Food Chemistry 231 (2017) 131–140

Contents lists available at ScienceDirect

Food Chemistry

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

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

Dan Cristian Vodnar [a,b,*], Lavinia Florina Călinoiu [a,b], Francisc Vasile Dulf [c,*], Bianca Eugenia Ştefănescu [b,d], Gianina Crişan [d], Carmen Socaciu [a,b]

[a] Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, 400372 Cluj-Napoca, Romania
[b] Institute of Life Sciences, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, 400372 Cluj-Napoca, Romania
[c] Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, 400372 Cluj-Napoca, Romania
[d] Department of Pharmaceutical Botany, Iuliu Haţieganu University of Medicine and Pharmacy, 12 I. Creangă Street, Cluj-Napoca 400010, Romania

ARTICLE INFO

Article history:
Received 6 October 2016
Received in revised form 21 March 2017
Accepted 22 March 2017
Available online 24 March 2017

Keywords:
Fresh
Thermally processed
Agro-food waste
Phenolic content

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.

* Corresponding authors at: University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, 400372 Cluj-Napoca, Romania (D.C. Vodnar).

E-mail addresses: dan.vodnar@usamvcluj.ro (D.C. Vodnar), francisc_dulf@yahoo.com (F.V. Dulf).

http://dx.doi.org/10.1016/j.foodchem.2017.03.131
0308-8146/

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 (4000g 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