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The Use of Enzyme Systems of the Genus *Prunus* for the Production of Benzaldehyde

Original Paper

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Abstract **Aim:** Benzaldehyde occurs in a number of plants, especially in the family *Rosaceae* and in particular in the genus *Prunus*. In nature, there are more than 100 genera and 3,000 species in the *Rosaceae* family. The objective of this study was to investigate the chemical composition of leaf essential oil of peach (*Prunus persica* L.) and cherry laurel (*Prunus laurocerasus* L.) as a new potential source of natural benzaldehyde.
Methods: The essential oil was prepared by hydrodistillation, and chemical constituents were determined by GC-FID, GC-MS and chromatographic profiles were compared with each other.
Results: The results show that essential oil obtained from peach and cherry laurel leaves appear to be a promising source of natural benzaldehyde. Under laboratory conditions the benzaldehyde content in peach and cherry laurel leaves reached 95.5% and 99.7%, respectively.
Conclusions: Laboratory and pilot experiments confirmed that by processing of 200–300 kg of green leaves of various species of the genus *Prunus*, especially peach and cherry laurel, 1 kg of benzaldehyde can be obtained.

Keywords *Amygdalin – benzaldehyde – biotechnology – essential oil – hydrodistillation*

INTRODUCTION

In recent times, the consumer preferences for natural products have led to an increasing demand for natural food additives. Benzaldehyde belongs to the group of such desired products (Verma et al., 2017). To judge by consumption, benzaldehyde is the second most important molecule in the flavour industry after vanillin (Prasad et al., 2018). Benzaldehyde is a significant aromatic substance, with a hint of almonds and cherries and a characteristic sweet aroma. It is widely used as a food and flavouring ingredient and can be found in many foods, including pastries, frozen dairy products, fruit juices, soft candies, gelatine pudding, soft drinks, alcoholic beverages, hard sweets, and chewing gum (Burdock, 2010), as a natural ingredient in *cherry* and other natural fruit *flavours* and as an additive to one or more types of tobacco products (Kosmider et al., 2016). Benzaldehyde is also used in dye (Yen, 2012), fragrances (perfumes, deodorants, etc.) (Davidson, 2017), pharmacy/medicines (Wen et al., 2021), and personal care items (shaving gels, moisturizing gels/creams, bath soaps, etc.) (Natsch, 2010). It is also used as a solvent for oils, resins, and cellulose fibres (Bishop, 1990). However, most benzaldehyde is of synthetic origin. Global annual production of synthetic and natural benzaldehyde is 7,000 tons and 100 tons, respectively. About 20% of natural benzaldehyde is obtained from the kernels of the apricots, peaches, prunes, and bitter almonds by enzymatic hydrolysis, which also produces toxic HCN (Brenna et al., 2016). Natural benzaldehyde extracted from fruit kernels has a market of around 20 tonnes per year and a price of approximately EUR 240 per kg (Dionísio et al., 2012).

There are three general procedures used worldwide to produce of nature-identical benzaldehyde (Remaud et al., 1997): (a) from toluene (direct oxidation); (b) from benzalchloride (hydrolysis); (c) from cinnamaldehyde (retroaldol reaction). The cinnamaldehyde itself may be extracted from natural material (cinnamon oil), leading to the so-called benzaldehyde ex-cassia. In this case the resulting benzaldehyde is classified as semisynthetic and not natural, as specified in the European directive (Commission Directive 91/71/EEC of 16 January 1991).

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Table 1. Harvesting time, leaf essential oil yield, and benzaldehyde content in different varieties of Prunus from Southwestern Slovakia.

Plant	Date of harvest	Essential oil yield (ml/100 g fresh leaves)	Benzaldehyde content in leaf essential oil %
Wild peach 1*	27.07.2021	0.347	92.93
Wild peach 2*	27.07.2021	0.327	96.44
Redhaven*	04.08.2021	0.288	95.56
Firehaven*	05.08.2021	0.301	94.94
Groshaven*	06.08.2021	0.309	94.11
Flamingo*	06.08.2021	0.384	95.94
Bergeval**	06.08.2021	not detected	not detected

**peach variety; ** apricot variety*

A common natural source for benzaldehyde production is almond kernels. Almonds are the most widely produced tree nut in the world, reaching over 1.2 million metric tons during the 2017/ 2018 season (INC statistical yearbook, 2017/2018).

Different extraction procedures of peach kernels, including soxhlet extractions with various solvents, hydrodistillation, ethanolic maceration followed by fractionation with various solvents, and supercritical fluid extraction (SFE) performed with pure CO_2 and with a co-solvent were compared. It was concluded that the production of peach almond oil by any technique is substantially adequate and that SFE presented advantages, with respect to the quality of the extracts owing to the high oleic acid content, as presented by some soxhlet extract samples (Mezzomo et al., 2010).

Almond kernels contain varying amounts of amygdalin, a diglucoside that is broken down into hydrogen cyanide and benzaldehyde in response to crushing of the kernel and exposure to water or saliva. Due to the high amygdalin content (>3%), bitter almonds are a significant source of benzaldehyde, which is an important flavouring substance also known as almond oil or almond essence (Franklin & Mitchell, 2019).

Amygdalin is important as an aromatic compound with anti-cancer properties; however, it is also a controversial compound because the by-product, hydrogen cyanide, is toxic and can cause both acute and subacute health problems (Jaszczak-Wilke et al., 2021). For these reasons, it is recommended to be very careful in the isolation of benzaldehyde from *Prunus* species (leaves and kernels). Figure 1 illustrates amygdalin hydrolysis and the conversion pathway to benzaldehyde and hydrogen cyanide (Luo et al., 2018).

Biotechnological production is a particularly attractive alternative for flavour production, since it occurs under mild conditions, presents high regio- and enantio-selectivity, does not generate toxic wastes, and offers products that may be labelled as 'natural' (Bicas et al., 2010). The bioproduction of aroma compounds may be achieved in two basic ways: through *de novo* synthesis (Craig, 2013; Xing et al., 2019) or by biotransformation (Dionísio et al., 2012; Jain et al., 2010; Okrasa et al., 2004), both being possible with genetically modified organisms.

Plants or parts thereof, e.g. leaves, can also be a useful and inexpensive renewable source of benzaldehyde. Benzaldehyde belongs to the most single compound in floral scent (Knudsen et al., 2006), occurring in a number of plants, especially in the *Rosaceae* family and in particular in the genus *Prunus* (Andersen, 2006). In nature, there are more than 100 genera and 3,000 species in the *Rosaceae* family (Soundararajan et al., 2019), which is one of the most abundant families of flowering plants. It can be divided into four subfamilies, according to the type of fruit:

1. *Amygdaloideae,* with stone fruit;
2. *Maloideae*, with kernels (fruits in which the flower hypanthium becomes fleshy);
3. *Rosoideae*, with aphids (dry fruits that do not open) or stone fruit (small, aggregate stone fruit);
4. *Spiraeoideae*, with follicles (dry fruits which splits on one side only).

These types include for example: almonds (*Prunus dulcis* L.); apricot (*Prunus armeniaca* L.); nectarine and peach (*Prunus persica* L.); plum (*Prunus domestica* L.); blackthorn (*Prunus spinosa* L.); laurel (*Prunus nobilis* L.) and cherry laurel (*Prunus laurocerasus* L.).

Benzaldehyde has been found to occur naturally in various fruits such as peaches (Verma et al., 2017), black currants (Eksi Karaagac et al., 2021), strawberries (Klatt et al., 2013), grapes (Petretto et al., 2021), and raspberries (Aprea et al., 2015).

Benzaldehyde has been reported to occur in several essential oils: hyacynth, citronella, orris, cinnamon, sassafras, labdanum, and patchouli (Burdock, 2010), bitter almond oil (Geng et al., 2016) and cananga oil (Kristiawan et al., 2008). It is a colourless liquid with a characteristic almond scent.

This essential oil, isolated by hydrodistillation from apricot seeds (*Armeniacae* Semen), was analysed by gas chromatography-mass spectroscopy (Lee et al., 2014). Benzaldehyde (90.6%), mandelonitrile (5.2%), and benzoic acid (4.1%) were identified as the main components.

The leaf essential oils of *Prunus phaeosticta* var. *phaeosticta* were isolated by hydrodistillation and analysed using headspace-GC methods to determine their composition and yield.

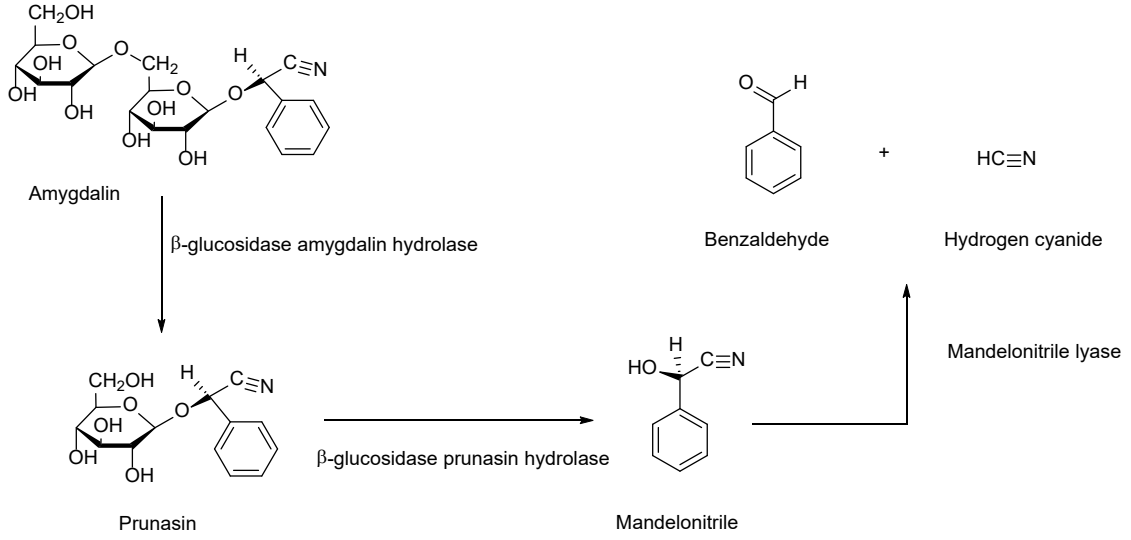


Figure 1. Amygdalin hydrolysis and conversion pathway to benzaldehyde and hydrogen cyanide (Luo et al., 2018).

Seventy-six compounds were identified in the hydrodistilled leaf oil, and 58 compounds were identified by the headspace, respectively, using GC and GC-MS. The main components of the oils were benzaldehyde (73.3%), 1,8-cineole (5.4%), and α-terpinyl acetate (4.4%) (Ho et al., 2009).

Large amounts of peach leaves and stems are by-products derived from peach tree cultivation and canned industries. Benzaldehyde was detected as the major volatile leaf component (70%–95%), whereas myrcene (18%–21%) and terpinolene (18%–26%) were found to be the most important compounds in stems (Maatalah et al., 2020).

The plum seed oil contained benzaldehyde as the main volatile compound, but 50 or more other volatile compounds were also analysed (Picuric-Jovanovic & Milovanovic, 1993).

Benzaldehyde was identified in branch enclosure experiments from 66 vegetation species sampled at three US sites (Helming et al., 1999).

Since the preparation of natural benzaldehyde from fruit stones/kernels (almonds, peaches) is relatively laborious and requires careful processing and on the other hand, fermentation and biotransformation have limits in economic efficiency, we tried to isolate benzaldehyde from leaves (peach and cherry laurel). This approach is very promising, because by processing other secondary parts of plants (leaves of fruit trees), it is possible to achieve some additional benefits in terms of profit. An even more important advantage is the possibility of placing new food products on the market based on natural compounds (Veličkovic et al., 2016).

MATERIALS AND METHODS

Plant material

The plants we investigated originated from Southwestern Slovakia. The agricultural cooperative Vajnory (near Bratislava) offered twigs with leaves of peach and apricot, namely: three varieties of "Haven" peach group: Firehaven, Groshaven, Redhaven (Owens, 2019); one Slovak peach variety, Flamingo (Benediková & Zetochová, 2018): and one apricot variety, Bergeval (Benediková, 2017). *Young twigs were harvested by hand at the time,* in August 2021.

The Research Institute of Plant Production *in Piešťany offered a variety of* cherry laurel that is one of the *most* popular and versatile evergreen *hedging* species. *Young twigs with leaves were harvested by hand at the time of* in the period from October 2021 to January 2022. The fresh plant material was stored at 4°C until further uses (no longer than 24h).

Apricot kernels were obtained from the Pezinok distillery.

Chemicals

The organic solvent ethyl acetate was p.a. grade (Sigma-Aldrich, USA). All other the synthetic standards were 99% purity (Sigma-Aldrich, USA).

Extraction of essential oils from leaves

Essential oils of *Prunus* species were extracted using a Clevenger apparatus as follows: 100 g of fresh leaves cut into 2 cm pieces were put into a 2,000 ml round-bottom flask and 1,000 ml of distilled water was added. The mixture was heated in a bath of stirred oil with a temperature of 130°C. The hydrodistillation was carried out during 2 hours from the first drop of distillate appeared in condenser. The yield of essential oil (ml/100 g) was determined on fresh weight basis. The isolated oils were stored in tightly closed vials at 4°C until analysis.

Pilot experiments

Pilot-scale experiments were performed under batch regime, processing 200 kg of twigs with leaves in a mixer after the addition of 1,000 litres of water, followed by distillation for 45 minutes. The obtained distillate was transferred into a separatory funnel, where essential oil was separated from aqueous phase. The hydrosol (aqueous phase below the layer of essential oil) was extracted by ethyl acetate. The extract was distilled in a vacuum, and organic phases were collected and dried with anhydrous magnesium sulfate. The samples of recovered essential oil were analysed by GC-FID and GC-MS.

Essential oil isolation from apricot kernels

Apricot kernel essential oil was prepared under laboratory conditions as follows: after grinding the apricot kernel using Philips blender, type HR2860, 100 g of flour (obtained from 390 g of crushed kernels) was homogenised with 1,000 ml of water and hydrodistilled in a Clevenger apparatus for 2 hours, followed by GC-FID analysis.

Essential oil analysis by GC-FID and GC-MS

The essential oil was analysed by gas chromatography with flame-ionisation detection (GC-*FID*) using Agilent 7,890N apparatus with a column DB WAX (methyl dodecanoate PEG film, length 30 m, thickness 0.15 µm, diameter 0.25 mm).
The constituents of essential oil were identified using gas chromatography-mass spectrometry (GC-MS) by means of the Agilent 5,977C single quadrupole GC/MSD instrument, using the same column (DB WAX), as for the GC- FID analyses. Mass spectra were compared with the NIST Spectral Database.
The operating conditions of GC-FID analysis were as follows: hydrogen was used as a carrier gas (1.5 ml/min); split mode (20:1); 1 µl of injection volume; oven temperature program: 95°C for 6 min, 10°C/min up to 120°C, 20°C/min up to 230°C, 230°C for 17 min; an injector temperature of 250°C; a detector temperature of 300°C.
The operating conditions of GC-MS analysis were as follows: helium was used as a carrier gas (1.5 ml/min), with split mode (50:1); 0.2 µl of injection volume; oven temperature 40°C for 5 min, 10°C/min up to 220°C, with 220°C for 5 min; an injector temperature of 250°C; and a detector temperature of 270°C. Electron impact mass spectra were acquired in scan mode in m/z range 27–330.

RESULTS AND DISCUSSION

In view of the data published recently by Tunisian and Italian authors (Bonesi et al., 2019; Maatalah et al., 2020), natural benzaldehyde is naturally *found* in the composition of essential oils extracted from leaves of different species of the genus *Prunus* by hydrodistillation in concentrations from 75% to 95%. Therefore, the peach and cherry laurel leaf oils investigated in this study may serve as a potential natural source of benzaldehyde for flavours, perfumery, and food ingredients.

Laboratory preparation of benzaldehyde from *Prunus* species leaves

At the beginning of the testing, we processed wild peach leaves grown in vineyards on the Carpathian slopes near Bratislava, Slovakia. Given the preliminary positive results (92%–96% benzaldehyde content in essential oil), we decided to perform a series of experiments using leaves of cultivated peach varieties. We evaluated four peach varieties (Flamingo, Redhaven, Groshaven, and Firehaven) and one apricot variety (Bergeval), to compare the percentage of representation of benzaldehyde in the essential oils studied. The summary of experiments is summarized in the Table 1. No essential oils appeared during distillation of the apricot leaves of the Bergeval variety or while processing peach leaves of all varieties; the average yield of essential oil was 0.326% V/w and with 94.99% benzaldehyde content. GC-MS identification of volatile organic compounds in the essential oil of processed peach leaves is illustrated in the Table 2 and Figure 2.
The results confirmed that benzaldehyde is the most abundant volatile organic compound of peach leaf essential oil, while the content of the other compounds is significantly lower (individual peaks of the chromatogram are less than 1%). Based on the results reached, it was calculated that approximately 300–400 kg of fresh peach leaves are needed to produce 1 kg of benzaldehyde.
As no separation of essential oil was observed during the hydrodistillation of apricot Bergeval variety leaves, the hydrosol (condensed water after hydrodistillation in Clevenger apparatus) was extracted with ethyl acetate in the feed to solvent volume ratio of 3:1. In this way, benzaldehyde dissolved in water (benzaldehyde solubility is 0.3%–0.7% in water) and other components, as (*E*)-2-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenol were extracted as shown in Figure 3.

Laboratory preparation of benzaldehyde from apricot seeds

The essential oil obtained from the apricot and plum seeds can also be the alternative source of benzaldehyde. The process of benzaldehyde releasing from apricot seeds consist in the biochemical transformation (Figure 1) of amygdalin by β-glucosidase hydrolysis during milling (Tunçel et al., 1998).
The results of the hydrodistillation of apricot kernels are presented in Figure 4. The concentration of benzaldehyde reached only about 10%, and various unidentified polyunsaturated fatty acids (22%) can be seen in the chromatogram. In addition, the yield of the essential oil was only 0.03% w/w, which is about ten times less compared to the hydrodistillation of peach leaves.

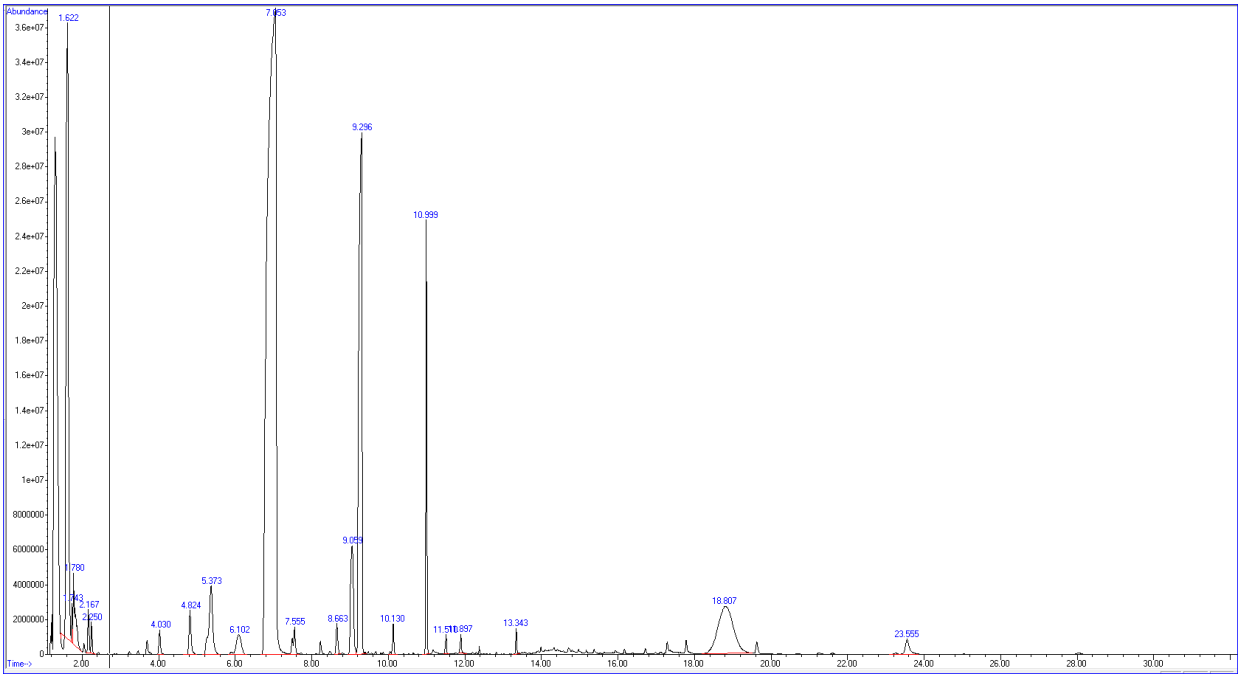


Figure 2. GC-MS chromatogram of the peach leaves essential oil (variety Flamingo). The individual compounds are described in table 2.

Table 2. GC-MS identification of volatile organic compounds in the essential oil of peach leaves (variety Flamingo).

Retention time (min)	Library/ID
1.6219	acetic acid, butyl ester
1.7429	1-butanol, 2-methyl-, acetate
1.7800	acetaldehyde, butylhydrazone
2.1674	butanoic acid, butyl ester
2.2504	(*E*)-2-hexenal
4.0296	(*E*)-2-hexen-1-ol
4.8244	1-octen-3-ol
5.3735	l-menthone
6.1015	menthone
7.0526	benzaldehyde
7.5547	menthyl acetate
8.6627	cyclooctyl alcohol
9.0594	levomenthol
9.2964	acetophenone
10.1304	piperitone
10.9990	benzyl alcohol
11.8972	benzyl nitrile
13.3430	4-imidazolidinone, 1-acetyl-2-thioxo-
18.8074	1,4-diphenylpyrazole
23.5554	mandelamide

Pilot testing of benzaldehyde preparation from peach leaves

Hydrodistillation of fresh peach twigs with leaves (Figure 5) in pilot conditions using SCC (spinning cone column) resulted in lower yields compared with those of laboratory experiments. Up to about 600 kg of twigs with leaves were needed to obtain 1 kg of benzaldehyde. The purity of the benzaldehyde reached about 96%, which is in good agreement with the laboratory experiments. The large losses of benzaldehyde (up to 42% of the feed material) were observed during the subsequent long-term evaporation of the ethyl acetate extract of distillate on a laboratory evaporator after 2 hours (55°C–60°C/30–35 kPa). Thus, the crude concentrate containing benzaldehyde will have to be purified by rectification in an inert atmosphere to avoid losses and spontaneous oxidation of benzaldehyde to benzoic acid. Pilot experiments also showed that no essential oil and targeted product – benzaldehyde – occurred in the stems or occurred only in insignificant quantities.

Laboratory preparation of benzaldehyde from cherry laurel leaves

Cherry laurel leaves (Figure 6) have been tested as another promising source of benzaldehyde. The advantage of this plant is the rapid growth of its green phytomass, because up to 30% of pruned green leaf mass can regrow again within 3 months. Shrubs can be grown, machine cut, or rowed, which facilitates the collection of green material for industrial

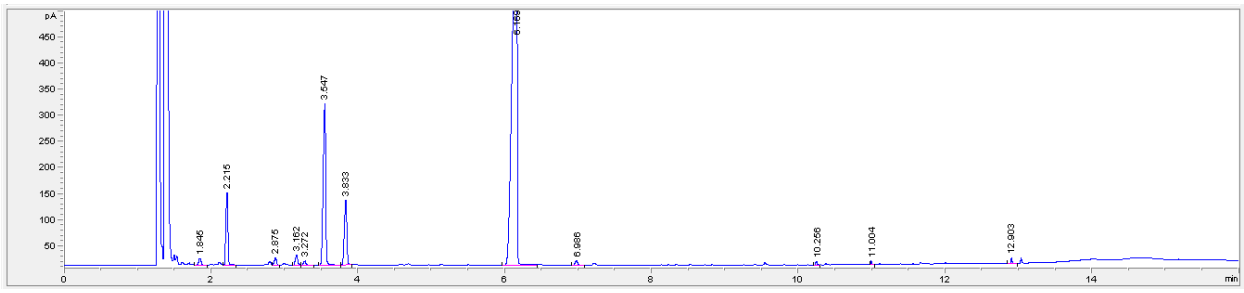


Figure 3. GC-FID chromatogram of hydrosol extracted with ethyl acetate, after hydrodistillation of apricot Bergeval leaves in Clevenger apparatus. The major constituents are representing by peaks at the following retention times (RT): 2.21 min (E)-2-hexenal; 3.54 min (Z)-3-hexenol); 3.83 min (E)-2-hexenol) and 6.17 min (benzaldehyde).

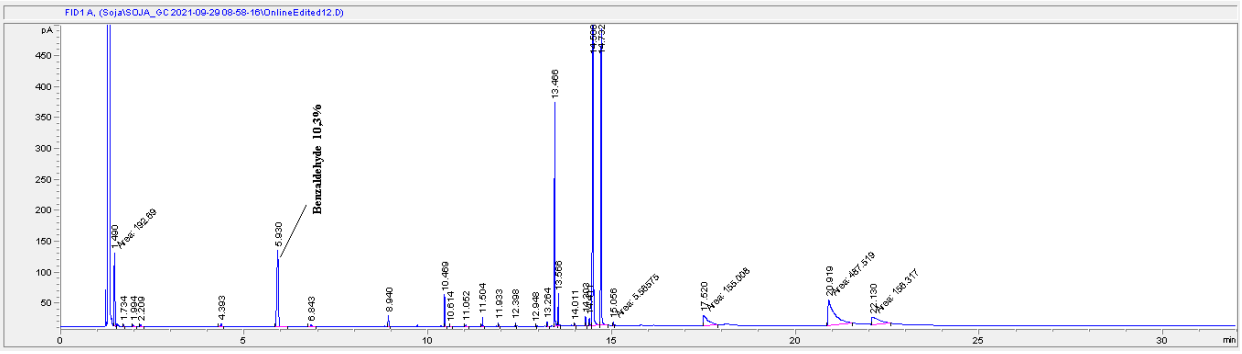


Figure 4. GC-FID chromatogram of the essential oil obtained from apricot seeds (Prunus armenica).



Figure 5. Peach leaves with twigs.

processing. According to the literature (Stanisavljevic et al., 2010), up to 99.7% of cherry laurel leaf essential oil is made up of benzaldehyde and insignificant quantities of (*Z*)-ocimenone (0. %) and (*E*)-2-hexenal (0.1%).



Figure 6. Cherry laurel growing in Piešťany, October 2021.

In Table 3 the results are presented of the essential oil extraction from cherry laurel leaves by hydrodistillation *in the Clevenger apparatus.* The isolated essential oil was of high purity, that is, 99.7% benzaldehyde. The results obtained are in good agreement with the data of Stanisavljevic et al. (2010). Cherry laurel leaves have been shown to be a more effective source of essential oil because they have a high benzaldehyde content compared with peach leaves. The yield of cherry laurel leaf essential oil reached about 0.463%, which means that approximately 200 kg of cherry laurel leaves are sufficient to obtain 1 kg of benzaldehyde. Moreover, peach trees are leafless in winter, while cherry laurel is an evergreen shrub, resistant at temperatures as low as minus 20°C. The

Table 3. Differences in essential oil yield and benzaldehyde content in cherry laurel depending on harvesting dates.

Plant	Date of harvest	Essential oil yield (ml/100 g fresh leaves)	Benzaldehyde content %
Cherry laurel*	10.11.2021	0.463	99.70
Cherry laurel**	11.01.2022	0.155	99.51
Cherry laurel*	17.03.2022	0.328	98.31
Cherry laurel**	25.03.2022	0.433	97.04
Cherry laurel**	06.04.2022	0.547	94.51

**Piešťany location; **Svätý Jur location*

harvesting of laurel leaves at minus 5°C in January 2022, followed by processing in a Clevenger apparatus gave lower benzaldehyde yields, which were still of relatively high purity.

CONCLUSIONS

As mentioned above, benzaldehyde is one of the most desired and important additives of food and cosmetics.

The results obtained confirmed the high content of benzaldehyde in the peach leaves and cherry laurel leaves grown in the Southwestern region of Slovakia. Exploiting the biochemical pathway of benzaldehyde formation in leaves of the genus *Prunus* makes it possible to obtain 1 kg of benzaldehyde from approximately 200–300 kg of fresh material.

For further development, it is necessary to carry out a series of experiments aimed at screening different species of the genus *Prunus*, especially different varieties of cherry laurel.

In the future, the effect of seasons and the irrigation regime on the benzaldehyde content of the leaves should also be investigated.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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