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Polyphenol-rich extracts from essential oil industry wastes

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The essential oil industry generates every year large amounts of waste, due to the low quantities of essential oils in the raw materials [8]. The wastes are usually discarded or used as biofertilizer, although they are rich in biologically active substances. Besides, these simple approaches are leading to ecological problems in the places where the wastes are dumped. Nowadays, the valorization of agricultural and food wastes became a priority and is a base for the successful implementation of circular economy. For this reason, the present study is focused on the utilization of essential oil industry waste as a source of polyphenolic compounds. Five residues from industrially processed raw materials (2018 harvest): one of *Lavandula angustifolia, Melissa officinalis* and *Ocimum basilicum*, and two of *Rosa alba* were collected and used for preparation of polyphenol-rich extracts. The extracts were analyzed for their polyphenol content (total and individual compounds), antioxidant activity, neutral sugars and uronic acids. The investigations suggested that the wastes were a rich source of polyphenols (flavonoids and phenolic acids) and could be used as supplements for increasing antioxidant activities in various food systems.

Keywords: Polyphenols, antioxidant activity, essential oil industrial waste, waste valorization.

INTRODUCTION

The essential rose oil industry is a key manufacturing field in Bulgaria. Besides the most important crops, such as *Rosa Damascena* and *Lavandula angustifolia* there are many others plants which are industrially processed. Because of the relatively low concentration of aroma compounds and pigments in the fresh plants, large quantities of wastes remain after the extraction or distillation of the important biologically active substances. The most common procedures for eliminating these wastes include simply throwing them away or composting. However, the waste could also serve as initial material for extraction of valuable by-products, such as polysaccharides, dietary polyphenols, aroma substances, etc. The beneficial economic effect of this approach has been recently outlined at the 8th World Congress on Polyphenols Applications [1] by the 5-Stages Universal Recovery Strategy of biologically active substances from waste biomass. The results of the previous studies on other industrial wastes: *Rosa damascena* [2] and *Calendula officinalis* [3] suggested that they could be successfully utilized as a source of polyphenols. Besides, investigation of the effect of lavender waste on the quality and safety of breads showed the potential of essential oil residues as natural bio preservatives [4]. Hence, the aim of the present study was to evaluate the potential of several industrial wastes of essential oil industry as a source of dietary polyphenols.

MATERIALS AND METHODS

Materials

The *Rosa alba* waste (steam distilled, RA_SD) was provided by Enio Bonchev distillery (Tarnichene, Bulgaria, 2018). The *Rosa alba* waste (obtained after supercritical CO_2-extracted fresh flowers, RA_CO2) was obtained from EKOMAAT distillery (Mirkovo, Bulgaria, 2018). The *Lavandula angustifolia* waste (after steam distillation, L_SD), the *Melissa officinalis* waste (after steam distillation, M_SD) and the *Ocimum basilicum* waste (after steam distillation, B_SD) were obtained from Zelenikovo distillery (Zelenikovo, Brezovo region, Bulgaria, 2018).

After treatment the steam distilled wastes were cooled down, inspected for elimination of impurities and dried under vacuum at 50°C. The CO_2-extracted waste was removed from the extraction cylinder and checked for impurities. Both wastes were stored at -18 °C until further treatment. All the solvents used were of analytical grade and purchased from local distributors.

Methods

The 70% ethanolic extracts from wastes were

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obtained according to [5] with small modifications: The dry wastes were ground and sieved (0.5 mm). 300 g of the dry residues were treated with 2000 mL of 70% ethanol for 1 h at 60°C, then left for 24 h at room temperature at constant stirring. The mass was filtered through nylon cloth (250 mesh), and the insoluble residue was extracted with additional 1000 mL of 70 % ethanol at the same conditions. The total polyphenol content of ethanolic extracts was determined using the method described by Singleton and Rossi [6]. The antioxidant activity was measured by ORAC and HORAC assays as described in [7] and the values were expressed as μmol Trolox equivalents per liter (μmolTE/L) and as μmol gallic acid equivalents (μmol GAE/L) per liter, respectively. The DPPH and FRAP analyses were performed according to the procedure described by Slavov *et al.* [2] and the results were expressed as μmolTE/L.

The individual phenolics and flavonoids were analyzed on Agilent 1220 HPLC system (Agilent Technology, USA), equipped with binary pump and UV-Vis detector. Wavelength of λ= 280 nm was used. Separation was performed using Agilent TC-C18 column (5 μm, 4.6 mm × 250 mm) at 25ºC. Mobile phases consisted of 0.5 % acetic acid (A) and 100% acetonitrile (B) at a flow rate of 0.8 mL/min. The gradient conditions started with 14% B, between 6 and 30 min linearly increased to 25% B, then to 50% B at 40 min. The standard compounds (gallic acid, 3,4-dihydroxy benzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, catechin, epicatechin, rutin, naringin, myricetin, quercetin, naringenin and kaempherol) were purchased from Sigma-Aldrich (Steinheim, Germany).

The amounts of acetaldehyde, ethyl acetate, methanol and higher alcohols were determined by GC-FID analysis by injecting 1μL on Shimadzu GC-17A (Shimadzu, Japan) equipped with capillary column TEKNOKROMA TRB-WAX (30 m; ϕ 0.32 mm; 0.25 μm thickness) and software GC Solution (Shimadzu, Japan). The injector and detector temperatures were 229 °C and 250 °C, respectively, the carrier gas pressure and speed were 32 κPa and 1 mL/min, respectively. The column temperature regimen was: starting at 40 °C, hold for 1 min, then increase with 5 °C/min until 100 °C, hold for 10 min, and finally increase with 15 °C/min until 220 °C.

The individual volatile compounds in the ethanolic extracts were determined according to the following procedure: 1.0 ml ethanolic extract was treated with 1.0 ml of dichloromethane (triple). The combined organic layers were dried under vacuum at 30 °C. To the dry residue 100 μL of dichloromethane was added. For analysis 1.0 μL from the solution was injected on the gas chromatograph Agilent GC 7890 with mass-selective detector Agilent MD 5975 and column HP-5ms. The following temperature regimen was used – initial temperature was 40 °C and then increase to 300 °C with 5 °C/min (hold for 10 min); injector and detector temperatures – 250 °C, helium was used as carrier gas at 1.0 ml/min. The scanning range of the mass-selective detector was m/z = 40 – 400 in splitless mode. The individual compounds were identified comparing the retention times and the relative indices (RI) with those of standard substances and mass-spectral data from libraries of The Golm Metabolome Database (http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html) and NIST'08 (National Institute of Standards and Technology, USA).

Statistical analysis

The analyses were run three times, and the data were given as mean values. Statistical significance was detected by analysis of variance (ANOVA) and Tukey's test; value of $p<0.05$ indicated statistical difference).

RESULTS AND DISCUSSION

Obtaining of ethanolic extracts from wastes

Treatment of the wastes with aqueous-ethanolic solutions is usually applied before extraction of polysaccharides from the raw materials. It aims at removing some low-molecular substances (pigments, sugars, etc.) which will hamper the future extraction. In our case it also aimed at obtaining of extracts rich in polyphenolic substances. In previous experiments we have investigated the influence of the ethanol concentration on the extractability of polyphenols and the subsequent polysaccharide extractions [2]. Our findings showed that extraction with 70% ethanol solutions gave the optimum results for possibilities of combined valorization of the waste materials of *Rosa damascena* and for this reason we have decided the treatment of the five residues after extraction or steam distillation to be performed with 70 % ethanol. The extracts obtained were subjected to preliminary analysis for their phenolic substances, polar aroma metabolites and antioxidant activity.

Total phenolic substances, individual phenolic acids and flavonoids, and antioxidant activity of ethanolic extracts from the wastes

The extracts were subjected to analysis for polyphenol content in order to assess the potential of each waste. This information, confronting the results of the two residues from industrial processing of *Rosa alba*, could also reveal the influence and extractability of different substances with different methods for essential oil crops treatment [8]. The results from the analysis are presented in Table 1. The highest polyphenol content exhibited the extract from RA_CO2 residue followed by the melissa waste (M_SD). These results suggested that supercritical CO_2 selectively extracts mostly the essential oil but not the polyphenols which are mostly solubilized when steam distillation is applied as method of processing. Nevertheless, the results for polyphenols, as well as the results for antioxidant capacity suggested that the wastes had potential to be used as a source of dietary antioxidants [9]. In the next experiments the individual substances – flavonoids and phenolic acids, contributing to the observed high antioxidant capacity, were determined by liquid chromatography (Tables 2 and 3). The RA_CO2 waste had a higher flavonoid content. This is the only residue obtained after supercritical CO_2 extraction process included in the study and these results suggested that this kind of extraction did not solubilize more hydrophilic compounds such as flavonoids. They remain in the waste and could be recovered as extracts in further treatment. In the subsequent experiments the amounts of major phenolic acids in the extracts were determined (Table 3).

Confronting the results for the phenolic acids the L_SD and RA_SD were found to be rich source of neochlorogenic acid, 3,4-dihydroxy benzoic acid, ferulic acid and gallic acid. In the B_SD extract the predominant phenolic acid was rosmarinic acid. Furthermore, the extracts were subjected to GC-MS analysis for content of polar volatile metabolites (Table 4).

Table 1. Polyphenol content and antioxidant capacity of ethanolic extracts from wastes

Waste	Polyphenols, mg/L	ORAC, µmolTE/L	HORAC, µmolGAE/L	FRAP, µmolTE/L	DPPH, µmolTE/L
RA_SD	2588±155^{d}	49203.9±1989.7^{c}	**23279.8±893.6^{a}**	1636.7±47.9^{c}	1176.7±48.5^{c}
RA_CO2	**11061±799^{a}**	**209214.4±4749.3^{a}**	**22361.4±1782.8^{a}**	**6000.0±43.7^{a}**	**5833.3±37.2^{a}**
L_SD	1453±91^{e}	48750.0±1480.1^{d}	16879.9±972.6^{b}	659.2±47.8^{d}	356.3±32.6^{d}
M_SD	**6630±292^{b}**	**225041.2±13683.3^{a}**	**24507.5±1117.8^{a}**	3331.3±33.9^{b}	2668.8±47.6^{b}
B_SD	3229±160^{c}	99635.3±5735.2^{b}	12834.7±1276.4^{c}	1745.0±58.3^{c}	1206.7±57.2^{c}

TE – Trolox® equivalents; GAE – Gallic acid equivalents; The results are presented as mean values of three replicates ± SD; $^{a, b, c, d, e}$ – different letters in a column mean statistical difference (one-way ANOVA, Tukey's test; $p<0.05$).

Table 2. Flavonoids (mg/L) in ethanolic extracts obtained from wastes

	RA_SD	RA_CO2	L_SD	M_SD	B_SD
Quercetin	170.67±2.48^{b}	185.37±1.93^{a}	37.41±1.86^{d}	53.26±1.99^{c}	nd
Quercetin-3-β-glucoside	157.14±1.90^{b}	**755.58±3.15^{a}**	49.28±2.24^{d}	102.79±1.83^{c}	154.52±2.59^{b}
Rutin	159.18±2.70^{d}	**1223.76±2.99^{a}**	nd	186.42±3.07^{c}	258.58±3.15^{b}
Myricetin	14.37±1.11^{e}	**114.19±1.39^{a}**	23.18±1.47^{d}	57.19±1.26^{b}	34.71±1.68^{c}
Kaempferol	**65.37±1.82^{a}**	28.68±1.34^{c}	12.46±1.27^{d}	34.12±1.56^{b}	25.19±1.47^{c}
Naringenin	nd	nd	nd	62.33±1.68^{a}	20.52±1.09^{b}
Apigenin	nd	nd	nd	153.99±2.17^{a}	20.56±1.67^{b}
Catechin	84.39±2.08^{e}	**1104.09±3.64^{a}**	413.14±2.84^{c}	439.18±2.73^{b}	204.78±3.64^{d}
Epicatechin	66.18±1.94^{c}	nd	**316.71±1.84^{b}**	**367.96±2.31^{a}**	nd

nd – not determined; The results are presented as mean values of three replicates ± SD; $^{a, b, c, d, e}$ – different letters in a row mean statistical difference (one-way ANOVA, Tukey's test; $p<0.05$).

Table 3. Phenolic acids (mg/L) in ethanolic extracts obtained from wastes

	RA_SD	RA_CO2	L_SD	M_SD	B_SD
Chlorogenic acid	nd	nd	nd	49.19±1.64	nd
Neochlorogenic acid	**162.92±2.10^{b}**	57.21±1.38^{c}	**197.66±2.17^{a}**	47.61±1.96^{d}	54.55±2.34^{c}
3,4-dihydroxy benzoic acid	**171.87±2.17^{b}**	76.22±2.08^{c}	**637.96±2.68^{a}**	57.08±1.84^{d}	30.39±2.07^{e}
Caffeic acid	32.27±1.59^{b}	nd	nd	49.19±1.60^{a}	nd
p-Coumaric acid	**42.42±2.08^{a}**	8.08±1.14^{c}	nd	9.68±1.10^{c}	12.01±1.06^{b}
Ferulic acid	**88.96±1.82^{b}**	8.32±1.17^{e}	**194.76±1.36^{a}**	28.32±1.27^{c}	24.35±1.48^{d}
Gallic acid	**159.39±1.67^{b}**	98.62±1.39^{c}	**365.56±2.43^{a}**	35.49±2.08^{e}	69.71±2.34^{d}
Rosmarinic acid	126.48±2.08^{c}	10.93±1.07^{e}	**154.83±1.29^{b}**	69.42±1.37^{d}	**1232.26±2.03^{a}**
Cinnamic acid	26.09±1.27^{b}	nd	nd	nd	43.38±1.56^{a}

nd – not determined; The results are presented as mean value of three replicates ± SD; $^{a, b, c, d, e}$ – different letters in a row mean statistical difference (one-way ANOVA, Tukey's test; $p<0.05$).

Table 4. Volatile metabolites (expressed as % of total ion current (TIC) in the extracts

Compound	RI	RA_SD	RA_CO2	L_SD	M_SD	B_SD
α-Pinene	940	0.45±0.07^{b}	0.48±0.06^{b}	0.25±0.06^{b}	0.36±0.06^{b}	0.68±0.07^{a}
β-pinene	980	0.32±0.05^{c}	0.33±0.05^{c}	1.54±0.08^{a}	0.91±0.09^{b}	0.31±0.04^{c}
β-Myrcene	991	0.18±0.04^{b}	0.19±0.04^{b}	1.19±0.09^{a}	0.25±0.08^{b}	nd
p-Cymene	1019	nd	nd	0.54±0.07^{a}	nd	0.72±0.06^{a}
Limonene	1025	nd	nd	**3.55±0.15^{a}**	**1.84±0.10^{b}**	0.22±0.05^{c}
Eucalyptol	1031	nd	nd	3.18±0.16^{a}	nd	2.91±0.10^{a}
cis-β-Ocimene	1040	nd	nd	**5.41±0.21^{a}**	**2.23±0.20^{b}**	0.21±0.06^{c}
trans-β-Ocimene	1050	nd	nd	3.37±0.19^{a}	1.65±0.15^{b}	0.35±0.10^{c}
γ-Terpinene	1062	0.47±0.07^{a}	0.50±0.07^{a}	0.38±0.06^{a}	nd	0.41±0.07^{a}
cis-Linalool oxide	1073	nd	nd	0.19±0.05^{a}	nd	0.16±0.04^{a}
trans-Linalool oxide	1078	nd	nd	0.29±0.05^{a}	nd	0.25±0.04^{a}
Terpinolene	1087	0.35±0.04^{a}	0.37±0.06^{a}	nd	nd	0.18±0.03^{b}
β-Linalool	1097	0.89±0.10^{c}	0.94±0.09^{c}	**18.91±0.15^{a}**	0.97±0.11^{c}	**5.92±0.12^{b}**
Phenethyl alcohol	1110	**8.70±0.18^{b}**	**11.10±0.16^{a}**	nd	nd	nd
cis-Rose oxide	1112	0.22±0.04^{a}	0.23±0.04^{a}	nd	0.33±0.05^{a}	nd
trans-Rose oxide	1127	0.15±0.04^{b}	0.16±0.04^{b}	nd	0.46±0.05^{a}	nd
Verbenol	1134	nd	nd	nd	0.33±0.06	nd
Camphor	1146	nd	nd	0.48±0.07	nd	nd
Citronellal	1151	nd	nd	nd	2.97±0.10	nd
Borneol	1169	nd	nd	0.58±0.10	nd	nd
Lavandulol	1171	nd	nd	**6.12±0.21**	nd	nd
Menthol	1173	nd	nd	nd	0.92±0.11	nd
Terpin-4-ol	1178	0.27±0.04^{c}	0.29±0.05^{c}	**3.10±0.11^{a}**	nd	0.77±0.09^{b}

Isomenthol	1180	nd	nd	nd	0.61±0.06	nd
Methyl chavicol	1182	nd	nd	nd	nd	2.79±0.10
α-Terpineol	1189	0.55±0.06^{c}	0.62±0.05^{b,c}	**3.13±0.09^{a}**	0.42±0.05^{c}	0.68±0.06^{b}
trans-Carveol	1195	nd	nd	nd	0.38±0.05^{a}	0.24±0.04^{a}
Myrtenol	1198	nd	nd	nd	1.33±0.09	nd
β-Citronellol	1228	**18.62±0.15^{b}**	**20.25±0.21^{a}**	nd	nd	nd
Nerol	1230	**3.81±0.11^{b}**	**4.02±0.12^{a}**	nd	2.65±0.12^{c}	nd
Neral	1240	nd	nd	nd	**15.78±0.21**	nd
Geraniol	1255	**8.51±0.14^{b}**	**10.37±0.16^{a}**	0.28±0.10^{d}	**3.43±0.09^{c}**	nd
Geranial	1270	nd	nd	nd	**18.11±0.23**	nd
Linalyl acetate, dihydro-	1275	nd	nd	**18.14±0.16**	nd	nd
(±)-Lavandulyl acetate	1290	nd	nd	**4.93±0.13**	nd	nd
Citronellyl acetate	1354	0.38±0.04^{a}	0.40±0.04^{a}	nd	0.42±0.05^{a}	0.15±0.03^{b}
Eugenol	1356	0.26±0.04^{a}	0.27±0.05^{a}	nd	nd	0.36±0.05^{a}
Neryl acetate	1364	0.55±0.09^{c}	0.58±0.08^{c}	0.95±0.08^{b}	**3.00±0.10^{a}**	nd
Geranyl acetate	1383	**4.51±0.15^{a}**	**3.68±0.13^{b}**	**2.94±0.11^{c}**	**4.42±0.16^{a}**	nd
Methyl eugenol	1401	nd	nd	nd	nd	0.61±0.03
β-Bourbonene	1383	0.28±0.07^{a}	0.30±0.08^{a}	0.20±0.09^{a}	nd	nd
β-Cubebene	1389	0.28±0.10^{a}	0.29±0.07^{a}	nd	nd	0.26±0.05^{a}
β-Elemene	1390	0.17±0.04^{b}	0.18±0.05^{b}	nd	nd	0.41±0.06^{a}
β-Caryophyllene	1419	2.66±0.10^{d}	**5.98±0.15^{c}**	**7.20±0.18^{b}**	**18.03±0.16^{a}**	2.07±0.16^{d}
α-Humulene (α-Caryophyllene)	1454	2.36±0.08^{b}	2.50±0.11^{b}	**5.06±0.12^{a}**	0.59±0.10^{c}	0.56±0.08^{c}
Germacrene D	1479	1.64±0.08^{c}	0.68±0.07^{d}	2.76±0.09^{b}	**4.33±0.11^{a}**	0.21±0.06^{e}
α-Farnesene	1508	0.63±0.05^{a}	0.66±0.05^{a}	0.27±0.04^{b}	nd	0.59±0.03^{a}
β-Bisabolene	1510	0.20±0.03^{a}	0.22±0.03^{a}	0.20±0.03^{a}	nd	nd
***trans*-Nerolidol**	1564	**2.90±0.12^{a}**	**3.06±0.15^{a}**	0.27±0.08^{b}	nd	nd
Spathulenol	1575	1.82±0.09^{a}	1.92±0.09^{a}	0.19±0.07^{b}	nd	nd
Caryophyllene oxide	1580	0.36±0.04^{c}	0.38±0.04^{c}	0.30±0.04^{c}	1.14±0.08^{a}	0.62±0.07^{b}
tau-Cadinol	1627	nd	nd	nd	1.16±0.09^{a}	0.31±0.06^{b}
tau-Muurolol	1629	nd	nd	nd	1.88±0.11^{a}	0.48±0.09^{b}
γ-Eudesmol	1631	0.33±0.04^{b}	0.35±0.04^{b}	0.42±0.05^{b}	1.17±0.08^{a}	nd
β-Eudesmol	1649	0.28±0.04^{b}	0.30±0.04^{b}	0.22±0.03^{b}	1.02±0.06^{a}	nd
α-Eudesmol	1651	0.99±0.06^{a}	1.04±0.06^{a}	0.34±0.05^{b}	nd	nd
α-Cadinol	1653	nd	nd	nd	**4.24±0.16^{a}**	1.64±0.10^{b}
Farnesol	1714	**4.37±0.09^{b}**	**4.62±0.11^{a}**	0.55±0.07^{d}	nd	1.51±0.08^{c}
n-Nonadecane	1901	0.14±0.06^{b}	0.15±0.05^{b}	0.17±0.04^{b}	0.81±0.07^{a}	0.28±0.06^{b}
n-Eicosane	2000	0.31±0.06^{b}	0.32±0.08^{b}	0.32±0.08^{b}	0.11±0.02^{c}	0.62±0.04^{a}

n-Heneicosane	2100	**12.80±0.16^{a}**	**7.10±0.21^{b}**	0.34±0.08^{d}	0.23±0.06^{d}	3.11±0.05^{c}
n-Docosane	2200	0.83±0.09^{b}	0.89±0.06^{b}	nd	nd	2.34±0.04^{a}
n-Tricosane	2300	**7.98±0.18^{a}**	**5.27±0.29^{b}**	nd	nd	2.10±0.16^{c}
n-Tetracosane	2400	3.15±0.12^{a}	2.87±0.13^{b}	nd	nd	1.31±0.10^{c}
n-Pentacosane	2500	1.95±0.16^{a}	2.06±0.10^{a}	nd	nd	1.63±0.09^{b}
n-Hexacosane	2600	2.69±0.14^{a}	2.84±0.17^{a}	nd	nd	1.84±0.13^{b}

nd – not determined; The results are presented as mean value of three replicates ± SD; $^{a, b, c, d}$ – different letters in a row mean statistical difference (one-way ANOVA, Tukey's test; $p<0.05$).

It is not surprising that the rose residues were rich in β-phenethyl alcohol, β-citronellol, geraniol and geranyl acetate. These compounds give the pleasant rose-like aroma of the extracts. The lavender waste was rich in β-linalool, linalyl acetate, lavandulol and lavandulyl acetate. The mellisa waste was among the richest in aroma metabolites waste – β-citral/neral, α-citral/ geranial, geranyl acetate, β-caryophyllene and germacrene D.

CONCLUSIONS

The results from the present study suggested that the wastes from the essential oil industry are rich in polyphenol and aroma substances. To the best of our knowledge, for the first time industrial wastes from *Rosa alba* (obtained from two different processing techniques) and *Ocimum basilicum* were investigated for their polyphenol and aroma substances. The obtained polyphenol- rich extracts could be purified and serve as source of dietary polyphenolic compounds. Besides, the extracts could additionally serve as a new type of aromatizing agents. The high amounts of polyphenolic compounds, known for their antimicrobial activity, also suggested that the wastes could successfully be used in the food industry as natural bio preservatives.

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