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Evaluation of bioactive compounds and antioxidant activity of ten seeds residue from oil processing

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INTRODUCTION & AIM

Currently, the linear economy model is the predominant scenario worldwide. this model is based on "produce, use and throw away". The ecological and economic waste disposal problems that have affected society in recent years are pushing towards the adoption of alternative models based on the circular economy. A waste production system is reduced to a minimum, thanks to the recycling and reuse of residues to produce new products. In this way, waste is converted into resources and this is particularly interesting in terms of sustainability [1]. Recent studies highlight that food industries wastes are often rich in bioactive compounds that can be recovered and used again in a different market, always boosting the food economy thanks to their residual potential [2]. In this study we have investigated the fatty acids profile and total phenols content (TPC) of extracts obtained from exhausted matrix of oil extraction from *Cannabis sativa* (hemp), *Juglans regia* (nuts), *Prunus dulcis* (almonds), *Coriandrum sativum* (coriander), *Triticum aestivum* (wheat germ), *Nigella sativa* (cumin), *Avena sativa* (avena), *Glycine max* (soy), *Pimpinella anisum* (anise), and *Pinus pinea* (pine-nut) [2]. The antioxidant potential was also investigated using a multitarget approach (ABTS, DPPH, and β-carotene bleaching test) [2].


Hemp


Nuts


Almonds


Coriander


Wheat germ


Cumin


Avena


Soy


Anise


Pine nuts

METHODS

Samples and extraction procedures

Exhausted matrix of oil (250 g) were subjected to extraction procedure using *n*-hexane and ethanol as solvent (200 mL) (48h x 3 times).

Gas-chromatography mass spectrometry analysis of n-hexane extracts

In order to determine *n*-hexane extracts composition, GC system (Hewlett-Packard Co., model 6890 N) coupled to a selective mass detector (Hewlett Packard 5973 N) was used. Electron impact ionization was carried out at energy of 70 eV. Injector and detector were maintained at 250 °C and 280 °C, respectively. The analytical conditions were: oven temperature 5 min isothermal at 50 °C, 50-250 °C at a rate of 5 °C/min, then held isothermal for 10 min. Constituents were identified by comparison of their GC retention indices with those of the literature or with those of standards [2].

Total Polyphenols Content (TPC) Ethanolic extracts TPC was assessed using Folin-Ciocalteu [3]. Briefly, extract was mixed with Folin-Ciocalteu reagent (0.2 mL), sodium carbonate 15% (1 mL), and water (2 mL). After incubation at 25 °C for 2 h, the absorbance was read at 765 nm. Result was expressed as milligrams of chlorogenic acid equivalents (CAE)/g of extract.

Antioxidant Activity

The antioxidant potential was assessed by using different methods. In 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, DPPH radical solution was mixed with extracts diluted in methanol at concentration ranging from 1 to 1000 μg/mL. After 30 min, the absorbance was read (517 nm). For 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, a solution of $ABTS^{\cdot+}$ radical (with an absorbance of 0.70 at 734 nm) was made and then extracts, diluted in methanol at different concentration (1-400 μg/mL), were added and left to react (25°C, 6 min). The absorbance was measured at 734 nm. Ascorbic acid was employed as positive control in both radical scavenging tests [4]. In β-carotene bleaching test, a β-carotene solution, linoleic acid, 100% Tween 20 and extracts diluted in methanol at different concentrations (2.5-100 μg/mL) were mixed. The absorbance was measured at 470 nm. Propyl gallate was used as positive control.

Statistical Analysis

Experiments were performed in triplicate. Prism GraphPad Prism Software (San Diego, CA, USA) was used to calculate the concentration causing 50% inhibition (IC_{50}). Data were analyzed by One-way analysis of variance (ANOVA) and significant differences were calculated according to Tukey's multiple range tests. The global antioxidant score (GAS) was calculated as previously described [5].

RESULTS & DISCUSSION

Gas-chromatography-mass spectrometry analysis revealed that walnut's *n*-hexane extract exhibited the highest linoleic acid content (87.13%) followed by hemp sample (51.2%). Cumin h-hexane extract showed an high oleic acid content with percentage of 40.4%.

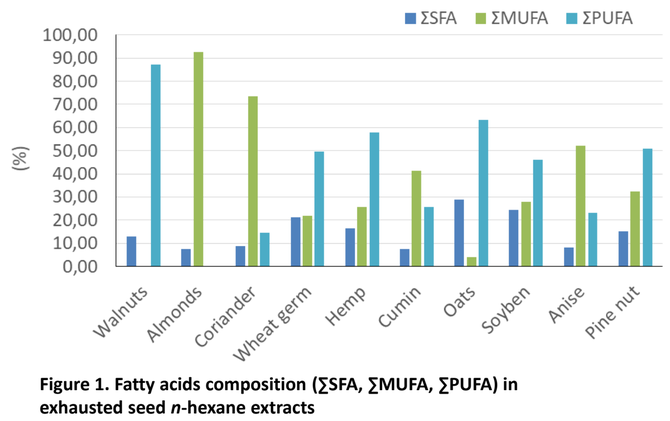

Figure 1. Fatty acids composition (∑SFA, ∑MUFA, ∑PUFA) in exhausted seed *n*-hexane extracts

The comparison of the sum of PUFA, MUFA and SFA highlights that extract deriving from exhausted walnuts has the highest PUFA content (87.1%) followed by oats (63.2%) (Figure 1). Almond sample showed the highest ∑MUFA with percentage of 92.5 followed by coriander (73.6%). Oats, soyben and wheat germ are particularly rich in SFA with percentage of 28.8, 24.4 and 21.3%, respectively.

Anise by-products ethanol extract was richest in TPC (35.8 mg gallic acid equivalent GAE/g extract).
A promising TPC was observed also in the extract of the cumin oil extraction residue with a value of 28.4 mg GAE/g extract.
The other samples exhibited a TPC in the range from 1.9 to 19.4 for *Glycine max* and *Avena sativa*, respectively (Figure 2).

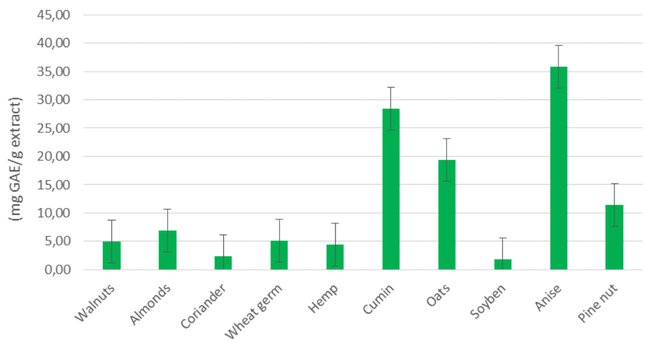

Figure 2. Total phenols content (TPC) in exhausted seed ethanol extracts

Almond's seed exhausted matrix extract exhibited the highest radical scavenging activity against $ABTS^{+\cdot}$ with IC_{50} value of 2.8 μg/mL followed by hemp extract (IC_{50} of 18.8 μg/mL) whereas anise showed the highest radical scavenging potential in DPPH test with IC_{50} of 27.2 μg/mL. A promising DPPH radical scavenging effect was observed , also, with almonds and pine nut samples (IC_{50} values of 28.9 and 29.1 μg/mL, respectively). The most promising protection from lipid peroxidation was obtained with anise ethanolic extract with IC_{50} of 14.1 μg/mL after 30 min of incubation.
The global antioxidant score (GAS) revealed that among the tested samples almonds exhibited the highest antioxidant activity followed by pine nuts. Collectively our results confirmed that the oil industry's waste can still be a source of useful compounds to be reintroduced into the industrial cycle.

Table 1. Antioxidant activity of exhausted seed ethanol extracts

Samples	DPPH	ABTS	β-Carotene bleaching test	
			30 min incubation	60 min incubation
Walnuts	46.5±3.4^{c}	NA	NA	NA
Almonds	28.9 ±2.3^{a}	2.8±0.0^{a}	45.7±3.8^{b}	48.6±4.7
Coriander	56.5±3.9^{d}	38.8±3.1^{d}	45.7±3.1^{b}	48.9±4.9
Wheat germ	56.9 ±3.8^{d}	52.16±4.3^{e}	47.3±3.4^{b}	44.6±4.1
Hemp	37.5±2.7^{b}	18.8±2.2^{b}	NA	NA
Cumin	32.2±2.6^{b}	31.0±3.2^{d}	43.5±3.0^{b}	47.4±4.3
Oats	28.6±2.7^{a}	114.2±9.8^{g}	NA	NA
Soyben	36.7±2.5^{b}	115.9±10.2^{h}	NA	NA
Anise	27.2±2.0^{a}	25.3±2.9^{c}	14.1±1.6^{a}	48.9±4.5
Pine nut	29.1±2.7^{a}	81.5±6.5^{f}	49.0±5.3^{c}	48.4±4.2
Sign.	**	**	**	*ns*

Data are expressed as means ± Standard Deviation (SD). NA: Not active. The following positive controls were used: Ascorbic acid in 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (IC_{50} value of 5.03 ± 0.79 μg/mL) and 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) (IC_{50} value of 1.72 ± 0.09 μg/mL) test; Propyl gallate in β-carotene bleaching test (IC_{50} value of 0.09 ± 0.004 μg/mL). Results followed by different letters in a same column are significantly different (**$p < 0.05$) by Tukey's multiple range test.

Figure 3. GAS of exhausted seed ethanol extracts

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