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Composition and properties of the polyphenolic extracts obtained from industrial plum pomaces

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The polyphenol composition of purified extracts obtained from plum pomace gathered from production lines of a modern fruit transformation plant was characterized. The extraction of polyphenols from pomaces was performed using water. These water extracts were purified on an Amberlite polymer bed and freeze-dried. The resulting preparations were characterized by high polyphenol contents (up to 50 g/100 g) determined using spectrophotometric method with Folin–Ciocalteu reagent. The selected plum preparations were characterized by high flavanol contents (up to 10 g/100 g) and high antioxidant capacities. Additionally, significant amounts of hydroxycinnamic acids and flavonols were detected in the plum preparations. The bacteriostatic effects of the extracts were observed against *Salmonella*, *Listeria* and *E. coli* O157:H7. Two of the extracts had high bactericidal effects against *Listeria*. This research showed that plum pomaces are a good raw material for the production of highly-concentrated polyphenol preparations with potential biological properties.

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1. Introduction

A considerable volume of published research indicates that increased consumption of fruits and their polyphenols is beneficial to human health (Lee, Kang, & Cho, 2007). Stone fruits, including plums, are polyphenol-rich. Plums are grown all over the world. According to FAO (2012), the yearly production of plum over the last 10 years surpassed 9 million tons. Plum fruits are desired products in many markets, including Europe, for their taste, nutritional value and as a raw-material for many products, such as juices, fruit drinks, alcoholic drinks, jams, and dried fruits (Hooshmand & Arjmandi, 2009; Satora & Tuszyński, 2010; Tarhan, 2007; Will & Dietrich, 2006). Plums are characterized by a high concentration of phenolic compounds ranging from 138 mg/100 g to 684 mg/100 g, depending on the cultivar (Cevallos-Casals, Byrne, Okie, & Cisneros-Zevallos, 2006; Chun, Kim, Moon, Kang, & Lee, 2003; Kim, Jeong, & Lee, 2003).

The most important phenolic compounds in plums are hydroxycinnamic acids, mainly four isomers of caffeoylquinic acid, where neochlorogenic acid is predominant. In addition to caffeoylquinic acids, significant amounts of *p*-coumaroylquinic acids are present. In addition to the

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presence of anthocyanins, flavonol glycosides, such as quercetin, flavanols and procyanidins, were detected (Nunes et al., 2008; Slimestad, Vangdal, & Brede, 2009). According to numerous reports, these substances are characterized by beneficial health properties, such as cancer prevention (Kim, Yu, & Lee, 2008; Nandakumar, Singh, & Katiyar, 2008), including breast cancer (Noratto, Porter, Byrne, & Cisneros-Zevallos, 2009). Moreover, they are able to prevent heart diseases, digestive system illnesses and osteoporosis (Franklin et al., 2006; Hooshmand & Arjmandi, 2009). Another interesting issue is the potential technological uses of plums, e.g., the possibility of applying fruits or plum products as meat additives for preventing lipid oxidation (Nunez de Gonzalez et al., 2008, 2009).

Press cake residue (pomace) is a by-product of the industrial transformation of fruit to juice. The amount of pomace depends on the type of transformed fruits and technological conditions (grinding size, enzyme treatment and pressing conditions), and may reach up to 25% of the transformed raw material (Buchert et al., 2005; Fronc & Nawirska, 1994). The potential uses of pomace are composting or use as a fuel (Schaub & Leonard, 1996). The use of pomace as an animal food component (Joshi & Sandhu, 1996), dietary supplements as fiber preparations (Larrauri, 1999), and as anthocyanins extracts (Kapasakalidis, Rastall, & Gordon, 2006; Landbo & Meyer, 2001) are alternative pomace uses. Plum pomace is a less recognized raw material, and there are few literature references on its polyphenol composition and properties.

Several works have demonstrated the antimicrobial activity of flavonoids extracted from bergamot (Mandalari et al., 2007), *Garcinia* spp. (Negi, Jayaprakasha, & Jena, 2008), pome fruits including apples, pears, quinces (Alberto, Canavosio, & Manca de Nadra, 2006; Fattouch et al., 2007, 2008), and grapes (Baydar, Özkan, & Sağdiç, 2004), among others. Polyphenols extracted from plants and fruits could be an alternative to chemical disinfectants and preservatives as consumers demand for more natural and fresh foods with fewer synthetic additives but increased safety and longer shelf life (Negi et al., 2008). Currently, chlorine in the form of sodium hypochlorite is commonly used as a disinfectant in agro-food industries, which is also used to wash fresh and fresh-cut fruits and vegetables. Nevertheless, concerns about its limited efficacy and the toxicity of chlorination by-products formed in the presence of organic matters have prompted the search for alternative, safer, more effective and environmentally friendly sanitation agents. Concerning microbial preservatives, traditional antimicrobials, such as acetic, benzoic, lactic, propionic and sorbic acids, nitrites, sulphites, have been used for many years to control the growth of microorganisms in food (Sofos, Beuchat, Davidson, & Johnson, 1998).

The aim of the work was to obtain concentrated polyphenol preparations from industrial plum pomaces using a low-pressure chromatography method with solvents applicable in food production. The resulting preparations were qualitatively and quantitatively characterized, and their antioxidant activities measured. Moreover, with the aim of replacing chemical disinfectants in agro-food industry processes, the bactericidal effects of the polyphenol extracts were determined against the foodborne pathogens *Salmonella*, *Listeria* and *E. coli* O157:H7.

2. Material and methods

2.1. Chemicals

Ultrapure water (Millipore System, GmbH, Vienna, Austria) and HPLC gradient-grade methanol (J.T. Baker, Deventer, Holland) were used to prepare all of the solutions. HPLC gradient-grade acetonitrile and formic acid were purchased from J.T. Baker (Deventer, Holland). Cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, peonidin-3-O-glucoside, quercetin-3-O-rutinoside, quercetin-3-O-galactoside, quercetin-3-O-glucoside, kaempferol-3-O-rutinoside, kaempferol-3-O-glucoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside, quercetin, kaempferol, isorhamnetin from Extrasynthese (Genay, France) and (+)-catechin, (–)-epicatechin, chlorogenic acid from Sigma-Aldrich Chemie GmBH (Steinheim, Germany) were used as standards for MS spectral comparisons. Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals to determine antioxidant activities were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

2.2. Plant material

Stoned pomaces of plums were the material for the research. The pomaces were collected in September 2009 from Alpex (Łęczeszyce, Poland), a modern fruit processing company. Ten tons of plums of various dark blue cultivars (Najbolia, Dabrowicka, Promis) were used to produce juice on a belt press. Detailed data on the technological conditions of the plum pressing are not given because they are confidential to the company. A representative sample of the obtained pomace was submitted for analysis in a laboratory.

One kilogram of the pomace was freeze-dried and 3 kg of fresh pomace was extracted with water to obtain raw polyphenol extracts and post-extraction pomace after drying. The raw extracts were then purified on a polymer bed (Amberlite XAD-7HP) resulting in three types of purified extracts, which differed in polyphenol contents.

For the determination of the phenolics in the starting material, 50 g of a representative sample of fresh plum pomace was ground with liquid nitrogen in an IKA A11 (IKA-Analytical Mill, Staufen, Germany) laboratory mill. The analytical sample (2 g) was then extracted, as described in our previous work (Sójka & Król, 2009).

2.3. Preparation of plum extracts

Fresh plum pomaces (3 kg) were subjected to water extraction in three steps at temperatures of 70–75 °C for 30 min. In the first step, the weight ratio of fresh pomace to water was 1:4 (w/v), while in second and third extraction steps, the volume of the water was equal to the volume of the extracts from the previous extraction step. The first and second extracts were collected by soaking on filtration cloth, while the third extract was pressed on a laboratory hand screw press (homemade, Lodz University of Technology, Poland) after soaking the pomace. All three extracts were combined and filtered on cellulose sheet Hobrafilt S40 N – 5 μm nominal retention, 3.6-mm thickness

(Hobra-Školnik S.R.O., Broumov, Czech Republic). The filtered extract was purified on 80 × 2.5-cm column (Pharmacia Fine Chemicals, Uppsala, Sweden) filled with Amberlite XAD-7HP (Sigma-Aldrich, Steinheim, Germany). The extract was applied at a flow rate of 250 mL/h. Afterwards, the column was washed with 300 mL of water and 150 mL of 10 % ethanol, with a flow rate of 150 mL/h. The elution of polyphenols was performed with 300 mL of 20% ethanol and 750 mL of 60% ethanol at a flow rate of 150 mL/h. Seven fractions of 150 mL each were collected. The fractions were pulled as follows: 1 + 2, 3 + 4, and 5 + 6 + 7. The ethanol was removed from each of the pulled fractions using a laboratory vacuum rotary evaporator (type 350P Unipan – Scientific Instruments, Warsaw, Poland) at 50 °C; these fractions were then freeze-dried. This procedure resulted in three concentrated preparations from the pomace. The resulting plum preparations were labeled as follows: PPE1 (1 + 2), PPE2 (3 + 4), PPE3 (5 + 6 + 7).

2.4. Sample preparation for quantification

The concentrated polyphenol preparations were diluted in a 50% solution of methanol and then centrifuged at 3600 × *g* for 5 min. The as-prepared samples were used to determine the total polyphenol content (TCP), the antioxidant activity, and the individual polyphenol contents, i.e., anthocyanins, hydroxycinnamic acids and flavonols, via an HPLC method.

2.5. HPLC conditions for identification and quantification

High performance liquid chromatography (HPLC) coupled with a DAD and an electrospray ion (ESI) trap mass spectrometer was used for the identification of the hydroxycinnamic acids, flavonols, proanthocyanidins, and anthocyanins. The HPLC system was equipped with a SCM1000 membrane solvent degasser (ThermoQuest, San Jose, CA, USA), a binary high pressure gradient pump (1100 Series; Agilent Technologies, Santa Clara, CA, USA), autosampler, and a column oven (Surveyor Series, Thermo-Finnigan, San Jose, CA, USA).

A Gemini C18 110A 250 mm × 4.6 mm i.d. (Phenomenex, Torrance, CA, USA) 5 μm column was used. The column temperature was 30 °C and the injection volume was 10 μL. The chromatographical data were collected using Xcalibur software, version 1.2 (Thermo-Finnigan, San Jose, CA, USA).

The solvents used and the gradient for hydroxycinnamic acids and flavonol separation were as follows: solvent A, 0.25% (v/v) formic acid in water; solvent B, 85:15 (v/v) acetonitrile:methanol, the gradient programme (time in min – % (v/v) B) was 0–16, 6–18, 9–18, 14–20, 30–20, 42–26, 50–29, 52–42, 62–42, 66–54, 68–80, 70–80, 72–16, and 83–84; the flow rates were 0.4 mL/min for 0–9 min, followed by a flow-rate gradient increase to 0.6 mL/min for 9–14 min and 14–82 min, followed by a flow-rate gradient decrease to 0.4 mL/min for 82–83 min.

The solvents and the gradient used for anthocyanin separations were as follows: solvent A, 0.25% (v/v) formic acid in water; solvent B, 0.25% (v/v) formic acid in acetonitrile, the flow rate was 12 mL/min; the gradient programme (time min – % (v/v) B): 0–5, 2–5, 32–20, 37–70, 42–70, 45–5, and 55–5.

The MS system coupled to the HPLC was an LCQ DECA ion trap mass spectrometer (Thermo-Finnigan, San Jose, CA, USA) equipped with an ESI source used in the negative mode. The source parameters were as follows: ion spray voltage, 4.50 kV; capillary voltage, –23 V; capillary temperature, 240 °C; and sheath nitrogen gas flow rate, 80 (arbitrary units).

The phenolics were quantified using a KNAUER Smartline chromatograph (Berlin, Germany) equipped with a degasser (Manager 5000), two pumps 1000, autosampler 3950, column oven Jetstream Plus 2 and detector PDA 2800. The phenolics extracts were separated on a 150 mm × 4.6 mm i.d., 5 μm, Gemini C18 110A column (Phenomenex, Torrance, CA, USA) using gradient elution with 10% (v/v) formic acid in water (solvent A) and 50:40:10 (v/v/v) acetonitrile:water:formic acid (solvent B). The column temperature was set to 40 °C, the flow rate was 1 mL/min and the gradient program was as follows (time min – % (v/v) B): 0–12, 0.6–12, 16–30, 20.5–100, 22–100, 25–12, 35–12. The injection volume was 20 μL. The data were collected using the Eurochrom 2000 software (Knauer, Berlin, Germany). The hydroxycinnamic acids were detected at 320 nm. Quercetin, kaempferol and isorhamnetin glycosides, and their aglycones were detected at 360 nm, while anthocyanins were detected at 520 nm. Standard curves using cyanidine-3-glucoside, rutin, quercetin, chlorogenic and *p*-coumaric acids were used for quantification. Cyanidin-3-glucoside was used to assay for anthocyanins, rutin was used to assay for quercetin, kaempferol and isorhamnetin glycosides, chlorogenic acid was used to assay for chlorogenic acid isomers, and *p*-coumaric acid was used to assay *p*-coumaryl derivatives.

2.6. Flavanols determination via vanillin–HCl assay

To estimate the content of flavan-3-ols in the extracts, the vanillin–HCl assay was performed, according to Nakamura et al. (2003).

2.7. HPLC analysis of flavanols

A method for applying the acid-catalyzed degradation of polymeric proanthocyanidins in the presence of toluene-α-thiol was used for the determination of flavanols. The crude methanol solutions for HPLC determination of free (+)-catechin and (–)-epicatechin were prepared by diluting 5 mg of the extract in 1.2 mL of dry methanol and sonication at room temperature for 30 min. The thiolysis reaction was performed according to the procedure described by Guyot, Marnet, Sanoner, and Drilleau (2001): Five milligrams of dry extract was mixed with 800 μL of benzylthioether (5% (v/v)) in anhydrous methanol and 400 μL of 0.4 M HCl in anhydrous methanol and the reaction was performed at 40 °C for 30 min. The thiolysis reactions were performed in triplicate for each sample. The samples were filtered through a 0.45-μm Teflon membrane (Millipore, Bedford, MA, USA) and then analyzed using RP-HPLC coupled to UV–visible detection according to the method described by Guyot et al. (2001). The 280 nm HPLC response factors of (–)-epicatechin, (+)-catechin were obtained from calibration curves of the respective commercial standards (Sigma Aldrich) whereas the 280-nm calibration curve of (–)-epicatechin benzylthioether was noted by the use of the standard additionally purified (Guyot,

Marnet, Laraba, Sanoner, & Drilleau, 1998). The response factors were next used both for the quantification of the total flavanols in the extracts and for the determination of their average degree of polymerization (DP) (Guyot et al., 1998). The average DP was measured by calculating the molar ratio of all of the flavan-3-ol units (thioether adducts + terminal units) to (–)-epicatechin and (+)-catechin corresponding to terminal units.

2.8. Calculations

For the resulting extracts (PPE1 – 3), the phenolic results were converted to starting material (pomace) weight according to Hager, Howard, and Prior (2008). The following calculations were used:

$$C_{pomace} = C_{product} \cdot R$$

$C_{product}$ – concentration of extract, R – ratio of the mass of extract to the mass of the fresh pomace, and C_{pomace} – concentration based on fresh pomace weight.

2.9. Total phenolics content

The total phenolics were measured using the method described by Singleton and Rossi (1965) with some modification. The volumes 0.25 mL of phenolics extracts were placed in 25-mL flasks and 0.25 mL of the Folin–Ciocalteu reagent was added and mixed. After 3 min, 2.5 mL of a 20% Na_2CO_3 solution was added to the reaction mixture. The flask was filled with water to the graduation mark, and the solution was stirred. The incubation was performed at room temperature for 1 h. The absorbance of the solutions was measured at a wavelength of 720 nm. The results were expressed as mg of (–)-epicatechin equivalents per 100 g of extract. All of the samples were analyzed in duplicate.

2.10. DPPH radical-scavenging activity

The DPPH scavenging activity was determined using the method described by Kim, Lee, Lee, and Lee (2002).

The phenolic extract (0.05 mL) was added to 1.95 mL of a methanolic (60 μM) DPPH solution. The mixture was shaken vigorously and incubated at room temperature in the dark for 30 min. The decrease in absorbance of the resulting solution was monitored at 515 nm for 30 min. A concentration response curve was prepared for the absorbance of the DPPH radical as a function of different Trolox concentrations. The results of the DPPH radical-scavenging activities of the plum phenolics extracts were expressed as mM TE/g of extract (TE – Trolox equivalent antioxidative capacity).

2.11. Antimicrobial tests

The microorganisms for antimicrobial tests were obtained from public collections: CECT – Spanish Type Culture Collection, NCTC – National Collection of Type Cultures (Porton Down Salisbury, UK), and ATCC – American Type Culture Collection (Manassas, VA, USA). The strains used for antimicrobial testing were a non-pathogenic strain of *Escherichia coli* O157:H7 (NCTC 12900), *Listeria monocytogenes serovar* 1a (CECT-4031), *L. monocytogenes serovar* 4d (CECT-940), *L. innocua* (CECT-910) and the strains of *Salmonella enterica* subsp. *enterica* (Smith) Weldin serotype Michigan (ATCC BAA-709) and Montevideo (ATCC BAA-710).

They were maintained at –20 °C on nutrient agar slants with glycerol. Prior to use, they were subcultured on Tryptone Soy Agar (TSA, Oxoid, Basingstoke, UK) or tryptone soy agar plus yeast extract (TYSEA, TSA plus 6 g/L of yeast extract) and incubated at 37 °C for 20–24 h. The bacterial cells were harvested by centrifugation at 9820 × *g*, 10 min at 10 °C and then resuspended in saline peptone (SP; 8.5 g/L NaCl and 1 g/L peptone). For the inoculum preparation, the bacterial concentration was estimated using a spectrophotometer set at $\lambda = 420$ nm according to standard curves. A suspension of 10^8 cfu/mL of each strain was prepared.

To test if plum polyphenol extracts could be used for disinfection in water, the bactericidal effects were assayed by placing the indicator microorganism in contact with an aqueous solution of the polyphenol extract. In a first experiment, the plum polyphenol extracts were tested at 10 mg/mL and 5 min of contact. If they did not show any antimicrobial effects at such high concentration, they would be rejected for further experiments. Therefore, 1 mL of each plum polyphenol extract (PPE1, PPE2 and PPE3) at 10 mg/mL was prepared in duplicate in sterile 1.5 mL microcentrifuge tubes. Afterwards, 100 μL of the pathogen suspensions was added to the tubes containing the 10^7 cfu/mL suspensions. After 5 min of contact, the populations in the tubes were determined by diluting 10-fold on SP and plating (20 μL) onto Sorbitol MacConkey Agar (SMAC, Biokar Diagnostics, Beauvais, France) supplemented with Cefixime-Tellurite (CT-SMAC, Biokar) for *E. coli* O157:H7, Xylose-Lysine-Desoxycholate agar (XLD, Oxoid Ltd., Basingstoke, England) for *Salmonella* or onto Palcam agar (Palcam Agar Base with selective supplement, Biokar Diagnostics, Beauvais, France) for *Listeria* spp. Plates were incubated at 37 ± 1 °C for 24 ± 2 h (*E. coli* and *Salmonella*) and 48 ± 2 h (*Listeria* spp.). Deionized water was used as a control (CK). In this experiment, the detection limit was 2.5×10^3 cfu/mL (3.40 log cfu/mL).

In a second experiment, different concentrations (1, 5 and 10 mg/mL) of the selected plum polyphenol extracts were tested against a cocktail of the 3 strains of *Listeria* using the same methodology. In this experiment, the detection limit was 25 cfu/mL. The data were transformed to log cfu/mL. When no colonies were detected, the half detection limit value was used for further calculations (1.10 log cfu/mL). The bactericidal experiments were repeated twice.

2.12. Statistical analysis

The influence of PPE2 and PPE3 extract doses on population reduction of *Listeria* spp. was determined using one-way analysis of variance, and significant differences between doses were determined using Duncan's multiple range test. The differences were considered significant at $p \leq 0.05$. The statistical analysis was performed using Statistica Version 7 software (StatSoft, Tulsa, OK, USA).

3. Results and discussion

3.1. *Identification of the components*

Purified plum pomace extracts were characterized by the presence of many polyphenol components (Table 1). The composition variability of the individual polyphenol groups in the researched extracts resulted from the sorption properties of the applied bed, which is characterized by good retention of the groups. In the desorption process, an increasing ethanol concentration was used, which resulted in the differentiation of the polyphenol content in the collected fractions. The most differentiated in number of identified polyphenol components was plum extract PPE3, which contained chlorogenic acids isomers, *p*-coumaroylquinic acids, quercetin and kaempferol glycosides.

The PPE1 extract was the poorest among plum pomace extracts analyzed; the presence of neochlorogenic acid was identified there. The extract contained some other components (most likely hydroxycinnamic acid oxidation products), but their detailed identification was not possible using the MS detector.

Significantly higher amounts of polyphenols were present in the PPE2 extract, which contained anthocyanins with cyanidin and peonidin rutinosides as the main components, and cyanidin and peonidin glucosides present in lower amounts. The identification of anthocyanins was performed using the

Table 1 – Phenolics identification in plum pomace extracts.

Compound	RT[a] [min]	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS[b] fragmentation (*m/z*)	Identification[c]	PPE1	PPE2	PPE3
Anthocyanins								
A1	18.08	280, 518	447	**285**	Cyanidin-glucoside		+	+
A2	19.70	280, 516	593	**285**	Cyanidin-rutinoside		+	+
A3	21.92	280, 516	461	**299**	Peonidin-glucoside		+	+
A4	23.07	280, 507	607	**299**	Peonidin-rutinoside[c]		+	+
Hydroxycinnamic acids								
H1	12.33	323	353	**191**, 179	Neochlorogenic acid[c]	+	+	+
H2	15.08	314	337	**163**, 191	3-*p*-coumaroylquinic acid[c]		+	+
H3	15.08	285, 324	353	**191**, 179	Chlorogenic acid		+	+
H4	16.30		367	**193**	3-O-feruloylquinic acid[c]		+	+
H5	20.30	312	337	**173**, 191	4-*p*-coumaroylquinic acid[c]		+	+
H6	20.30	326	335	**179**	3-O-caffeoylshikimic[c]		+	+
Proanthocyanidins								
P1	12.20	280	577	289	Procyanidin dimer[c]		+	+
	13.90	280	1153	**865**, 577, 289	Procyanidin tetramer[c]		+	+
	14.20	–	865	577, 289	Procyanidin trimer[c]			+
	14.94	–	1441		Procyanidin pentamer[c]			+
	15.50	–	577	289	Procyanidin dimer[c]			+
	18.12	–	865	577, 289	Procyanidin trimer[c]			+
	20.40	–	577	289	Procyanidin dimer[c]			+
Flavonols								
F1	31.15	255, 353	609	**301**, 271, 255	Quercetin-rhamnosylgalactoside[c]			+
F2	32.03	255, 354	609	**301**, 271, 255	Quercetin-rutinoside			+
F3	34.53	255, 350	463	**301**, 271, 255	Quercetin-galactoside			+
F4	36.52	255, 348	463	**301**, 271, 255	Quercetin-glucoside			+
F5	43.43	265, 341	593	**285**, 255, 227	Keampferol-rutinoside			+
F6	43.88	249, 351	623	**315**, 314, 300	Isorhamnetin-deoxyhexose-hexoside[c]			+
F7	45.22	26, 350	623	**315**, 300	Isorhamnetin-rutinoside			+
F8	59.25	314	593	**285**, 447	Kaempferol-coumaroylhexoside[c]			+
F9	59.93	314	593	**285**, 447	Kaempferol-coumaroylhexoside[c]			+
Others								
O1	10.67	331	705	**339**, 513	Unknown	+	+	+
O2	11.32	279	513	**205**, 247	Unknown	+	+	+
O3	14.38	285, 325	705	**513**, 339, 295	Unknown		+	+
O4	15.98	282	705	**513**, 339, 295	Unknown			+
O5	16.30	326	705	**513**, 339, 295	Unknown		+	+
O6	17.53	323	705	**513**, 339, 295	Unknown		+	+
O7	18.73	292, 316	705	**513**, 339, 295	Unknown		+	+
O8	21.02	273	403	**241**	Unknown		+	+
O9	25.43	273	718	–	Unknown			+
O10	49.55	268	913	–	Unknown			+

[a] RT – retention time (min).
[b] The *m/z* values of the predominant ions are given in bold type.
[c] Tentative identification.
+ - presence of the compound detected using MS.

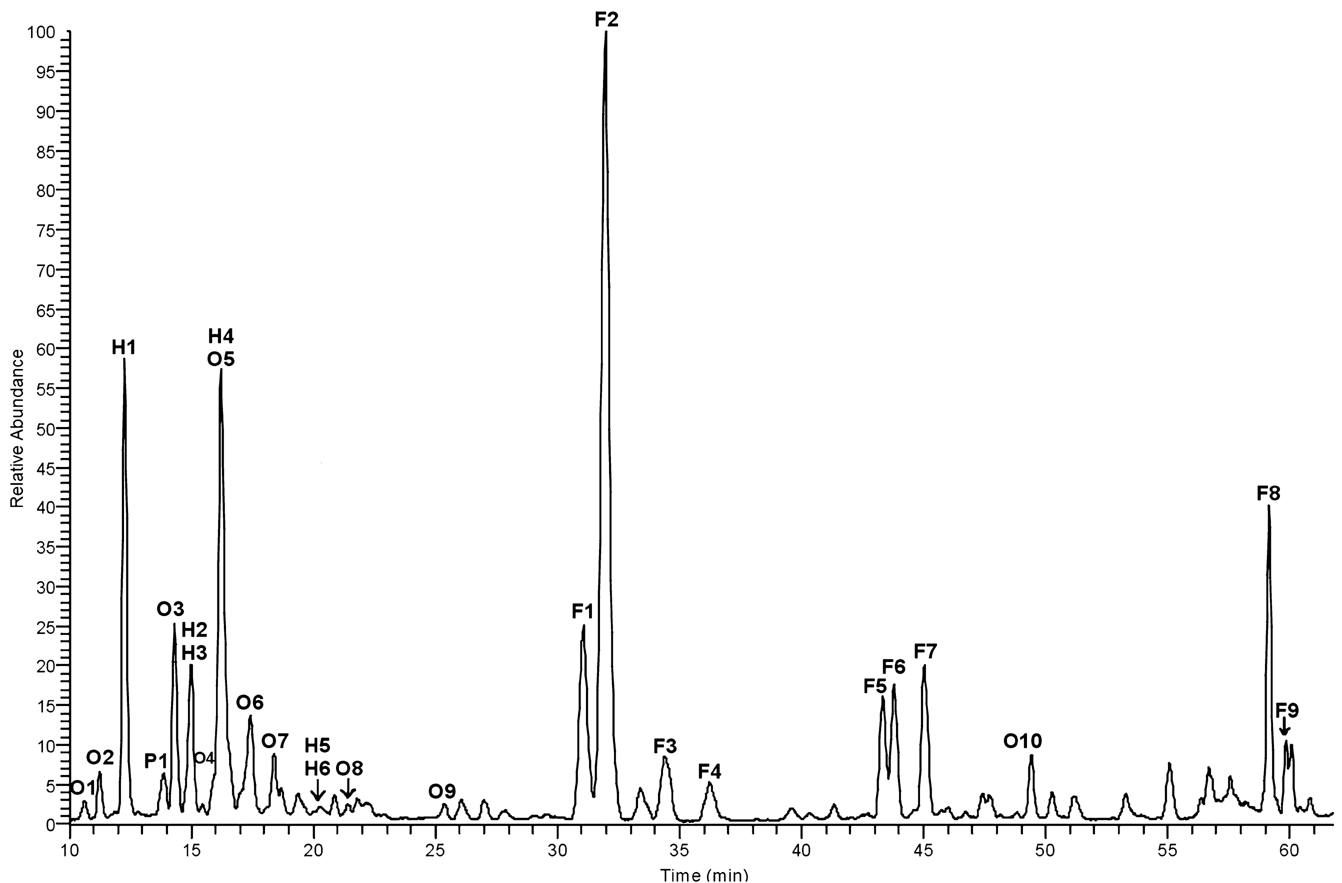

Fig. 1 – HPLC chromatogram TIC (base peaks) of PPE3 plum pomace extract. Peak codes correspond to those in Table 1.

UV–Vis spectra, MS detector data comparison with available standards and comparison with the literature reference data. The research by Tomás-Barberán et al. (2001), Slimestad et al. (2009) and Treutter et al. (2012) also proved that the anthocyanins discussed earlier were the primary plum anthocyanins. In addition to anthocyanins, neochlorogenic and chlorogenic acids, 3-*p*-coumaroylquinic and 4-*p*-coumaroylquinic acids were also present. These hydroxycinnamic acids were identified based on the UV–Vis spectra, MS/MS data and reference data (Clifford, Johnston, Knight, & Kuhnert, 2003; Fang, Yu, & Prior, 2002; Nakatani et al. 2000). Chlorogenic acid was also confirmed by comparison with a commercial standard. The presence of 3-*O*-feruoylquinic and 3-*O*-caffeoylshikimic acids was identified as well using MS/MS. The first acid gave deprotonated molecule $[M-H]^-$ at *m/z* 367 fragmented to *m/z* 193, which matched the deprotonated ferulic moiety. The second one showed a deprotonated molecule $[M-H]^-$ at *m/z* 335 with a product ion at *m/z* 179 corresponding to deprotonated shikimic acid. These ion fragmentations were similar to those described by Clifford et al. (2003) and Fang et al. (2002). The presence of these two acids in plums was proved by Nunes et al. (2008). The presence of traces of the two procyanidin was found in PPE2. The deprotonated procyanidin $[M-H]^-$ at *m/z* 577 with a product ion at *m/z* 289 proved the presence of the dimer, the second procyanidin was a tetramer, the deprotonated molecule $[M-H]^-$ at *m/z* 1153 fragmented into *m/z* 865, 577 and 289. A large number of unidentified components were present in the extract. The components had UV–Vis maxima at 320–330 nm and a deprotonated molecule $[M-H]^-$ at *m/z* 705, which fragmented into *m/z* 513 and then to *m/z* 339. The UV spectra of these components may be hydroxycinnamic acid derivatives. The same fragmentation was found in the research by Guyot, Bernillon, Poupard, and Renard (2008) where MS/MS fragmentation of the deprotonated molecule $[M-H]^-$ at *m/z* 513 (*m/z*) is linked to the loss of one quinic acid moiety. According to these researchers, the compounds are most likely products of caffeoylquinic acids oxidation and were identified as caffeoylquinic acid dehydrodimers.

PPE3 extract was the most differentiated by the presence of polyphenol components (Fig. 1). In addition to the components discussed earlier in PPE2, the presence of other proanthocyanidins and quercetin glycosides with predominantly rutinoside was found in the extract. Quercetin glucoside and galactoside were identified as well by comparison with standards. Another compound, i.e., quercetin diglycoside, was also recorded. This compound, most likely ramnosylgalactoside, was eluted before rutin and gave a deprotonated molecule $[M-H]^-$ at *m/z* 609 fragmenting to *m/z* 301, which was fragmented similar to rutin. The same quercetin glycosides, excluding ramnosylglucoside, were identified in plums by Slimestad et al. (2009) and Tomás-Barberán et al. (2001).

In addition to quercetin glycosides, two isorhamnetin diglycosides were identified. One was rutinoside, which was identified by comparing with the standard, the other one was

a diglycoside composed of deoxyhexose and hexose parts. The last identified flavonoid group was the kaempferol glycosides. The presence of kaempferol rutinoside was identified by comparison with standard. The presence of two acylated glycosides was identified as well; both gave a deprotonated molecule [M-H]⁻ at *m/z* 593, which fragmented to *m/z* 447 and 285 and corresponded to the loss of *p*-coumaric acid and hexose, respectively. Moreover, extra slopes at ~314 nm were found in the UV spectra of the components, which proved the presence of hydroxycinnamic acid derivatives. Similar compounds of identical fragmentation were found by Seeram, Rupo, Scheuller, and Heber (2006) in his research on strawberries, where the compound was identified as kaempferol–coumaroyl–glucoside. The acylated kaempferol glycosides and one isorhamnetin diglycoside was confirmed in plum extract for the first time.

The obtained extracts were diversified in these polyphenols. The main identified polyphenols in the extracts were identical to those from plum fruits, which were confirmed using available references results (Nunes et al., 2008; Slimestad et al., 2009; Treutter et al., 2012; Usenik, Štampar, & Veberič, 2009). In addition to native polyphenols, additional components were identified in the extracts. They were designed as polyphenol oxidation products. These compounds can appear during fruit storage as a result of enzymatic processes, i.e., polyphenoloxidase (PPO) activity, or as the result of heat processes (Guyot et al., 2008).

3.2. Polyphenol content, antioxidant activity

The results of the polyphenol determination using the Folin–Ciocalteu method showed the high polyphenol contents in the selected plum preparations (Table 2). The highest polyphenol content was found in PPE3; the value was greater than 50 g/100 g, followed by PPE2 with 38 g/100 g of phenolics. PPE1 was characterized by significantly lower polyphenol contents of approximately 5 g/100 g. Those data indicate that the technology applied for extraction and purification of pomace is a good method for the production of high concentration polyphenol extracts.

The phenolic contents (hydroxycinnamic acids, flavonols and anthocyanins) determined using HPLC in polyphenol concentrates obtained from plum pomace are presented in Table 2. The PPE2 and PPE3 preparations contained the most polyphenols (the same as for the FC assay).

The main PPE1 component was neochlorogenic acid, which had 0.47 g/100 g. No anthocyanins, flavonols and flavanols were found in the PPE1 extract.

Table 2 – Phenolic composition (expressed as mg/100 g of fresh pomace or g/100 g of dry extract) of plum pomace phenolic extracts and antioxidant activity (AA) determined using the DPPH method.

Compound	Content expressed in milligrams per 100 grams of fresh pomace [mg/100 g of fresh pomace]								Content expressed in grams per 100 grams of dry extract [g/100 g of dry extract]		
	Pomace		PPE1		PPE2		PPE3		PPE1	PPE2	PPE3
	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD	Mean	Mean	Mean
Neochlorogenic acid	6.42	0.88	0.31	1.5	2.79	0.9	1.57	0.7	0.47	6.97	3.14
3-*p*-coumaroylquinic acid	0.95	3.87	0.00	–	0.41	2.1	0.44	1.1	0.0	1.02	0.88
Chlorogenic acid	2.20	2.83	0.00	–	0.65	1.4	1.07	2.1	0.0	1.62	2.14
Quercetin galactoside	0.75	2.07	0.00	–	0.00	–	0.62	0.1	0.0	0.0	1.24
Quercetin glucoside and rutinoside	2.60	2.72	0.00	–	0.06	0.8	1.43	0.1	0.0	0.15	2.86
Kaempferol-rutinoside	0.32	1.77	0.00	–	0.00	–	0.16	0.1	0.0	0.0	0.32
Isorhamnetin-rutinoside	0.36	2.83	0.00	–	0.00	–	0.24	0.0	0.0	0.0	0.47
Quercetin	0.03	4.71	0.00	–	0.00	–	0.07	0.0	0.0	0.0	0.14
Kaempferol	0.00	–	0.00	–	0.00	–	0.02	0.0	0.0	0.0	0.03
Cyanidin-glucoside	0.09	0.90	0.00	–	0.03	2.9	0.03	0.1	0.0	0.08	0.06
Cyanidin-rutinoside	0.27	3.71	0.00	–	0.16	0.1	0.10	0.5	0.0	0.40	0.20
Peonidin-glucoside	0.01	3.29	0.00	–	0.00	3.9	0.00	8.5	0.0	0.01	0.01
Peonidin-rutinoside	0.28	1.50	0.00	–	0.10	0.3	0.07	1.6	0.0	0.24	0.14
Sum of hydroxycinnamic acids	9.57	1.15	0.31	1.5	3.84	1.1	3.09	1.2	0.47	9.61	6.17
Sum of flavonols	4.03	1.76	0.00	–	0.06	0.8	2.45	0.1	0.0	0.15	4.89
Sum of flavonol aglycons	0.03	4.71	0.00	–	0.00	–	0.09	0.0	0.0	0.0	0.18
Sum of anthocyanins	0.65	2.25	0.00	–	0.29	0.4	0.21	0.7	0.0	0.72	0.41
Sum of flavanols[a]	135.24	4.59	n.a.	–	2.53	2.2	5.06	4.3	n.a.	6.30	10.10
mDP[b]	n.a.	–	–	–	–	–	–	–	n.a.	1.7 (0.85)	4.1 (0.35)
Total phyto-compounds HPLC	149.52	4.22	0.31	1.5	6.72	1.4	10.88	1.7	0.47	16.79	21.75
Total phyto-compounds FC[c]	n.a	–	–	–	–	–	–	–	5.10 (4.31)	38.53 (1.74)	50.27 (5.75)
AA [mM TE/g][d]	n.a	–	–	–	–	–	–	–	0.138 (0.72)	0.746 (1.34)	1.036 (2.32)

Values are means [mg/100 g] $n = 2$; RSD – relative standard deviation [%]; values in parentheses are RDS.

[a] The content of flavanols determined by the thiolysis method.

[b] Mean degree of flavanols polymerization.

[c] Total phenolics content determined by the FC method calculated on (–)-epicatechin.

[d] Anti-oxidant activity determined by the DPPH method, calculated as mM TE/g.

The PPE2 extract was rich in polyphenols and had approximately 17 g/100 g of polyphenols, which was determined using HPLC. Hydroxycinnamic acids (9.61 g/100 g) were the primary components of the extract, and neochlorogenic acid had the highest concentration of 6.97 g/100 g. Chlorogenic acid was at the level of 1.62 g/100 g, and 3-*p*-cumaroylquinic acid was present at 1.02 g/100 g. The amount of anthocyanins was 0.72 g/100 g. The share of hydroxycinnamic acids were ~57% of total polyphenols determined using HPLC in PPE2 extract.

The PPE3 extract contained somewhat more polyphenols (22 g/100 g) with a significantly lower contribution of hydroxycinnamic acids compared with PPE2. Their concentration was 6.17 g/100 g of primarily neochlorogenic acid. In addition to hydroxycinnamic acids, flavonol glycosides were present in the extract; their concentration was 4.89 g/100 g, where quercetin galactoside, glucoside and rutinoside were the primarily components, and kaempferol and isorhamnetin rutinosides were present in lower amounts (Table 2).

The use of thiolysis showed that the largest fraction of flavanols in the extracts were condensed tannins (proanthocyanidins). The levels of the proanthocyanidins in the preparations were 6.30 g/100 g and 10.1 g/100 g for PPE2 and PPE3, respectively. The flavanols were not determined in PPE1 because the vanillin test showed only traces of these compounds. The average degree of polymerization of the flavanols was 4.1 for PPE3 and 1.7 for PPE2. According to Milala et al. (2013), proanthocyanidins of plum pomace compose more than 85% of the polyphenols. Despite the use of water, which is a weak extractant for proanthocyanidins (García-Marino, Rivas-Gonzalo, Ibáñez, & García-Moreno, 2006; Hellström & Mattila, 2008), the extracts were characterized by a high content of proanthocyanidins, compared to other polyphenols. In addition to the determined components, the plum extracts contained smaller amounts of the other polyphenol components, which are described in "Identification" section (Table 1).

Considering the content of polyphenols in starting material (fresh pomace) and the content in resulting extracts (PPE1-3) calculated on fresh pomace (Table 2), the different transfer of compound groups to final product can be noticed. The sum of recovery of both the hydroxycinnamic acids and anthocyanins was 76%; the compounds had the highest concentration in PPE2. The significant recovery yield may result from their good water solubility. For flavonol glycosides, the recovery was 62%, and the compounds were accumulated in the PPE3 extract. Furthermore, the PPE3 extract was characterized by a high concentration of flavonol aglycones, which may result from the partial hydrolysis of the glycosides under the extraction conditions. The most difficult group of compounds to extract were the flavanols. The recovery of the flavanols was 6%, even though the group was dominant in the PPE3 extract and the second most abundant in the PPE2 extract. The high concentration of flavanols in PPE2 and PPE3 results from the richness of the starting material in flavanols (135 mg/100 g), which are ~90% of the plum pomace polyphenols. The high flavanol contents in plum pomaces were confirmed by Milala et al. (2013). Because the starting material used is characterized by a large concentration of peel, the quantitative and qualitative composition of plum extracts depends on the cultivar. According to Treutter et al. (2012), the total polyphenol content in plum peels depends on the cultivar and varies between 40 and 2990 mg/100 g of fresh weight, where blue and dark blue cultivars are characterized by a high proportion of flavanols and hydroxycinnamic acids. In the research by Tomás-Barberán et al. (2001), the values for flavanols and hydroxycinnamic acids in peels of various plum cultivars were reported to be 66–183 and 11.5–37.4 mg/100 g FW, respectively.

The antioxidant activities of the preparations (Table 2) were correlated with the polyphenol contents. PPE3 showed the highest antioxidant activity, 1.036 mM TE/g, while PPE2 was 0.746 mM TE/g. The antioxidant activity of these preparations was approximately 3 times lower, compared to the extracts obtained from black currant pomace, which were purified on octadecylsilane bed, as previously described (Sójka, Guyot, Kołodziejczyk, Król, & Baron, 2009). The activity of PPE1 preparation was significantly lower (0.138 mM TE/g). In previous research on extracts prepared in the same way from sour cherry pomace (Kołodziejczyk et al., 2013) antioxidant activity was comparable and between 1.2 and 1.6 mM TE/g.

3.3. Antimicrobial activity

Preliminary studies to determine the antimicrobial activity of polyphenol extracts to evaluate their possible use as bactericides in industrial processes involving disinfection have been performed. In the first experiment, the results demonstrated that PPE1 did not reduce the population of any of the studied pathogens (data not shown). However, PPE2 and PPE3 tested at 10 mg/mL reduced *Listeria* spp. populations below the detection limit (data not shown), which meant a reduction of >4.00 log cfu/mL. For this reason, PPE2 and PPE3 were selected and tested at different concentrations against *Listeria* spp. The results demonstrated that both extracts at lower concentration also reduced the *L. monocytogenes* population below the detection limit (Fig. 2). The PPE2 and PPE3 extracts demonstrated antimicrobial effects against the gram-positive *Listeria* spp. strains tested but not against the gram-negative bacteria *Salmonella* and *E. coli* O157:H7. Those extracts contained more phyto-compounds than the PPE1 extract. In a similar study, we demonstrated that some fractions of sour cherry polyphenol extracts also have high bactericidal effects against *Listeria* spp. but no activity against *Salmonella* and *E. coli* O157:H7 (Kołodziejczyk et al., 2013). The high reduction values were obtained at concentrations above 500 μg/mL of the cherry polyphenol extract. In other studies, Fattouch et al. (2007) found that aqueous acetone quince peel extracts inhibited the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and *Candida albicans* when tested using the agar diffusion agar; *P. aeruginosa* and *S. aureus* were more susceptible than *E. coli* and *C. albicans*. In a similar study, Fattouch et al. (2008) also found that apple, pear and quince peels and pulp extracts had bacteriostatic effect against different pathogenic and spoilage microorganisms, such as *S. aureus*, *P. aeruginosa* and *B. cereus*, which were the most susceptible, while a lower or no effect was found against *E. coli*, *C. albicans* and *Aspergillus niger*. Similar trends were observed in other studies using crude hexane and chloroform extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* (Negi et al., 2008) and extracts from some Asian edible plants (Alzoreky & Nakahara, 2002). Jayaprakasha, Negi, Sikderm, Mohanrao, and Sakariah (2000) demonstrated that hexane, chloroform and acetone extracts of peels of Citrus

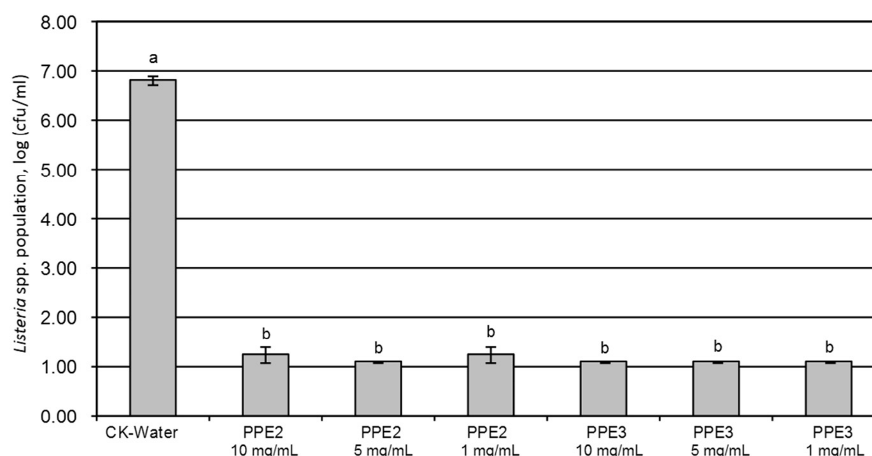

Fig. 2 – Listeria spp. population (log cfu/mL) after 5 min contact with selected plum polyphenol extracts (PPE2 and PPE3) at different concentrations. The results are the mean of 4 replications and the vertical bars indicate the standard deviation. The different letters above the bars indicate the significant (p < 0.05) differences between the doses used.

reticulate, which are rich in flavones, possessed antibacterial activity and had the potential to be used as biopreservatives.

Generally, gram-negative bacteria are more resistant to bactericidal polyphenols than gram-positive bacteria (Fattouch et al., 2007). The reason for the higher sensitivity of the gram-positive bacteria could be attributed to the differences in their cell membrane constituents and arrangements. The gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier (Scherrer & Gerhardt, 1971), and the resistance of gram-negative bacteria toward antibacterial substances may be due to the outer phospholipidic membrane carrying the structural lipopolysaccharide components, which makes it impermeable to lipophilic solutes and porins constitute a selective barrier to the hydrophilic solutes (Nikaido & Vaara, 1985). However, nature bergamot (*Citrus bergamia* Risso) peel fractions were inhibitory to gram-negative bacteria only (Mandalari et al., 2007) and the gram-positive *Bacillus subtilis* at high concentrations (<1000 µg/mL). Park, Biswas, Phillips, and Chen (2011) also found that the growth of *Salmonella* was more susceptible than *Listeria* to blueberry and Muscadine water and ethanol phenolic extracts. Ikigai, Nakae, Hara, and Shimamura (1993) also proposed that this difference is caused by repulsion between the phenolics and the surfaces of the gram-negative bacteria, which are coated with lipopolysaccharide.

At the studied concentrations, we did not find any differences in the microbial activity of PPE2 and PPE3, even though they showed different polyphenol compositions. Fattouch et al. (2007) found that, among the standard polyphenols tested, chlorogenic acid exhibited the greatest antibacterial activity, especially against *S. aureus*, and this polyphenol is present in the PPE2 and PPE3 extracts. Other authors (Hakkinen, Karenlampi, Heinonen, Mykkanen, & Torronen, 1999) reported that the inhibitory effect of berry extracts was a synergistic effect between various phenolic compounds. We have demonstrated that plum polyphenol extracts could be used to reduce *Listeria* spp. in water used for industrial processing; however, more studies have to be performed to determine the optimal dose and evaluate the possibility of use as biopreservatives in the food industry.

4. Conclusions

Fresh industrial plum pomaces are a good raw material for the preparation of polyphenol-rich extracts. The application of a purification technology using raw water extraction on a polymer-bed-type Amberlite XAD makes it possible to easily obtain polyphenol concentrates rich in anthocyanins, hydroxycinnamic acids, flavanols, and flavonol glycosides. Due to the high antioxidant and antilisterial capacity and beneficial biological properties of the components present in the plum extracts and corresponding pomaces, the product can be classified as valuable, and after safety tests, i.e., the determinations of the pesticide residues and other contaminants, could possibly be used as a potential food product supplement.

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