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Utilization of essential oil industry chamomile wastes as a source of polyphenols

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The annual global production of chamomile (*Matricaria Chamomila*) essential oil was roughly estimated between 80 and 100 tonnes. Due to the low content of essential oil in the chamomile (around 0.30-0.45 % on dry basis) large quantities of wastes are generated every year by the essential oil industry. The aim of the present work was to investigate the possibility for valorization of chamomile waste. Three wastes were investigated – after extraction of chamomile with 1,1,1,2-tetrafluoroethane (F), after hydrodistillation (HD) and after steam distillation (S). It was found that extraction of the raw materials by fluorocarbons preserves to a large extent the polyphenols – F wastes had 2697.81±84.90 mg/L total polyphenols compared to 1500.30±36.22 mg/L and 366.24±26.37 mg/L for S and HD wastes, respectively. Similar trend was observed for antioxidant activity results: F wastes showed 31235.42±238.21 μmol TE/L (by ORAC method) and 12235.23±267.68 μmol GAE/L (by HORAC method) while S showed 2 times (by ORAC method) and 3 times (by HORAC method) lower values. The individual phenolic acids and flavonoids were determined by HPLC and the main compounds found were *p*-coumaric acid, chlorogenic acid, catechin, quercetin, and naringin. The polar metabolites and aroma compounds in the ethanolic extracts were investigated by GC-MS. The results suggested that the chamomile waste could be a valuable and cheap source for obtaining of by-products with pronounced antioxidant activity.

Keywords: *Matricaria Chamomila* (chamomile), waste valorization, antioxidant activity, polyphenols.

INTRODUCTION

Valorization of biodegradable agricultural and food wastes became a priority in the last years. Novel promising and uninvestigated waste materials are residues from the essential oils industry. Such underexplored waste is obtained from *Matricaria Chamomila* (chamomile) which is the fifth top-selling herb / essential oil plant with wide application in cosmetics, foods, aromatherapy, and as pharmaceutical additive [1]. Among the main biologically active substances giving the chamomile its beneficial effects are terpenoids (α-bisabolol and its oxides, chamazulene, farnesene, etc.), lactones, glycosides ((Z)- and (E)-2-β-D-glucopyranosyloxy-4-methoxy cinnamic acids), phenolic compounds – apigenin, quercetin, luteolin, etc. [2].

The annual global production of chamomile essential oil was roughly estimated between 80 and 100 tonnes [3]. Industrially the main approach for obtaining of chamomile essential oils is by steam distillation. The hydrodistillation process is an alternative method which involves covering the plant tissues with water during the distillation and hence some part of the polyphenols could be extracted. In the last years supercritical CO_2 extraction also became an alternative approach having the advantage to operate at lower temperatures but the initial investments are much higher compared, for example, to steam distillation facilities [4, 5]. Treatment of essential oil crops with fluorocarbons resembles to a large extent CO_2 extraction although the pressure applied is lower. The solvent has the ability to penetrate in the cells and disrupts the plant cell walls, which facilitates further extraction of biologically active substances from the waste material.

Due to the low content of essential oil in the chamomile plants – around 0.30-0.45 % on dry basis [6] large quantities of wastes are generated every year. Approaches and methods for valorization of chamomile residues in the scientific literature are relatively scarce [7, 8]. Recently it was demonstrated that the waste obtained after steam distillation of chamomile could be a source of water-soluble pectic polysaccharides [9].

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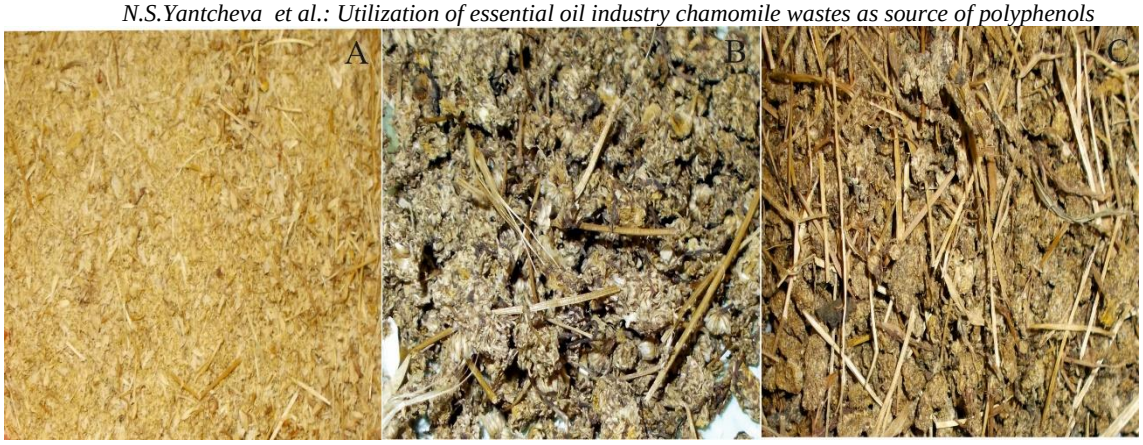

Figure 1. Chamomile wastes investigated: A. after extraction with 1,1,1,2-tetrafluoroethane (F); B. after hydrodistillation (HD); C. after steam distillation (S)

Nevertheless, information about polyphenol content, antioxidant activity and aroma substances in the chamomile waste could not be found in the literature, to the best of our knowledge.

This observation gave ground to the purpose of this study: to investigate chamomile wastes as a source of polyphenol-rich extracts, as a novel approach for valorization of chamomile waste biomass. Three residues were examined: generated by industrial steam distillation (S), by hydrodistillation (HD) and by extraction with 1,1,1,2-tetrafluoroethane (F) of chamomile flowers (Fig. 1).

EXPERIMENTAL

Waste materials

The chamomile wastes (*Matricaria chamomilla*) – after steam distillation and hydrodistillation (water distillation), were obtained from Strelcha distillery, (Strelcha, region of Plovdiv, Bulgaria, 2016 harvest). The chamomile wastes obtained after extraction with 1,1,1,2-tetrafluoroethane [10] of the fresh *Matricaria chamomilla* flowers were provided from Zelenikovo distillery (region of Plovdiv, Bulgaria, 2016 harvest). The wastes were inspected for impurities, dried at 50°C under vacuum, and kept at -18°C.

Extraction with 70% ethanol

Prior to extraction the dry wastes were ground and sieved (0.5 mm). 100 g of dry chamomile residues were treated with 500 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 and the insoluble residue was extracted with additional 500 mL of 70 % ethanol at the same conditions.

Analytical methods

The total polyphenol content of ethanolic extracts was determined as described by Singleton and Rossi [11]. The antioxidant activity by Oxygen Radical Absorbance Capacity (ORAC) and Hydroxyl Radical Averting Capacity (HORAC) assays was measured according to Číž *et al*. [12]. The results of ORAC analysis were expressed as μmol Trolox® equivalents per liter extract (μmol TE/L) and the results of HORAC: as μmol gallic acid equivalents per liter extract (μmol GAE/L).

The content of individual phenolic and flavonoid components was analyzed on an Agilent 1220 HPLC system (Agilent Technology, USA), equipped with binary pump and UV-Vis detector. Detection was performed at a wavelength of λ= 280 nm. Separation was carried out on an 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 min and 30 min linearly increased to 25% B, then to 50% B for 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 from Sigma-Aldrich (Steinheim, Germany).

The individual volatile (aroma) and non-volatile polar compounds in the ethanolic extracts were determined as described by Yantcheva *et al*. [13].

Statistical analysis

All analyses were triplicated and the results were presented as mean values. Statistical differences were detected by analysis of variance (ANOVA, Tukey's test) and a value of $p < 0.05$ indicated statistical significance.

RESULTS AND DISCUSSION

The three waste materials: 1) obtained after extraction of chamomile with 1,1,1,2-tetrafluoroethane (F); 2) obtained after hydrodistillation of chamomile (HD) and 3) obtained after steam distillation of chamomile (S), were subjected to extraction with 70% ethanol and ethanol extracts and alcohol insoluble residues (AIR) were obtained. In a previous study [14] the influence of the ethanol concentration on the extractability of polyphenols and polysaccharides from waste rose petals was investigated and it was found that the 70% ethanol extraction gave the best results. For this reason, in the present study extraction with 70% ethanol solution was chosen. Besides, this procedure allows the AIRs to be further used as a source of pectic polysaccharides with minimum losses during ethanol pretreatment [14]. The extracts obtained were subjected to preliminary analysis for their total phenolic substances and antioxidant activity. The results from the analysis are shown in Table 1.

The results from the preliminary experiments suggested that steam distillation led to significant reduction of polyphenols due to their partial extraction compared with 1,1,1,2-tetrafluoroethane (freon) extraction. The hydrodistillation includes utilization of more water during the process and hence the extraction of polyphenols from the material was much more pronounced. Extraction with halocarbons (freons) preserves to a large extent the polyphenolic substances: their amount is almost two and seven times higher than S and HD wastes, respectively. The higher is the concentration of polyphenols, the higher is the antioxidant activity measured by ORAC and HORAC methods, and the highest results were observed for the F waste: 31235.42±238.21 TE/L (by ORAC method) and 12235.23±267.68 GAE/L (by HORAC method). It seems also that the extraction with 1,1,1,2-tetrafluoroethane led to better disintegration of the cell walls of the plant materials, which is beneficial for the further extractions of biologically active substances. In this regard extraction of the plant materials by halocarbons resembles the extraction with supercritical CO_2 although the state of the extractant used is above its critical point.

Furthermore, we investigated the content of phenolic acids and major flavonoids of the 70 % ethanolic extracts and the results are presented in Table 2.

Table 1. Polyphenols and antioxidant activity of 70 % ethanol extracts of wastes

Waste	Total phenolics, mg/L	ORAC, μmol TE/L	HORAC, μmol GAE/L
F	2697.81±84.90 [a]	31235.42±238.21 [a]	12235.23±267.68 [a]
S	1500.30±36.22 [b]	14985.66±196.85 [b]	4562.32±201.56 [b]
HD	366.24±26.37 [c]	3982.25±168.82 [c]	1142.39±157.84 [c]

[a, b, c] – different letters signify statistical significance.

Table 2. Phenolic acids and flavonoids in 70% ethanolic extracts

Phenolic acids, mg/100 mL	F	S	HD
Chlorogenic acid	21.62±0.42 [a]	17.60±1.39 [b]	2.79±0.65 [c]
Neochlorogenic acid	8.37±0.36 [a]	-	1.48±0.12 [c]
Vanillic acid	1.55±0.11 [a]	1.23±0.10 [a]	-
Caffeic acid	1.58±0.14 [a]	0.59±0.09 [b]	-
p-Coumaric acid	21.42±1.65 [a]	6.47±1.02 [b]	4.85±0.89 [b]
Ellagic acid	2.76±0.45 [a]	1.42±0.12 [b]	1.28±0.08 [b]
Cinnamic acid	2.88±0.68 [a]	1.17±0.54 [b]	0.70±0.08 [b]
Gallic acid	0.88±0.03 [a]	1.28±0.05 [b]	0.37±0.04 [c]
TOTAL, mg/100 mL	61.06±1.85 [a]	29.76±1.45 [b]	11.47±1.11 [c]
Flavonoids, mg/100 mL			
Quercetin	24.26±1.25 [a]	27.25±1.85 [a]	13.47±1.42 [b]
Quercetin-3-β-glucoside	29.22±1.37 [a]	17.07±1.14 [b]	5.83±1.08 [c]
Myricetin	16.47±0.98 [a]	5.66±0.87 [b]	8.83±0.99 [c]
Kaempferol	1.24±0.08 [a]	1.82±0.10 [b]	1.23±0.05 [a]
Naringin	28.56±1.06 [a]	8.64±0.95 [b]	9.86±0.96 [b]
Naringenin	3.29±0.58 [a]	3.44±0.65 [a]	3.47±0.44 [a]
Catechin	21.78±1.14 [a]	46.82±1.62 [b]	11.13±1.17 [c]
Epicatechin	-	-	17.74±1.26
TOTAL, mg/100 mL	124.82±1.52 [a]	110.70±1.96 [b]	71.56±1.75 [c]

[a, b, c] – different letters signify statistical significance.

The highest contents of total phenolic acids were observed for the F waste: 61.06±1.85 mg/100 mL extract and the main representative was *p*-coumaric acid. *p*-Coumaric acid was also present in significant amounts in the S and HD wastes but the total amounts of phenolic acids were considerably lower: 29.76±1.45 and 11.47±1.11 mg/100 mL for S and HD, respectively, compared to F. Chlorogenic acid, a compound whit pronounced antioxidant activity [15] was the most abundant one in the S waste. Other phenolic acids contributing to the higher antioxidant activity of the ethanolic extract from chamomile treated with halocarbons were neochlorogenic and chlorogenic acids. Comparing the total amount of flavonoids the same trend could be confirmed: the highest amounts were found in the F waste (124.82±1.52 mg/100 mL) while S and HD had 110.70±1.96 and 71.56±1.75 mg/100 mL, respectively. The most abundant flavonoids were quercetin-3-β-glucoside and quercetin, naringin, and catechin. These results suggested that the halocarbon extraction of plant material extracted mainly the essential oils and some non-polar metabolites but significant part of the phenolic compounds was preserved in the waste and it could be a promising source of polyphenols.

In the next analysis the non-volatile polar compounds present in the extracts were determined by GC-MS and the results are presented in Table 3.

Table 3. Polar non-volatile substances in ethanolic extracts. RI - relative index (Kovats retention index); % of TIC - total ion current.

Compound	RI	F	S	HD
		% of TIC		
L-Valine	1228	0.98±0.05 [a]	0.77±0.04 [b]	0.33±0.05 [c]
Glycerol	1266	4.13±0.21 [a]	4.31±0.31 [a]	4.52±0.28 [a]
L-Leucine	1272	0.20±0.01 [a]	0.35±0.05 [b]	0.05±0.01 [c]
L-Isoleucine	1299	0.69±0.07 [a]	0.56±0.02 [b]	0.09±0.01 [c]
L-Proline	1307	3.28±0.54 [a]	6.65±0.41 [b]	0.15±0.10 [c]
Succinic acid	1310	0.26±0.04 [a]	0.38±0.03 [b]	1.47±0.09 [c]
Fumaric acid	1355	0.40±0.02 [a]	0.47±0.03 [a]	0.87±0.05 [b]
Serine	1362	0.84±0.05 [a]	1.20±0.08 [b]	0.04±0.01 [c]
L-Threonine	1390	0.49±0.04 [a]	0.39±0.03 [b]	0.05±0.01 [c]
L-Homoserine	1446	0.14±0.02 [a]	0.11±0.01 [a]	0.05±0.01 [c]
Malic acid	1488	2.23±0.11 [a]	1.74±0.14 [b]	2.26±0.19 [a]
Salycilic acid	1516	0.16±0.01 [a]	0.05±0.01 [b]	0.09±0.01 [c]
L-Aspartic acid	1531	0.26±0.05 [a]	2.15±0.09 [b]	0.47±0.06 [c]
L-Threonic acid	1528	0.32±0.04 [a]	0.76±0.06 [b]	0.14±0.02 [c]
L-Phenylalanine	1646	0.55±0.09 [a]	0.63±0.08 [a]	0.19±0.04 [b]
L-Asparagine	1682	0.22±0.04 [a]	0.44±0.03 [b]	0.87±0.05 [c]
L-Lysine	1737	0.45±0.01 [a]	0.42±0.02 [a]	0.41±0.02 [a]
Vanillic acid	1758	0.18±0.01 [a]	0.28±0.01 [b]	0.15±0.01 [a]
Protocatechuic acid	1813	0.19±0.02 [a]	0.16±0.02 [a]	0.41±0.03 [b]
Quinic acid	1843	0.34±0.02 [a]	0.22±0.02 [b]	1.59±0.06 [c]
Syringic acid	1888	0.20±0.01 [a]	0.13±0.01 [b]	0.43±0.03 [c]
Gluconic acid	1991	1.40±0.21 [a]	1.68±0.12 [a]	1.58±0.15 [a]
Palmitic acid	2039	2.22±0.24 [a]	6.49±0.32 [b]	7.10±0.28 [b]
Glucaric acid	2013	0.68±0.09 [a]	1.65±0.08 [b]	1.55±0.10 [b]
Myo-Inositol	2090	0.68±0.07 [a]	0.42±0.04 [b]	0.39±0.04 [b]
Stearic acid	2132	0.20±0.01 [a]	0.12±0.01 [b]	0.89±0.02 [c]
Caffeic acid	2140	0.83±0.03 [a]	0.54±0.04 [b]	0.51±0.02 [b]
Linoleic acid	2209	1.60±0.24 [a]	6.53±0.32 [b]	8.14±0.38 [c]
α-Linolenic acid	2217	1.10±0.10 [a]	5.13±0.19 [b]	6.82±0.25 [c]
Stigmasterol	3315	1.27±0.09 [a]	0.95±0.08 [a]	1.89±0.12 [b]
β-Sitosterol	3355	1.17±0.14 [a]	1.35±0.09 [a]	1.66±0.12 [b]

[a, b, c] – different letters signify statistical significance.

Table 4. Aroma (volatile) substances in ethanolic extracts. RI - relative index (Kovats retention index); % of TIC - total ion current

Compound	RI	F	S	HD
		% of TIC		
β-Cubebene	1391	0.19±0.01 [a]	1.89±0.08 [b]	1.11±0.12 [c]
(Z)-β-Farnesene	1442	0.11±0.01 [a]	2.38±0.12 [b]	1.97±0.20 [b]
Decanoic acid	1449	2.22±0.11 [a]	1.40±0.14 [b]	4.68±0.32 [c]
(-)-Spathulenol	1578	0.14±0.02 [a]	1.24±0.15 [b]	1.15±0.14 [b]
Lauric acid	1622	2.09±0.13 [a]	1.27±0.15 [b]	1.53±0.18 [b]
α-Cadinol	1641	2.06±0.12 [a]	1.94±0.21 [a]	2.56±0.18 [b]
α-Bisabolol oxide B	1648	0.10±0.01 [a]	7.95±0.32 [b]	10.05±0.29 [c]
7-Methoxycoumarin	1737	0.08±0.01 [a]	10.23±0.62 [b]	3.57±0.52 [c]
α-Bisabolol oxide A	1792	0.10±0.01 [a]	8.11±0.41 [b]	10.29±0.55 [c]
7-Hydroxycoumarin	1813	0.09±0.01 [a]	0.65±0.05 [b]	2.45±0.15 [c]
Myristic acid	1839	10.72±0.27 [a]	7.94±0.36 [b]	5.08±0.44 [c]
(2E)-2-(2,4-Hexadiynylidene)-1,6-dioxaspiro[4.4]non-3-ene	1892	0.82±0.12 [a]	8.03±0.21 [b]	11.11±0.33 [c]
Phytol	2164	1.29±0.15 [a]	2.23±0.20 [b]	4.50±0.26 [c]

[a, b, c] – different letters signify statistical significance.

The results from the analysis suggested that even after extraction / distillation a high amount of linoleic and linolenic acids (which also includes amounts of calendic acid – a conjugated linoleic acid) [16] remain in the S and HD wastes.

Due to the nature of the halocarbon extraction process the less polar compounds were predominantly extracted and for this reason the amounts of linoleic and linolenic acids in the F residue were 4 to 6 times lower compared to S and HD residues. The extract from F waste was rich in malic, quinic, caffeic and syringic acid. The industrial essential oil processes (distillation or extraction) always left part of the aroma (volatile) and polar non-volatile substances in the residual materials [13, 14]. This is due to the nature of the raw materials, to the chemical bonding of the compounds in the plant matrix or to technological reasons. Although present in low amounts, a great number of these substances possess beneficial effects: antimicrobial, antioxidant, anti-inflammatory, etc. [1, 2]. For this reason, in the next experiments aroma (volatile) metabolites kept in the chamomile wastes were determined (Table 4).

The information derived from these analyses allowed comparing the extractability of different classes of biologically active substances, and advantages and disadvantages of the different techniques for essential oil production – steam distillation, hydrodistillation and extraction with halocarbons (freons). Considering the less polar terpenes (aroma compounds) it could be concluded that the freon extraction extract to a higher extent the non-polar substances. For this reason, β-cubebene, (Z)-β-farnesene, (-)-spathulenol, α-bisabolol oxide A and B were present in much higher concentrations in S and HD residues. From this point of view, having in mind the anti-irritant, anti-inflammatory, and anti-microbial properties of these substances, the S and HD wastes could be also used as sources of natural healing and bio-preservative substances [2, 17].

CONCLUSIONS

The present study focused on the investigation of the potential application of chamomile wastes as a source of biologically active substances – dietary polyphenols, volatile (aroma) and non-volatile polar metabolites. The 70% ethanolic extracts obtained from three wastes: generated by industrial steam distillation (S), hydrodistillation (HD) and extraction with 1,1,1,2-tetrafluoroethane (F), showed pronounced antioxidant effects and also were rich in phenolic acids and flavonoids. To a larger extent the extraction with non-polar solvents (halocarbons) led to significant preservation in the wastes of valuable biologically active substances (phenolic acids, flavonoids, etc.). Steam distillation and even more hydrodistillation, due to the formation of water phase in the distillation still, led to extraction and loss of some of the more polar substances but preserved to a higher degree the non-polar terpene compounds. The investigated approach of treatment of the wastes with 70% ethanol and obtaining of valuable by-products also have the advantage of possible combining with further extraction of the plant residues in order to

obtain polysaccharides and thus will lead to a better valorization of the wastes.

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