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Enriching absinthe bitters with stilbenes and lignans from waste plant materials

Hana Dočekalová [a], Ivo Soural [a], Josef Balík [a], Pavel Híc [a], Jan Tříska [b], Naděžda Vrchotová [b], Barbara Anna Kowalczyk [c], Daniel Seriš [a], Miroslav Horák [a,*]

[a] Department of Post-Harvest Technology of Horticultural Products, Faculty of Horticulture, Mendel University in Brno, Lednice, Czech Republic
[b] Laboratory of Metabolomics and Isotopic Analyses, Global Change Research Institute, Brno, Czech Republic
[c] Department of Ornamental Plants and Garden Art, University of Agriculture in Kraków, Krakow, Poland

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ABSTRACT

The study aimed to enrich absinthe bitters with stilbenes and lignans from waste plant materials, specifically Vitis vinifera and Picea abies. These bioactive compounds, analysed by liquid chromatography and gas chromatography, reached levels up to 580 ± 25 mg/L for stilbenes and 35.02 ± 1.21 mg/L for 7-hydroxymatairesinol. In primary absinthe, these values were below the detection limit. Total antioxidant capacity and polyphenol levels significantly increased, presenting the enriched absinthes as value-added products with enhanced bioactive content. Key aroma components remained unchanged, including terpenes fenchone and trans-anethole. Thujone concentrations complied with legal limits. The findings support the production of absinthe bitters with augmented bioactive substances without compromising key sensory attributes, offering a health-promoting alcoholic beverage.

1. Introduction

Absinthe is an alcoholic beverage with a distinctive bittersweet flavor reminiscent of anise, a herbal aroma, and a high alcohol content, typically ranging from 45% to 80% by volume (Ahmad, Nadeem, Al-Sabahi, & Umar, 2016). It is classified under Regulation (EU) 2019/787 of the European Parliament as a "bitter-tasting spirit or bitters" and under Regulation (EC) No 1334/2008 of the European Parliament and (EC) No 1334/2008, absinthe is monitored for the toxic substance thujone with a limit of 35 mg/L sum of *alpha* and *beta* thujone.

There are three varieties of absinthe on the Czech market. The initial one is absinthe, acquired through blending alcohol of food quality with coloring agents, sugar, flavoring agents, and a small portion of herbal macerate/essence (Pielech-Przybylska & Balcerek, 2019). The second is absinthe as a high-percentage alcoholic macerate in which it is important to use less wormwood because of its high bitterness (referred to as "absinthe bitters"). The third variant is distillate obtained by distilling the herbal macerate. Absinthes within the second and third variants are classified as aperitifs and their base consists of three herbs, including wormwood (*Artemisia absinthium* L.), aniseed (*Pimpinella anisum* L.) and fennel (*Foeniculum vulgare* Mill.). These herbs are typically macerated in grape distillate (grape spirit) or food-grade alcohol (ethyl alcohol of agricultural origin) to create the bitter macerate described earlier. Alternatively, this macerate can be distilled to yield a refined absinthe distillate (Kowalcze & Jakubowska, 2016). The second and third variants of absinthe do not contain added sugar and coloring/flavoring agents, and are therefore very special alcoholic beverages, containing, in addition to alcohol and water, only substances from the herbs used or other plant materials, as applicable. These three herbs have been shown to counteract digestive problems, prevent stomach cramps, are highly antimicrobial and also act as antioxidants (Kowalcze & Jakubowska, 2016)).

To enrich absinthe, it is possible to use biologically active substances of plant origin, which further extend the positive effects of the drink. Lignans, such as those offering protection against pathogens or participating in the regulation of plant growth, possess antimicrobial, antioxidant, and antiviral properties in the human body, guarding against heart disease and cancer. They are primarily concentrated in the wood and bark of trees, but also exist in lesser quantities in roots, leaves, flowers, fruits, and vegetables (Cui, Du, Liu, & Rong, 2020; Rodríguez-García et al., 2019). According to previous research (Holmbom et al., 2003), it was found that lignan concentrations in Norway spruce

* Corresponding author. Faculty of Horticulture, Mendel University in Brno, Valtická 337, 691 44, Lednice, Czech Republic.
E-mail address: miroslav.horak@mendelu.cz (M. Horák).

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(*Picea abies* L.) knots are as high as 5–10 %, with 70–85 % of these lignans being 7-hydroxymatairesinol (HMR). Lignans can be extracted from spruce bark knots using various techniques, which are presented in several papers (Balík et al., 2021; Patyra, Kołtun-Jasion, Jakubiak, & Kiss, 2022). Moreover, the use of knots is a favourable application of waste material from wood processing. Since lignans such as secoisolariciresinol, matairesinol and lariciresinol (red wine) (Nurmi et al., 2003) or lyoresinol (originating from barrels during the maturation of beverages e.g. rum, brandy and whisky) (Winstel & Marchal, 2019) are commonly found in beverages, the use of 7-hydroxymatairesinol appropriately complements the range of lignans in foods.

Substances from plants that can enrich absinthe also encompass stilbenes, pivotal in plants' reactions to biotic and abiotic stressors (Tuladhar, Sasidharan, & Saudagar, 2021). Stilbenes are highly antimicrobial and anti-inflammatory agents as well as potent antioxidants (Treml et al., 2019). They can be found, for example, in grapes and tissues of grapevine (*Vitis vinifera* L.); this mainly concerns *trans*-resveratrol and its oligomers – viniferins (Goufo, Singh, & Cortez, 2020). In wines or grapes, stilbene concentrations are in the order of mg/l; much higher stilbene concentrations (in order mg/L) are found in old stems and canes of *Vitis vinifera* L., which in the case of vineyard grubbing or winter pruning makes biological waste with a huge potential for stilbene utilisation by extraction (López-Hernández & Rodríguez-Bernaldo de Quirós, 2016; Soural et al., 2015). The aim of the work was to assess the use of waste material (old wood) from *Vitis vinifera* L. and waste knots of *Picea abies* L. for enrichment of an alcoholic beverage – absinthe bitters – with stilbenes and lignans.

2. Material and methods

2.1. Material

Plant material: cultived varieties from Bělá pod Pradědem village: common wormwood (*Artemisia absinthium* L.), anise (*Pimpinella anisum* L.), fennel (*Foeniculum vulgare* Mill.), For common wormwood, the entire aerial part of the plants connecting the root and leaves was used, harvested during the second harvest on August 10, 2022. Anise seeds were harvested during the first harvest on September 2, 2022. Fennel seeds were harvested during the second harvest on September 15, 2022. Waste woody biomass from grapevine (*Vitis vinifera* L.) from wine region Moravia, sub-region Slovacko, village Kostice, knots of Norway spruce (*Picea abies* L.) from Vysočina region.

Standards: thujone (α and β mixture, 90–98 g/100 g α-thujone and 2–10 g/100 g β-thujone, purity ≥99 g/100 g, Sigma Aldrich, Prague), (+)-fenchone (purity ≥97 g/100 g, Sigma Aldrich, Prague), *trans*-anethole (purity ≥99 g/100 g, Sigma Aldrich, Prague), 7-hydroxymatairesinol (purity ≥98 g/100 g, Sigma Aldrich, Prague), *trans*-resveratrol (purity ≥98 g/100 g, Sigma Aldrich, Prague), *trans*-ε-viniferin (purity ≥99 g/100 g, Sigma Aldrich, Prague), Trolox (purity ≥98 g/100 g, Sigma Aldrich, Prague) and gallic acid (purity ≥98 g/100 g, Sigma Aldrich, Prague).

Other chemicals - for TAC: $FeCl_3$ (purity ≥97 g/100 g, Sigma Aldrich, Prague), acetic acid (99 g/100 g, Lachema, Czech Republic), HCl (35 g/100 g, Penta, Czech Republic), sodium acetate (Penta, Czech Republic), TPTZ (Sigma Aldrich, Prague), for TPC: Folin-Ciocalteau solution (Penta, Czech Republic), Na_2CO_3 (Penta, Czech Republic), for mobile phases: acetonitrile (purity ≥99 g/100 g, Penta, Czech Republic), *o*-phosphoric acid pro HPLC (Penta, Czech Republic), deionised water (prepared using the device Aquaosmotic 0), helium (purity ≥5.5, Siad Czech, Czech Republic), for preparing of absinthes: food grade alcohol (96.1 g/100 g Fagron, Czech Republic).

2.2. Preparation of the primary absinthe bitters

The absinthe macerate was prepared by infusing the herbs in food grade alcohol (70 g/100 g). For 1 L, 2.5 g of wormwood, 25 g of aniseed and 25 g of fennel were used and the resulting mixture was stirred using a Multi–functional orbital shaker (PSU-20i, EastPort, Czech Republic) for 24 h at a constant laboratory temperature of 22 °C, 100 RPM at normal atmospheric pressure. Afterwards, the residual herbs from the herbal absinthe macerate were removed by filtration using filter paper (diameter 150 mm, KA 2 – M, Pernštejn paper mill, Czech Republic). The absinthe macerate – i.e. absinthe bitters – thus prepared was labelled as sample **A0**.

2.3. Preparation of enriched absinthes

2.3.1. Absinthe bitters with added stilbenes

Proportionally, 83.32 g of wooden mass (stilbene content of 10 g/kg) obtained from the old stems (older than 1 year) of the Cabernet Moravia vine. The vine stem was first initially sliced into strips with a width of 2–3 mm on table circular saw (JET JTS 315 LA, JET US). These strips were then placed into a grinder and ground into particles under conditions of 5000 RPM (IKA MF10 basic, IKA-Werke, Germany) through a sieve with a 1 mm mesh size and ground with a grinder IKA MF10 basic (IKA-Werke, Germany) was added to 1 L of primary absinthe macerate (A0).

The resulting mixture was heated to 50 °C under constant stirring for 165 min using a Thermomix (Vorwerk, Czech Republic). Subsequently, the woody mass was removed by filtration using filter paper (150 mm diameter, KA 2 – M, Pernštejn paper mill, Czech Republic) from the absinthe macerate enriched mainly with stilbenes; the resulting enriched absinthe bitters was left standing to cool down spontaneously to reach a room temperature and was stored at this temperature in a dark place for about 1 month until analysed. The resulting absinthe bitters is referred to as sample **AST**.

2.3.2. Absinthe with added lignans

For lignan enrichment, a spruce water extract from lignan-containing knots was used. The lignan extract was obtained using the methodology according to Híc et al. (2017).

The spruce knots were ground using a grinder Cutting Mill SM 100 (Retsch, Verder Ltd., Czech Republic) and the mixture was placed in water. The mixture was boiled at 100 °C for 60 min and then the solid part was separated from the liquid part by decantation. The solid part was dried in a vacuum spray dryer SDS-02 (Lab1st, USA) at a temperature of 60 °C–80 °C according to Ramos et al. (2019). The accompanying oligosaccharides were removed from the dried extract by dissolving the lignans in food grade alcohol (ethyl alcohol of agricultural origin) (96 g/100) under a reflux condenser. After concentrating by vacuum evaporation using by RV 10 C (IKA, Germany), according to Híc et al. (2017), the lignan solution content was 56.2 g HMR/L.

Proportionally, 0.624 mL of lignan solution was added to the resulting 1 L primary absinthe macerate (A0). The resulting extract was then allowed to stand for 24 h at room temperature in a dark place and stored in this way for about 1 month until analysed. The resulting absinthe bitters is referred to as sample **ALI**.

2.4. HPLC analysis of substances

The HMR and individual stilbene contents and the sum of stilbenes in the A0, AST and ALI samples were determined by LC-DAD/FLD, following the methodologies used earlier to determine these substances in previous studies for stilbenes by (Soural et al., 2015) and for lignans by (Balík et al., 2021). HMR determination was performed using an HPLC apparatus Hewlett Packard 1050 (Hewlett Packard, USA) with a diode array detector DAD Agilent G1315B (Agilent Technology, Czech Republic) and a Phenomenex Luna C18 (2) column (3 μm, 150 mm). The mobile phases consisted of water, acetonitrile and *o*-phosphoric acid. Mobile phase A consisted of 5 g/100 g acetonitrile and 0.1 g/100 g *o*-phosphoric acid and mobile phase B consisted of 80 g/100 g acetonitrile and 0.1 g/100 g phosphoric acid. A gradient from 20 % B to 80 %

B within 20 min was used for separation. Flow rate 0.25 mL/min. The temperature of the analysis was 25 °C. Detection at 220 nm. For stilbenes, their total content (of *trans*-resveratrol, *trans*-ε-viniferin and r2-viniferin) was also calculated and each stilbene was quantified separately.

2.5. GC analysis of terpenic substances

Selected volatile aromatics – thujone (α and β mixture, 90–98% α-thujone and 2–10% β-thujone), (+)-fenchone and *trans*-anethole were quantified based on calibration curves experimentally obtained from the above standards. Standards were diluted with ethanol solution at 70 g/100 g. The compounds in the samples and standards were determined using the GC-MS technique (Agilent Technologies 7890A, Inc., Santa Clara, CA, USA), where these compounds were determined under the conditions described in a previous study (Goliáš, Balík, & Létal, 2022). For detection, a quadrupole mass spectrometer Agilent GC MSD 5975 (Agilent, USA) and a 30 m × 0.25 mm I.D. quartz glass DB-WAX column were used. Helium was used as carrier gas at a flow rate of 1.2 ml/min.

2.6. TAC – total antioxidant capacity

The FRAP (Ferric Reducing Antioxidant Power) method was performed according to the methodology of Soural et al. (2015). The FRAP method was performed in acetate buffer at pH 3.6 (34 mmol/L sodium acetate in 281 mmol/L acetic acid solution). The reaction mixture contained 12 mmol/L $FeCl_3$, 10 mmol/L 2,4,6-tri (2-pyridyl)-*s*-triazine in 40 mmol/L of HCl solution and a buffer (the ratio was 1:1:10). A ten times diluted sample of absinthe macerate was mixed with the reaction mixture in volumes of 25 μL (sample) and 2 ml (reaction mixture). Mixing was carried out in a disposable plastic cuvette (10 mm). The obtained solution was measured after 10 min at the wavelength of 593 nm using a spectrophotometer Specord 50 Plus (Analytik Jena, Germany). The control sample was prepared in the same ratio of reaction mixture and sample except that 25 μL of deionised water was used instead of the sample. The antioxidant capacity was calculated from the calibration curve using Trolox (FRAP method), where it was expressed as mg of Trolox per litre of absinthe macerate.

The DPPH method was performed according to the methodology of (Seriš et al., 2014). The samples were diluted with distilled water (in a 1:10 ratio). A solution of DPPH in methanol, with a concentration of 0.1 mmol/L, was meticulously prepared in a 250 ml volumetric flask. Thereafter, 100 μL of the diluted sample and 2000 μL of the DPPH solution were transferred into a 10 mm cuvette. The cuvettes, containing the samples, underwent vigorous shaking for 20 s using an orbital shaker IKA MS 3 (IKA, Germany). Following a 30-min incubation period in darkness, absorbance readings were acquired using a spectrophotometer Specord 50 Plus (Analytik Jena, Germany) at a wavelength of 515 nm. A control sample of pure methanol was employed as a blank. The results were reported as mg of Trolox per litre of absinthe macerate.

2.7. TPC – total polyphenolic content

The total polyphenolic content (TPC) method was performed according to the methodology of (Singleton & Rossi, 1965) with modification according to (Snurkovič, 2015), using Folin–Ciocâlteu reagents (FCR), at a wavelength of 700 nm, using a spectrophotometer Specord 50 Plus (Analytik Jena, Germany). The absinthe bitters samples were diluted 10 times before using the method. A sample of absinth macerate (0.2 mL) was pipetted into a 50 ml volumetric flask and mixed with 20 mL of distilled water and 1 mL of FCR. After 3 min, 5 ml of a volumetric solution of 20 g/100 g Na_2CO_3 was added and then the flask was filled to the punch mark with distilled water and the solution was stirred. After 30 min, absorbance was measured in a spectrophotometer at 700 nm using a 10 mm cuvette. The result was compared with the absorbance of the blank (1 mL FCR, 5 mL 20 g/100 g Na_2CO_3, deionised water). Antioxidant capacity was calculated from the calibration curve using gallic acid.

2.8. Statistical analysis

ANOVA, Tukey multiple range test using Statistica 12 (StatSoft, Czech Republic) software was used to study statistical significance with $p < 0,05$. Mean and standard deviation were calculated for all measurements: antioxidant capacity (AC), total polyphenols (TPC), lignan (HMR), stilbenes (resveratrol and viniferins), terpenes (thujone, anethole and fenchone) from three replicates using MS Excel 2016 software.

3. Results and discussion

The values of bioactive substances measured in fortified absinthes were compared with the results achieved for other non-fortified beverages, (wine, rum, brandy, and grape juice) which naturally contain bioactive substances such as stilbenes (*trans*-resveratrol, *trans*-ε-viniferin, r2-viniferin) or lignans (lyoniresinol) and also were compared with fortified beers. No stilbenes (*trans*-resveratrol, *trans*-ε-viniferin, r2-viniferin) or lignans (HMR) were detected in the primary absinthe (A0). On the contrary, high levels of these substances (stilbenes 728 mg/L, lignans 35.02 mg/L) were found in both types of enriched absinthes (AST and ALI), many times higher than in normal unfortified foods or with comparable values in fortified foods (Fig. 1). The estimated content was 797 mg/L for stilbene, resulting in 91% yield. Such a high extraction rate was achieved due to the high ethanol content of absinthe, which extracts these substances better than water. The disadvantage of this method is the not inconsiderable reduction in the total quantity of the drink due to the soaking into the dry extracted material. This reduction was as high as 5,4 %. The comparison of absinthe with alcoholic beverages containing 4.5–40 g/100 g of alcohol was made on the basis of the correct serving of this beverage in the hospitality sector, when absinthe is diluted with water 1:5, causing the alcohol content to drop from the initial 70 to about 12 g/100 g when consumed, thus making it comparable to wine in terms of percentage.

Thus, it can be said that the consumption of 0.02 L of absinthe alone (the content before dilution with water, when served properly) enriched with stilbenes corresponds to 0.2–12 L of wine in the context of *trans*-resveratrol and 2 L of wine in the context of *trans*-ε-viniferin; consumption of absinthe enriched with lignans corresponds to 3 L of wine or 0.12 L of brandy/rum within the lignan content, where for 7-hydroxymataresinol (HMR) content it still represents only 1.4% of the recommended daily dose (FDA-USA, 2003).

3.1. Stilbenes

3.1.1. Trans-resveratrol

The resulting *trans*-resveratrol content in the enriched absinthe (AST) was 58 ± 4 mg/L, which presents significant values compared to the maximum levels in non-enriched beverages in studies by other authors, e.g., Chianti red wine with a maximum content of 2.5 mg/L (Stecher, Huck, Popp, & Bonn, 2001) or Cabernet Sauvignon red wine with a maximum content of 2.06 mg/L (Vitrac et al., 2005) or even other wines commonly up to 2 mg/L (Ribeiro de Lima et al., 1999), and even more significantly compared to grape must 0.09–0.79 mg/López--Hernández and Rodríguez-Bernaldo de Quirós (2016; Stecher et al., 2001). In a paper by Wang, Catana, Yang, Roderick, and van Breemen (2002), Italian wines were compared, where the *trans*-resveratrol content reached up to 3.16 mg/L in the cuvee (Negroamaro + Malvesia nero) and in the case of the sum of *trans*-and *cis*-forms together even 5.66 mg/L, but even this number is still 10 times lower than in an enriched absinthe bitters.

3.1.2. Trans-ε-viniferin

The resulting *trans*-ε-viniferin content of enriched absinthe (AST)

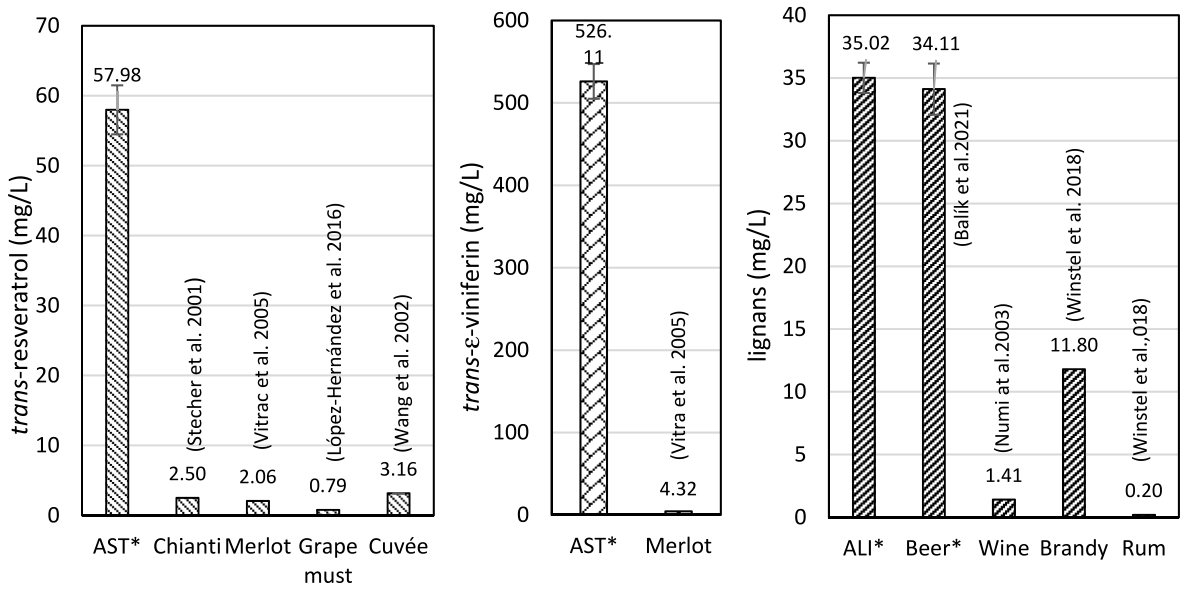

Fig. 1. Comparison of enriched absinthes (AST* enriched with stilbenes and ALI* enriched with lignans) with other beverages from the literature, showing mean values with standard deviation or maximum values. Enriched beverages are marked with *. For *trans*-resveratrol and trans-*ε*-viniferin, this involves red wines (Chianti and Merlot), or grape must and red cuvee wine (Negroamaro + Malvesia nero) as applicable. Beer enriched with lignans in a way similar to enriched absinthe (ALI) and non-enriched beverages: wine, brandy and rum.

was 526 ± 21 mg/L when compared to Merlot with a maximum content of 4.32 g/L, for example (Vitrac et al., 2005) it is a demonstrably higher value. There are more noticeable differences for *trans*-*ε*-viniferin compared to wines than for resveratrol.

3.1.3. Sum of stilbenes

The total stilbene content in the enriched absinthe (AST) was 728 mg/L, with r2-viniferin present next to *trans*-resveratrol and *trans*-*ε*-viniferin. This value is above average compared to other sources of these substances such as red wine or red grape must. From the above it can be deduced that by consuming 0.02 L of enriched diluted absinthe bitters, an intake of up to 14.6 mg of stilbene substances is achieved. Recommended daily dosages of stilbenes (specifically resveratrol) depend on various factors, including individual health status, age, weight, and any pre-existing medical conditions. However, a study by Novelle, Wahl, Diéguez, Bernier, and De Cabo (2015) specifies this dosage range from 5 mg to 5 g per day. When it comes to compound from the stilbenes viniferin, research is not as extensive as with resveratrol, and determining the daily dosage is still uncertain. However, it also depends on various factors such as age, gender, health status, and individual needs.

3.2. Lignans

7-hydroxymatairesinol (HMR) is practically not found in common foods, so there is an effort to deliberately enrich foods with this substance due to its positive effects on the human body. According to available sources, the recommended daily dose of 7-HMR for an adult is 50 mg per day (FDA-USA, 2003). Balík et al. (2021) conducted a beer fortification experiment using an identically prepared extract of Norway spruce (*Picea abies* L.) to obtain very significant results in terms of HMR (34 ± 2 mg/L beer); in the case of an even richer HMR sample for beer they achieved values up to 161 ± 9 mg/L but this beer already had an unpleasant woody aftertaste and received the worst sensory evaluation in this study.

For enriched absinthe (ALI), the resulting HMR content was 35 ± 1 mg/L, a very similar result to that of beer, which was also still a comfortable beverage according to the preliminary sensory assessment.

If lignans are added in the form of an extract, the amount can be very well controlled. At the same time, there is no loss of beverage due to absorption into the extracted material, as was the case with stilbenes.

Lignans are found in unfortified foods such as wine at levels as low as 0.8–1.4 mg/L (Nurmi et al., 2003), which is much lower than in the prepared enriched absinthe or fortified beer. Similarly, only 0.2–11.8 mg/L of lignans are found in brandy and rum (Winstel & Marchal, 2019). From that it can be deduced that by consuming 0.02 L of enriched, diluted absinthe bitters, an intake of up to 0.68 mg of HMR can be achieved.

3.3. Antioxidant capacity (AC) and total polyphenol content (TPC)

The enriched absinthe bitters beverages showed an increase in antioxidant capacity (Fig. 2) compared to the primary non-enriched bitters (A0) with an antioxidant capacity of 743 ± 14 mg/L (FRAP) and 623 ± 45 mg/L (DPPH), where both enriched variants had values above 1000 mg/L, i.e. 1201 ± 15 mg/L (FRAP) and 1125 ± 10 mg/L (DPPH), for the absinthe enriched with stilbenes (AST) and 1014 ± 62 mg/L (FRAP) and 979 ± 12 mg/L (DPPH), for the absinthe enriched with lignans (ALI). As both stilbenes and lignans are categorized as antioxidants, the authors anticipated an increase in antioxidant capacity upon their addition. This expectation aligns with the observed results.

Also, the polyphenol content increased from 55 ± 4 mg/L in the primary absinthe (A0) to 422 ± 64 mg/L in the stilbene-enriched absinthe (AST). On the contrary, in the case of lignan enrichment in ALI and A0 samples, there was no statistically significant increase in total polyphenols. The differences between the results for all polyphenols and the antioxidant capacity of the samples may be due to the lower selectivity of this method towards some phenolic compounds (Zekovič et al., 2017).

In summary, the increase in antioxidant capacity and polyphenol content in the enriched absinthe bitters samples can be attributed to the addition of stilbenes and, to a lesser extent, lignans, both of which are known for their antioxidative properties.

3.4. Terpene content

The enrichment of the primary absinthe with stilbenes or lignans did not lead to statistically significant changes in the content of the studied

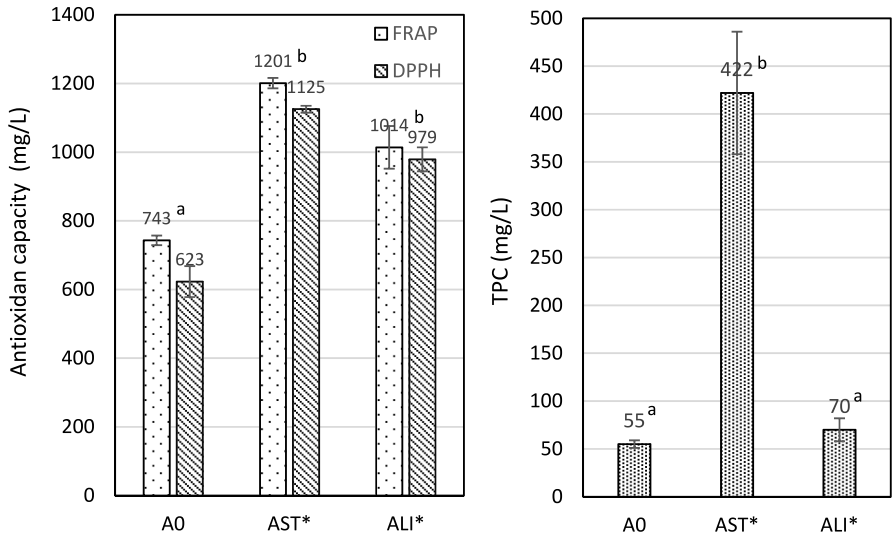

Fig. 2. Antioxidant capacity (AC) and total polyphenol content (TPC) in the primary absinthe (A0) and in the enriched absinthes (AST* enriched with stilbenes and ALI* enriched with lignans). Letters "a, b" indicate statistically significant differences (n = 3).

basic aroma components from the range of terpenes: anethole and fenchone originating from the two main absinthe herbs (anise and fennel) and thujone, the toxic substance sourced from wormwood (Table 1.). No statistically significant differences were found for the enriched absinthes. The results confirm that these terpenes are released into absinthe unevenly during production. The Fenchone and trans-Anethole contents are very similar in wormwood essential oil at 317 and 466 mg/g (Mujović et al., 2023), but the concentration of trans-Anethole is significantly higher in absinthe. Thujone, which is present in wormwood essential oil at a concentration of 66,8 mg/g (Zámboriné Németh & Thi Nguyen, 2020), is present in very low concentrations in the absinthe sample.

4. Conclusions

Absinthe bitters was enriched with stilbenes isolated from waste wood biomass of *Vitis vinifera* L. and with lignans from waste knots of *Picea abies* L. The stilbene-enriched absinthe bitters contained 58 mg/L of *trans*-resveratrol which is demonstrably higher than in other beverages such as ciders or wines where *trans*-resveratrol is naturally found at levels ranging from 0.09 to 5.66 mg/L. It was a similar case with *trans*-ε-viniferin, where the enriched absinthe reached values of up to 526 mg/L. Absinthes enriched with lignans reached values of up to 35 mg/l compared to non-enriched beverages such as wine with an average of 1.41 mg/L. Similarly, in brandy and rum, 0.2–11.8 mg/L of *trans*-ε-viniferin is found. The profile of the two basic terpenes (*trans*-anethole and fenchone) as well as of the monitored substance thujone, derived from the basic absinthe herbs, did not change statistically in the enriched absinthe bitters beverages. At the same time, however, the antioxidant capacity and polyphenol content increased compared to the primary, non-enriched bitters by 25 %–670 %. In this way, not only was there a significant enrichment of absinthes with biologically active substances, but also the use of stilbenes and lignans from waste plant materials. From a practical point of view, it is preferable to use extracts because there is no loss of beverage by soaking into the extracted material. Some of the bitter taste of absinthe from wormwood can be replaced by extracts from waste wood, and savings can be made on the amount of herb used to make absinthe.

Table 1
Contents of observed terpenes in absinthes.

	Thujone[a] (mg/L)	*trans*-Anethole (mg/L)	Fenchone (mg/L)
Absinth without addition **(A0)**	0.80 ± 0.07 [a]	348 ± 17 [a]	1.41 ± 0.09 [a]
Absinth with stilbenes **(AST)**	0.73 ± 0.03 [a]	356 ± 21 [a]	1.43 ± 0.06 [a]
Absinthe with lignans **(ALI)**	0.91 ± 0.06 [a]	351 ± 12 [a]	1.52 ± 0.08 [a]

[a] Content of thujone is sum of *alpha*- and *beta*-structure. Letters "a, b" indicate statistically significant differences (n = 3).

Ethics statement

The research was not carried out with animals and humans.

CRediT authorship contribution statement

Hana Dočekalová: Writing – original draft, Resources, Data curation, Conceptualization. **Ivo Soural:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Josef Balík:** Writing – review & editing, Supervision, Project administration. **Pavel Híc:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Jan Tříska:** Writing – review & editing, Supervision. **Naděžda Vrchotová:** Resources, Investigation, Data curation. **Barbara Anna Kowalczyk:** Writing – review & editing, Data curation. **Daniel Seriš:** Resources, Investigation. **Miroslav Horák:** Writing – review & editing, Visualization, Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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