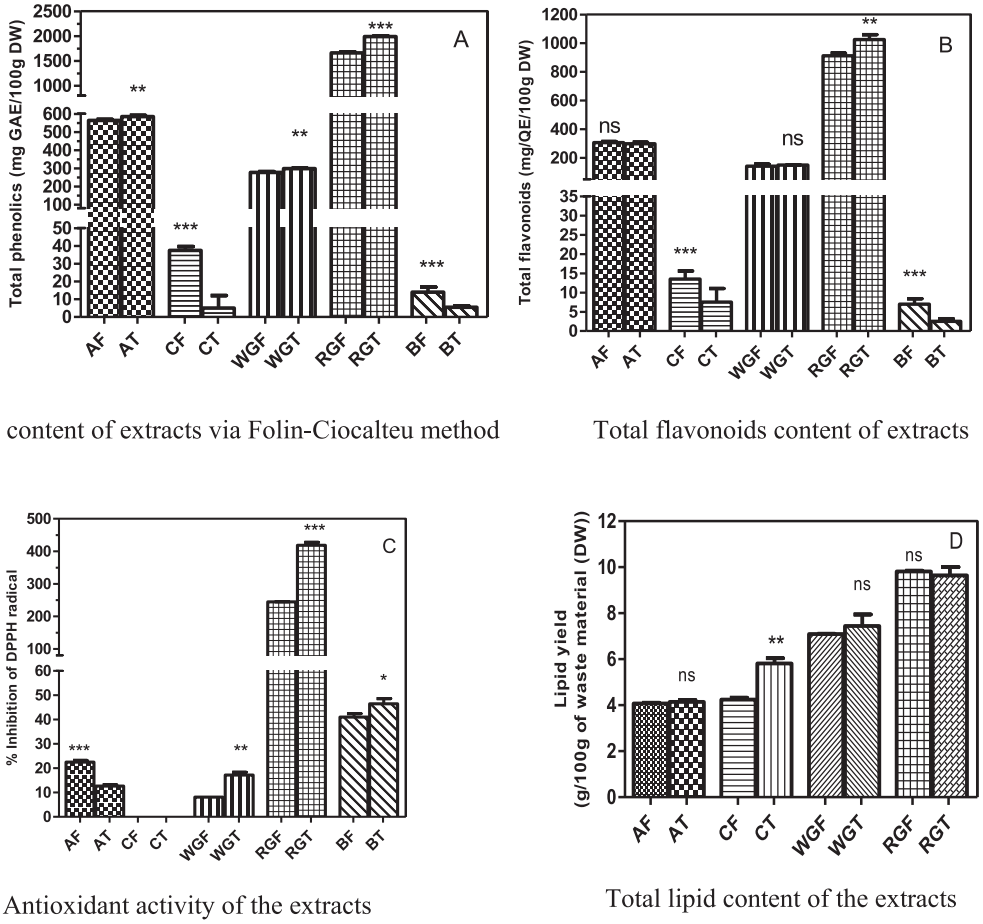

Fig. 1. Total phenolic content (Folin-Ciocalteu method) (A), total flavonoids content (B), antioxidant activity (C) and total lipid content (D) of the extracts, both fresh and thermally processed. Total phenolic content of the extract is expressed as gallic acid equivalents (GAE) in mg/100 g dry weight (DW) of waste. Total flavonoid content is expressed as quercetin equivalents (QE) in mg/g dry weight (DW). The percentage inhibition (I%) was calculated as [1 – (test sample absorbance/blank sample absorbance)] × 100. Values are reported as mean ± SD of triplicate determinations and different symbols (*, **, ***) indicate significant differences ($p < 0.05$) (paired *t*-test), while symbol (ns) indicate no significant difference. AF-apple waste fresh; AT-apple waste thermally processed; CF-carrot waste fresh; CT-carrot waste thermally processed; WGF-white-grape waste fresh; WGT-white-grape waste thermally processed; RGF-red-grape waste fresh; RGT-red-grape waste thermally processed; BF-red-beet waste fresh; BT-red-beet waste thermally processed.

3.3. Total lipid content

The total lipid yield of extracts was determined and the results are presented in Fig. 1(D). The red-beet waste material registered low content in lipids and so the results are not shown. No significant differences between fresh and thermally processed samples of apple, red and white grape waste were registered. The only significant difference is for carrot waste samples, where, after the thermal process, an increase of 2 g of lipids/100 g of waste material was reported. In the study of Shyamala and Jamuna (2010), the carrot pulp had a lipid content of 2.72%, a lower value than what we observed here.

3.4. HPLC-DAD-ESI-MS analysis of phenolic compounds

The analyzed samples contained sixteen phenolic compounds that originate from 5 phenolic groups: anthocyanins, cinnamic acids, dihydrochalcones, flavonols and flavan-3-ols. From the anthocyanins group, four phenols were identified: petunidin glucoside, malvidin glucoside, cyanidin-3-O-arabinoside and peonidin-3-O-glucoside. The five cinnamic acids identified were caffeic acid, caffeic acid-4-O-glucoside, two caffeoylquinic acids and a 3,4-dicaffeoylquinic acid. The dihydrochalcones identified were phloridzin and phloretin glucoside. From the flavanol group, two major phenols were detected: epicatechin and catechin-3-O-glucose. The flavonol group had three identified phenols: rutin, quercitrin, and quercetin glucoside (Table 1). In almost all phenolic cases, the quantities in the fresh and thermally processed samples substantially differed. The anthocyanin group was present only in the red grape waste samples, and the malvidin-3-O-glucoside from this group was the major phenolic compound identified in all the extracts (13.958 [mg/%DW] in RGT and 13.015 [mg/%DW] in RGF). The next predominant phenolic is also within the same group identified in the red grape waste sample, and, depending on the reference extract (fresh or thermally processed), could be either malvidin glucoside (8.365 [mg/%DW], when the reference is the thermally processed sample) or cyanidin-3-O-arabinoside (7.745 [mg/%DW], when the reference is the fresh sample). The flavonols are present in the apple peel tissues (apple samples), which agrees with a previous study by Rupasinghe et al. (2010). Additionally, the dihydrochalcone and flavonol groups were found only in the apple waste extracts, which agrees with a study that reported that apple peels' health advantages correlate with the presence of phenolic compounds, which include the flavonols and dihydrochalcones (Boyer & Liu, 2004). An interesting observation is the fact that, in some cases, the thermally processed samples show higher phenolic content in comparison with the fresh samples. This agrees with a Morales-de la Peña et al. (2011) study, which reported that thermal

Table 1
Identification of the phenolic compounds (mg/100 g DW) in the extracts via HPLC-DAD-ESI-MS method.

Phenolic compounds	$[M+H]^+$ ion fragments	Samples (mg/%DW)									
		AF	AT	CF	CT	WGF	WGT	RGF	RGT	BF	BT
Anthocyanins	Petunidin 3-O-(6′-*p*-coumaroyl)-glucoside)	625, *317*							2.454^{a}	2.356^{b}	
	Malvidin 3-O-(6′-*p*-coumaroyl)-glucoside	639, *331*							7.358^{b}	8.365^{a}	
	Cyanidin 3-O-arabinoside	419, *287*							7.745^{b}	7.625^{a}	
	Peonidin 3-O-glucoside	463, *301*							2.984^{a}	1.892^{b}	
	Malvidin 3-O-glucoside	493, *331*							13.015^{b}	13.958^{a}	
Cinnamic acid	Caffeic acid	181, *163*					2.138^{b}	2.56^{a}			
	Caffeic acid-4-O-glucoside	343, *181,163*	2.492^{b}	3.165^{a}							
	5-Caffeoylquinic acid	355, *181, 163*			14.146^{a}	4.265^{b}					
	3-Caffeoylquinic acid	355, *181,163*	1.27^{b}	2.65^{a}							
	3,4-Dicaffeoylquinic acid	515, *355*			2.457^{a}	0.263^{b}					
Dihydrochalcones	Phloridzin (Phloretin 2′-O-glucoside)	437, *275*	5.714^{a}	4.652^{b}							
	Phloretin 2′-O-xylosyl-glucoside	569, *437, 275*	1.125^{a}	0.958^{b}							
Flavan-3-ols	Epicatechin	291					2.368^{a}	2.261^{b}			
	Catechin 3-O-glucose	453, *291*					2.262^{a}	2.265^{a}			
Flavonol	Rutin (Quercetin 3-O-rutinoside)	611, *303*	4.318^{a}	4.316^{a}							
	Quercitrin (Quercetin 3-O-rhamnoside)	449, *303*	2.12^{b}	2.46^{a}							
	Quercetin 3,4′-O-diglucoside	627, *465,303*	0.903^{b}	1.362^{a}							
Betacyanins											
Betanidin	Betanidin	389,345								3.866^{b}	3.952^{a}
	Isobetanidin	389, 345								2.353^{b}	2.456^{a}
	Betanidin-5-O-β-glucoside	551, *389*								7.006^{b}	7.100^{a}
	Isobetanidin-5-O-β-glucoside	551, *389*								10.061^{a}	10.074^{a}

Values (mean ± SD, n = 3) in the same row followed by different superscript letters (a, b) indicate significant differences ($p < 0.05$) between fresh and thermally processed samples of the same extract (Student's *t*-test – GraphPad Prism Version 5.0, Graph Pad Software Inc., San Diego, CA).)

treatment can accelerate the activity of enzymes that participate in the biosynthesis of phenols, and with a Wang et al. (2014) study, which reported that high temperature increases the content of phenolic compounds because of the hydrolysis of polysaccharides. In accordance with this observation, in our study, a significant increase in malvidin glucoside (13.68%) was registered after thermal processing red grape waste; another major example was reported for caffeic acid 4-O-glucoside from apple waste, with a 27% (from 2.492 [mg%] to 3.165 [mg%]) increase after thermal treatment. On the other hand, samples of carrot waste contained only the cinnamic acid group and this phenolic content decreased significantly with 70–90% (from 14.146 [mg/%DW] to 4.265 [mg/% DW]) during thermal processing. This agrees with Chandrasekara, Naczk, and Shahidi (2012), who stated that thermal treatment can reduce the amount of phenolic compounds because the high temperature causes the polymerization or loss of thermolabile phenols. In both cases, a previous study (Rupasinghe, Laixin, Huber, & Pitts, 2008) demonstrated that processing technique (baking) and temperatures influence phenolic content by increasing or decreasing different phenolic compounds. For instance, a major, interesting finding of this study is that flavonols present in apple skin powder are relatively resistant to thermal degradation during baking, which is in agreement with our findings that flavonols are also not degraded during processing. Another example from the same literature study indicates that phloridzin values decrease during baking (56% less), which also agrees with our results when our thermal process (at just 80 °C) causes a loss of approximately 10% of the phloridzin.

The group of betacyanins contains four betanidin compounds that were all identified in the red-beet waste extracts. With one exception (isobetanidin-5-O-β-glucoside), the amounts of all the reported betanidin compounds significantly increase during the thermal process This could be explained by the fact that betanin can regenerate by recondensation of hydrolysis products associated with a recovery of color (Stintzing & Carle, 2007). As betacyanins undergo thermal treatment, it is known that pigments will experience degradation and fluctuating chromatic stability (Herbach, Rohe, Stintzing, & Carle, 2006). Our findings agree with those reported by Harivaindaran, Rebecca, and Chandran (2008); specifically, as the temperature increases, the yield of betacyanin content increases (samples of Dragon fruit yielded the highest betacyanin content at 100 °C for 5 min).

3.5. Fatty acid composition analyzed by GC–MS

The fatty acid profile of each sample is presented in Table 2. In almost all cases, the fatty acids were significantly different between fresh and thermally processed samples, with six exceptions whose differences were not significant. The exceptions are: palmitic acid (16:0), in the case of CW; palmitoleic acid (16:1n-7), in the case of RGW and WGW; 20:1n-9 acid, in the case of AW and RGW and 16:1n-9 acid, in the case of RGW. The first fatty acids analyzed were lauric (12:0), myristic (14:0) and pentadecanoic acid (15:0), all of which are more present in thermally processed samples (e.g., in the case of WGW for all the above mentioned acids, the fresh samples have a value of 0 while the TP samples have values ranging from 0.04 to 0.16% of TLs). A possible explanation for this observation is the fact that thermal treatment enhances the release of these fatty acids by breaking the cell walls. Apple waste that was thermally processed had the highest content of FAs. The dominant fatty acids in the TLs were palmitic acid (16:0), with a highest value of 19.98 in the CF sample; oleic acid (C18:1n-9), with a highest value of 19.32 in the RGT sample and linoleic acid (c18:2n-6), with the highest value of 66.3 in WGF sample.

With respect to palmitic acid (16:0), an interesting observation occurred: in the cases of apple and carrot waste, the highest content was registered for fresh samples (a difference of 3.9% for apple

Table 2
Fatty acid composition (molar% of total fatty acids) of total lipids for each sample, determined by GC–MS.

Samples		Fatty acids (%)																	
		12:0	14:0	15:0	16:0	16:1 n-9	16:1 n-7	17:0	18:0	18:1 n-9	18:1 n-7	18:2 n-6	18:3 n-3	20:0	20:1 n-9	21:0	22:0	22:1 n-9	23:0	24:0
TLs																				
A	F	0.17	0.42	0	19.67***	0.2	0.39***	0.35	4.76	9.7	0.83**	49.46***	6.83**	2.95	0.26 n^{s}	0.38**	2.16	0.34***	0	1.13
	T	0.33***	0.56**	0.161***	18.93***	0.64***	0.28***	0.27**	4.84**	14.74***	0.74	43.98***	5.9**	3.69**	0.21	0.29	2.77***	0	0.18***	1.48**
C	F	0	0	0.4	19.98***	0	0.8***	0.57***	1.21	3.26	1.12**	63.89***	6.62**	0.68***	0	0	0.88**	0	0.58**	0
	T	0.22***	0.21***	0.4 n^{s}	16.41	0.46***	0.32	0.24	1.45***	16.92***	1.02	54.61	5.87	0.46	0.09***	0.15***	0.5	0.08***	0.15	0.43**
WG	F	0	0	0	10.16	0.07	0.16	0	3.89	12.07	0.59	66.3***	2.97	1.77	0.14	0	1.24	0	0	0.63**
	T	0.05**	0.16***	0.04**	10.46***	0.13**	0.18 n^{s}	0.09**	4.95***	13.56***	0.8***	60.9	3.09**	2.42***	0.22**	0.18***	1.98***	0.03*	0.27***	0.49
RG	F	0	0.09	0	10.75	0.08 n^{s}	0.15 n^{s}	0.04	3.28	19.32	0.73	61.87***	2.15	0.49	0.22	0	0.63	0	0	0.22
	T	0.06**	0.13**	0.04**	11.75***	0.07	0.14	0.09*	3.96***	19.86***	0.87**	56.53	3.72***	0.91***	0.23 n^{s}	0.08**	1.15***	0.02***	0.12***	0.27*

Molar % values are the mean of three measurements (n = 3). Different superscript symbols (*, **, ***) in the same column indicate significant differences ($p < 0.05$) among TLs of the fresh and thermally processed samples of the same extract (Student's *t*-test – GraphPad Prism Version 5.0, Graph Pad Software Inc., San Diego, CA). TLs – total lipids, C12:0, lauric; C14:0, myristic; C15:0, pentadecanoic; C16:0, palmitic; C16:1n-9, cis-7 hexadecenoic; C16:1n-7, palmitoleic; C17:0, margaric; C18:0, stearic; C18:1n-9, oleic; C18:1n-7, vaccenic; C18:2n-6, linoleic; C18:3n-3, α-linolenic; C20:0, arachidic; C20:1n-9, 11-eicosenoic; C21:0, heneicosylic; C22:0, behenic; C22:1n-9, erucic; C23:0, tricosylic; C24:0,lignoceric.

and a difference of 21.75% for carrot), while in the cases of red and white grape waste thermally processed samples actually had the highest content. A similar result was observed for palmitoleic acid (16:1n-7), in which apple- and carrot-waste fresh samples registered the highest content. A possible explanation is that these FAs are sensitive to thermal treatment, thereby decreasing the percentage that remains after thermal processing. In the case of C16:1n-9, thermally processed samples of apple, carrot and white grape wastes registered the highest content. Stearic acid (18:0), as well as oleic acid (18:1n-9) were consistently higher in content in all thermally processed samples. Linoleic acid (18:2n-6) was found in a higher proportion in the fresh samples of each type of extract (an average of 60% in the fresh samples vs. 54% in thermally processed ones). A possible explanation for this is the fact that linoleic acid is very sensitive to heat; therefore, after a thermal treatment, the concentration decreases. The distribution of high levels of linoleic acid in each sample (fresh and processed) agrees with Kinsella's results (1974) on grape seed oil (also containing 60% linoleic acid). With respect to α-linolenic acid (18:3n-3), in apple and carrot waste samples (fresh and processed) the content of C18:3n-3 was high (6.83% in AF and 6.62% in CF), while in red and white grape waste samples the level was relatively low (3.09% in WGT and 3.72% in RGT), the latter results being similar to Kinsella's findings on grape seed oil (2.3%). An extremely interesting fact regarding the α-linolenic acid was that for apple and carrot waste the highest content was registered in the case of fresh samples, while for red- and white-grape waste the highest values were reported for thermally processed samples. According to Simopoulos (2002), nutrition societies recommend a precisely balanced ratio (<5:1) of these two types of FAs (18:2n-6 and 18:3n-3) to be necessary for optimal health. In Table 3, all the individual lipid classes are examined. All types of extracts were found to contain very-long-chain saturated fatty acids (VLCSFA), with chain lengths exceeding 20 carbons. According to Gurr, Harwood, & Frayn (2002), these VLCSFA have a major role in the cuticular surface layers of plant tissues; therefore, using only waste extracts (peels) in this study, our findings are in agreement with these works. Regardless of whether the heat treatment is applied or not, apple samples, having the highest content in SFA, contained a much higher amount of VLCSFA than the other extracts. According to Simopoulos (2002), the two types of PUFAs (polyunsaturated fatty acids), namely n-6 and n-3, are very important with respect to health and disease. A high ratio of n-6/n-3, ranging from 22.34 to 28.82, was found in WGF and RGF. Additionally, these two types of extracts show the highest ratio of PUFAs/SFAs, ranging from 3.92 to 4.13. The analysis of FAs show a high ratio of n-6/n-3 and much higher levels of n-6 PUFA in those specific cases in which a high value of 18:2n-6 was reported (Table 2). A low ratio of n-6/n-3 was found in apple and carrot, precisely 7.24 in AF, increasing 2.9% after the thermal process, and a percentage of 9.66 in CF, decreasing 3.72% after the thermal process. Therefore, according to Simopoulos (2002), who stated that a lower ratio of omega-6/omega-3 fatty acids is more desirable in reducing the risk of many chronic diseases of high prevalence in Western societies and in developing countries, apple and carrot wastes can be integrated into a recommended healthy diet in different forms. PUFAs have a major effect in reducing the incidence of cardiovascular disease and cancer (Banni & Martin, 1998) and are therefore desirable in the human diet. Simopoulos, in his study (2002), considers that the paper by Ahrens et al. from 1954 and subsequent work by Keys et al. firmly established the omega-6 fatty acids as the important fatty acid in the field of cardiovascular disease. According to Simopoulos (2002), the optimal ratio of omega-6/omega-3 varies from 1/1 to 4/1, depending on the disease, but it is essential to decrease omega-6 intake while increasing the amount of omega-3 to prevent and manage of chronic disease. Additionally, the bal-

Table 3
Individual lipid classes and the GC–MS analyzes of FAME.

		Fatty acid classes (%)							
		ΣSFAs	ΣMUFAs	ΣPUFAs	Σn-3 PUFAs	Σn-6 PUFAs	n-6/n-3	PUFAs/SFAs	VLCSFA (>20c)
Samples					TLs				
A	F	32 ± 0.9	11.72 ± 0.45	56.28 ± 2.75***	6.83 ± 0.45***	49.46 ± 1.9 ***	7.24	1.76***	6.62
	T	33.5 ± 0.88 n^{s}	16.62 ± 0.68***	49.88 ± 1.9	5.9 ± 0.3	43.98 ± 1.55	7.45 ***	1.49	8.41***
C	F	24.32 ± 1.00**	5.18 ± 0.2	70.51 ± 2.55***	6.62 ± 0.35***	63.89 ± 2.4***	9.66**	2.90	2.14***
	T	20.63 ± 1.13	18.89 ± 0.6***	60.48 ± 2.45	5.87 ± 0.6	54.61 ± 1.9	9.3	2.93**	1.69
WG	F	17.69 ± 0.63	13.04 ± 0.3	69.27 ± 2.4***	2.97 ± 0.1	66.3 ± 1.9***	22.34***	3.92**	3.64
	T	21.09 ± 0.3***	14.92 ± 0.6***	63.99 ± 2.2	3.09 ± 0.1**	60.9 ± 2.00	19.73	3.03	5.34***
RG	F	15.49 ± 0.65	20.49 ± 0.9	64.02 ± 2.1***	2.15 ± 0.1	61.87 ± 1.6***	28.82***	4.13***	1.34
	T	18.55 ± 0.25***	21.2 ± 0.5**	60.25 ± 1.5	3.72 ± 0.1***	56.53 ± 1.5	15.2	3.25	2.53***

Values (mean ± SD, n = 3) in the same column followed by different superscript symbols (**, ***, ns) indicate significant differences/no significant differences ($p < 0.05$) among fresh and thermally processed samples of the same extract (separately for each lipid class) (Student's *t*-test – GraphPad Prism Version 5.0, Graph Pad Software Inc., San Diego, CA). SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids, VLCSFA – very long chain saturated fatty acids.

Table 4
Antimicrobial activity of waste extracts before and after thermal process.

Test items		*L. monocytogenes*	*E. coli*	*S. typhimurium*	*P. aeruginosa*	*S. aureus*	*B. cereus*	*E. faecalis*	*F. nucleatum*	*P. anaerobius*
mg/mL										
AF	MIC	62.5	15.62	31.25	15.62	3.9	62.5	15.62	62.5	125
	MBC	125	31.25	62.5	31.25	7.81	125	31.25	125	250
AT	MIC	31.25	7.81	31.25	7.81	1.95	31.25	7.81	62.5	250
	MBC	62.5	15.62	62.5	15.62	3.9	62.5	15.62	125	500
CF	MIC	250	500	–	500	125	–	500	62.5	250
	MBC	500	1000	–	1000	250	–	1000	125	500
CT	MIC	–	–	–	–	–	–	–	250	–
	MBC	–	–	–	–	–	–	–	500	–
WGF	MIC	7.81	15.62	31.25	15.62	1.953	15.62	15.62	31.25	31.25
	MBC	15.62	31.25	62.5	31.25	3.9	31.25	31.25	62.5	62.5
WGT	MIC	3.9	3.9	15.62	7.81	1.953	7.81	7.81	15.62	15.62
	MBC	7.81	7.81	31.25	15.62	3.9	15.62	15.62	31.25	31.25
RGF	MIC	1.953	1.953	7.81	7.81	1.953	7.81	15.62	15.62	15.62
	MBC	3.9	3.9	15.62	15.62	3.9	15.62	31.25	31.25	31.25
RGT	MIC	1.953	1.953	3.9	3.9	1.953	3.9	7.81	7.81	7.81
	MBC	3.9	3.9	7.81	7.81	3.9	7.81	15.62	15.62	15.62
BF	MIC	1.953	1.953	7.81	7.81	1.953	15.62	31.25	31.25	31.25
	MBC	3.9	3.9	15.62	15.62	3.9	31.25	62.5	62.5	62.5
BT	MIC	15.62	7.81	15.62	3.9	31.25	31.25	15.62	15.62	15.62
	MBC	31.25	15.62	31.25	7.81	62.5	62.5	31.25	31.25	31.25
Streptomycin μg/mL	MIC	0.015	0.12	0.06	0.06	0.03	0.015	0.012	0.12	0.12
	MBC	0.03	0.24	0.12	0.12	0.06	0.03	0.024	0.24	0.24

ance of omega-6 and omega-3 fatty acids is very important for homeostasis and normal development. In general, in all analyzed fractions, the thermal process significantly increased ($p < 0.05$) MUFAs and SFAs (carrot being an exception and, in the case of apple, no significant difference was reported), while unsaturated fatty acids decreased (Table 3). These results partially agree with the previous findings of Dulf et al. (2016), who reported a decrease of PUFAs but also of MUFAs, after exposure to a fermentation process and an increase of SFAs. A clear explanation of this partial similarity is based on the different types of treatment (thermal and fermentation) within each study. PUFAs were the predominant fatty acids in the TLs because of the dominance of n-6 PUFAs, especially linoleic acid C18:2n-6 (ranging from 43.98 – the lowest value in AT – to 66.3 – the highest value in WGF).

3.6. Evaluation of antibacterial effects

The investigated extracts exhibit antibacterial activity against bacteria strains. The results are summarized in Table 4. A great range of bacteriostatic effects of the extracts were observed, depending on the strain studied. For *L. monocytogenes*, higher antibacterial activity was registered for RGF, RGT, and BF, with an MIC of 1.953 (mg/mL). This result may be due to the increased content of phenolic compounds like anthocyanidins in red grape wastes and betacyanins compounds in red beets. There are studies that state that polyphenols have diverse antibacterial activity based on the fact that they attack a large number of bacteria. Because of the high antioxidant activity of thermally processed red-beet waste (activity increased by the presence of betacyanins), it could be stated that the increased antibacterial activity that occurs in the presence of betacyanins directly correlates with their antioxidant activity. In all of the cases (except for the *L. nucleatum* anaerobic strain), CT extract has shown zero antibacterial activity, while CF had a very low inhibitory effect. By comparing the values, it can be stated that carrot extracts have no antibacterial effects. The *S. aureus* strain is much more sensitive to almost all the extracts. Gram-positive bacterial strain, *B. cereus*, exhibits a higher resistance in comparison with *S. aureus*; the highest sensitivity of *B. cereus* was to RGT (MIC = 3.9 and MBC = 7.81), RGF and WGT (MIC = 7.81 and MBC = 15.62). In the case of *E. faecalis*, the minimum inhibitory concentration registered was 7.81 (mg/mL) for AT and WGT extracts. A high inhibitory activity was registered against the Gram-negative bacterium, *E. coli*, by the following extracts: RGF, RGT, BF with an MIC of 1.953 (mg/mL) and an MBC of 3.9 (mg/mL), followed by WGT. Gram-negative bacteria, *S. typhimurius* and *P. aeruginosa*, were not as sensitive as *E. coli* toward extracts' antibacterial effects, but red grape waste and red-beet waste extracts, due to their high phenolic content and

high antioxidant activity, exhibited the highest inhibitory results against the studied strains (MIC = 3.9 and MBC = 7.81 for RGT). Against *P. aeruginosa*, BT extract had the same high inhibitory effect as RGT extract. In the case of anaerobic strains, RGT had the highest antibacterial effect. Against anaerobic bacteria, the investigated extracts have relatively small antibacterial effects. In almost all cases, the thermally processed samples have a better inhibitory activity than fresh samples against studied strains. The highest inhibitory activity against almost all the strains is attributed to the thermally processed red-grape waste sample. A possible explanation for this could be the release of phenolic compounds during heat treatment, which directly correlates with the antibacterial activity. When analyzing Table 4, it can also be concluded that the examined extracts had better inhibitory activity against Gram-positive bacterial strains than Gram-negative strains, with the least activity against anaerobic species.

3.7. Evaluation of antifungal activity

The antifungal activity of the investigated extracts against specific fungi is presented in Supplementary Table 1. The highest antifungal effect was exhibited by RGT extract against *Aspergillus flavus*, with an MIC of 7.81 and an MFC of 15.62, followed by RGF, WGT and AT. The *Penicillium furniculosum* and *Candida parapsilosis* strains were very resistant to the extracts' antifungal effects; in the case of thermally processed white- and red-grape waste extracts, no inhibitory activity is observed against *Penicilinium*. Red-beet waste extracts exhibit a very small antifungal effect against all studied strains.

3.8. Evaluation of mutagenic and antimutagenic activity

The extracts' potential antimutagenicity toward *S. typhimurium* TA98 and TA100 was investigated. The results are summarized in Table 5. With respect to *S. typhimurium* TA98, this assay indicates strong antimutagenic activity for AT, white- and red-grape waste extracts (both forms) and BF. A moderate inhibition is exhibited by AF and BT, with an inhibition of 37.95% and 34.35%, respectively. Regardless the type of extract, carrot had no antimutagenic activity toward *S. typhimurium* TA98, showing an inhibition of 0.52%. In the case of white- and red-grape waste samples, an interesting observation is noticed: in both cases, the higher inhibition is exhibited by thermally processed samples. This aspect can be explained by the fact that the thermal process increases antioxidant activity. RGT shows the highest antimutagenic potential, with an inhibition of 67.79% toward *S. typhimurium*. Some authors stated that plant compounds exhibiting antimutagenic properties have a series of possible applications in human health. With respect to TA100, apple, white- and red-grape wastes (in both forms: fresh and thermally processed) exhibit a strong antimutagenic activity, with higher inhibition in the case of thermally processed samples. Carrot and red-beet waste samples register low inhibition activity. The RGT sample shows again the highest antimutagenic activity, with an inhibition of 71.81%, followed by AF, AT, WGT and WGF.

Table 5
Antimutagenicity assay for *S. typhimurium* TA98 and TA100 bacterial strains.

Test item	Number of revertants			
	TA 98		TA100	
	Mean ± S.D.	Inhibition%	Mean ± S.D.	Inhibition%
Negative Control	9.45 ± 3.5[a]		9.45 ± 2.21[a]	
AF	121 ± 3.6	37.95	145 ± 6.5	58.8
AT	110 ± 2.1	43.58	136 ± 4.9	61.36
CF	194 ± 5.6	0.52	298 ± 7.2	15.34
CT	185 ± 4.7	5.12	295 ± 5.4	16.19
WGF	87 ± 3.76	55.38	189 ± 4.5	46.30
WGT	83 ± 2.54	57.43	174 ± 6.2	50.56
RGF	71 ± 3.16	63.58	115 ± 3.6	67.32
RGT	62.8 ± 3.0	67.79	99.2 ± 5.4	71.81
BF	113 ± 2.9	42.05	290 ± 6.8	17.61
BT	128 ± 3.4	34.35	310 ± 6.4	11.93
4-NPD[b]	195 ± 11.2	–	–	–
NaN_3[b]	–	–	352 ± 14.26	–

[a] Values expressed are means ± S.D. of three replicates.
[b] 4-NPD and NaN_3 were used as positive controls for *S. thyphimurium* TA98 and TA100 strains, respectively.

4. Conclusions

Overall, the results suggest that apple peel, carrot pulp, red and white grape peel and beetroot pulp/peel waste can be exploited for their nutrients and antioxidant components and used to add value in food formulations. Hence, these results pave the way for utilization of food industry bio-waste that has bioactive potential after thermal treatment (10 min at 80 °C).

The thermally processed samples, when compared with fresh samples, have a stronger inhibitory activity against the studied strains (bacteria and fungi). The carrot extracts have no antimicrobial effects, while the best inhibitory activity against almost all the strains was attributed to thermally processed red-grape waste.

The increase in total antioxidant activities of thermally processed samples shows that the antioxidant activity is enhanced by thermal processing. Red-grape waste extract exhibited the highest scavenging activity, with a significant increase of 58.37% ($p < 0.05$) in radical inhibition capacity after thermal processing.

The fatty acid content is significantly different between fresh and thermally processed samples. Among all fatty acids measured, linoleic acid (C18:2n-6) has the highest value in all analyzed samples, with a peak of 66.3% of TLs in the WGF sample; however, its content decreases during thermal processing.

The presence of bioactive compounds such as fatty acids and phenolic compounds in agro-industrial waste enables fruits and vegetable leftovers to become more valuable for the food industry. Use of these leftovers can provide an extra source of income, while in the same time reducing the waste disposal problem.

Conflict of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.03.131.

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