We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Comprehensive Insight into the Elderflowers and Elderberries (*Sambucus nigra* L.) Mono and Sesquiterpenic Metabolites: Factors that Modulate Their Composition

Ângelo C. Salvador, Armando J. D. Silvestre and
Sílvia M. Rocha

Additional information is available at the end of the chapter

Abstract

Plant secondary metabolites are synthesized for their protection and regulation pur-poses. Quite often, due to their properties, these metabolites have relevant organoleptic and biological properties and can play important roles in human health and general well-being. A relevant case study in this context is berries and flowers from *Sambucus nigra* L., which have been used for generations in folk medicine. Although those effects are mainly linked to phenolic compounds, mono and sesquiterpenic secondary metabolites may also play a key role. Despite their potential, *S. nigra* mono and sesquiterpenic compounds are yet largely unexplored. Complex and dynamic external and internal plant-related phenomena deeply affect terpenes profile, as metabolism, abiotic and biotic stresses, and understanding these phenomena is the first step for *S. nigra* berries and flowers' valua-tion. This chapter will cover aspects linked to elder plant uses, mono and sesquiterpenic composition, and the influence of preharvest and postharvest effects over these metabo-lites. This knowledge is crucial for scientists and industries to understand and improve the quality of *S. nigra*-based products.

Keywords: elderberry, elderflower, *Sambucus nigra* L., secondary metabolites, mono and sesquiterpenic compounds

1. Introduction

A growing interest in the exploitation of natural products as sources of bioactive compounds with potential health benefits has been observed, particularly in the consumption of plant-based products that are able to prevent, ameliorate, or even treat chronic diseases with increasing incidence in the advent of the twenty-first century [1]. The elderberry plant (*S. nigra* L.) may be reported to illustrate this trend, especially due to its potential impact on the local economies as a raw material used to produce, specially, food and beverages, but also nutraceutical and cosmetic products derived from berries and flowers.

S. nigra flowers and berries have been widely used in folk medicine for numerous applications that include antimicrobial, antiviral, antioxidant, anti-inflammatory, anticancer, antidiabetic applications, among others [2–6]. The detailed knowledge of the chemical composition of *S. nigra* is not only extremely important to understand its biological effects but also to improve its value and applicability. Elderberries and elderflowers received increased attention due to the presence of phytochemicals with many reported health benefits, comprising, among others, vitamins, terpenic, and phenolic compounds [5]. Volatile and semi-volatile terpenic components (mono and sesquiterpenics), in particular, are plant secondary metabolites that play key roles in their protection and communication processes. They are often reported as toxic to some microorganisms involved in plant-insect interactions [7, 8]. Additionally, they confer plant protection against oxidative stress, namely as thermo-tolerance mediators [9], playing important roles in their adaptation to biotic and abiotic stresses [10]. On the other hand, the transcriptional regulatory network to different biotic and abiotic stresses is reflected on the plant metabolism, as well as phenological processes, such as on ripening [11], which ultimately will impact the plant secondary metabolites' profile. Nonetheless, these effects are still poorly explored and understood, particularly in the case of *S. nigra.*

Understanding those effects could be of extreme importance, given that mono and sesquiterpenic-based extracts are commercially important, namely for pharmaceutical/nutraceutical, agronomic, food, sanitary, and cosmetic industries. For instance, limonene and linalool, two of the most used monoterpenic compounds, are often employed in perfumes, creams, soaps, as flavor additives for food, as fragrances for household cleaning products and as industrial solvents [8]. In the particular case of potential health benefits-related applications, these compounds have been reported as exhibiting hepatoprotection [12], anti-inflammatory [13], analgesic [14], and antioxidant [15] activities, among others [16]. These effects are strongly dose-dependent, which reinforce the need to study in detail the terpenic composition of *S. nigra* berries and flowers, in-depth, and the variables that have an impact on their composition.

The challenge to understand the impact of pre- and postharvest processes over the mono and sesquiterpenic compounds is the first step to further establish approaches that can control the variables that have a significant effect over these processes. Hence, the present chapter is devoted to a detailed discussion of *S. nigra* mono and sesquiterpenic composition, and of the parameters, such as pre- and postharvest conditions, that modulate their composition. Also, a general perspective of *S. nigra* L. berries and flowers relevance and uses is reported.

2. Elderberry and elderflower applications

Flowers and berries from *S. nigra* species have long been used in folk medicine for prophylactic and therapeutic purposes, being considered the medicinal "chest" from the days of Hippocrates [17]. The relief of early symptoms of common cold using elderflowers fulfills the requirement of medicinal use according to the Committee on Herbal Medicinal Products [3]. Elderflowers were also used to alleviate bronchial and pulmonary diseases, tumors, and ulcers [18]. Laxative [19], treatment of asthma [20], toothache, colic, cold, and rheumatic conditions [21, 22] have been reported, as well as bronchitis, whooping cough, hemorrhoids, expectorant [20], insect bites, and fever [21]. Elderberry preparations are traditionally used as laxative, diaphoretic [2], for fever reduction [19], anti-rheumatic, and to treat colic in infants [23]. Their juice has also been used to treat sciatica, headache, dental pain, heart pain, nerve pain, while the syrup was recommended to treat cough and cold [2].

This species has gained attention due to its diverse uses, assimilating in markets from food and herbal industries. In 2010, *S. nigra* (flowers and berries) was the most harvested medicinal plant intended for export trade and for infusions and phytopharmaceutical production in Bulgaria and Romania [24]. Additionally, elderberry was ranked on the Top 20 best-selling herbal dietary supplements in the medicinal, food, and mass market in the USA in 2011–2014, with increasing sales of 64% in 2014 [24, 25].

One of the main uses of elderberries is the production of natural food colorants, juices, and concentrates, due to their high content in phenolic compounds [26]. Those are also exploited for the formulations of decoctions [2, 19], infusions [23], juice and syrup/concentrate [2], extracts, supplements, pies, ice creams, jellies, juices, beverages, beers, wines, liqueurs, and fruit bars [27–29]. In addition to color, flavor (taste and aroma) is also an important parameter in the consumer perception and product acceptance [30]. Due to the pleasant and characteristic floral aroma, elderflowers are often used as flavoring agents [31] for the preparation of infusions, decoctions [19–22], pastry products [23], nonalcoholic cordials, and fermented beverages [18, 23]. Elderflowers are characterized by an intense, pleasant, and characteristic aroma, currently named as elderflower aroma [32, 33]. Despite the role of esters, alcohols, and aldehydes, monoterpenes, as limonene, terpinolene, and terpinene, present a relevant contribution for the elderflowers fruitiness aroma [34], and more exotic notes, such as woody and spicy, have been attributed to some mono and sesquiterpenic compounds [32, 34].

3. *S. nigra* mono and sesquiterpenic metabolites composition

Terpenic compounds form a large and structurally diverse family of secondary metabolites derived from C_5 isoprene units, with over 35,000 known structures [35]. The volatile and semivolatile ones, that is, mono and sesquiterpenic compounds, result from two main biosynthetic routes, starting from the mevalonate and the methylerythritol phosphate pathways (**Figure 1**). These are produced through the activity of a large family of enzymes, the mono and sesquiterpene synthases and cyclases, but others are formed through transformation of the initial
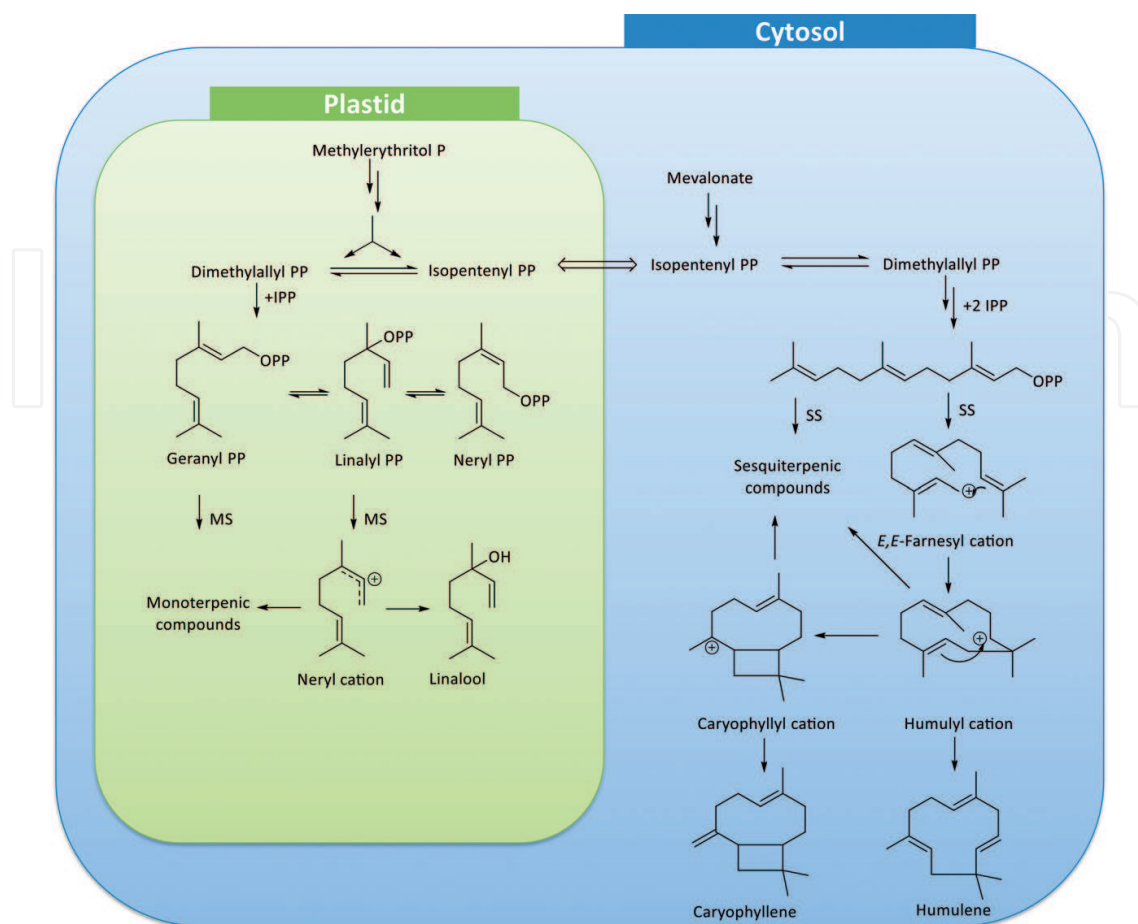
Figure 1. Simplified mono and sesquiterpenic compounds biosynthetic pathways, illustrated with the routes for linalool, caryophyllene and humulene found in *S. nigra*. MS, monoterpene synthases; SS, sesquiterpene synthases; PP, pyrophosphate.

products by acylation, dehydrogenation, oxidation, and other reaction types, such as acetylation [35, 36]. For instance, in **Figure 1**, illustrates the biosynthesis of linalool, caryophyllene, and humulene, three compounds present in *S. nigra* plant. The biosynthesis occurs from their respective linear precursors, geranyl pyrophosphate and farnesyl pyrophosphate, originating. Both linear and cyclic structures [35].

Information about mono and sesquiterpenic compounds from elderflowers and elderberries is still scarce and disperse. Thus, to systematize this data, the information related with ripe berries and fresh flowers and minimally processed products, such as infusions, syrups, and juices, is presented in **Table 1**. The reported studies are mainly focused on analytes' identification rather than on their quantification, however, when available, quantitative data is also provided.

So far, 89 mono and sesquiterpenic compounds are reported in elderflowers (64) and elderberries (61). Recent studies using an advanced gas chromatographic methodology (comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry detection—GC × GC-ToFMS) have contributed to substantially increase the knowledge about *S. nigra* berries and flowers volatile terpenic profile by reporting dozens

Metabolites	Elderflowers[a]	Elderberries[a]	References
Monoterpenic compounds			
Hydrocarbon type			
Camphene	—	✓	[37]
3-Carene	✓	✓	[31, 38, 39]
Cosmene	✓	—	[31, 40]
o-Cymene	—	✓	[41]
p-Cymene	✓	—	[31, 32, 37]
2,6-Dimethyl-2,6-octadiene	✓	—	[31]
ᴅ-Limonene[b]	✓	2.24–9.92	[31, 32, 37, 38, 41–43]
1,3,8-p-Menthatriene	—	✓	[37]
Myrcene	✓	✓	[31, 37, 38, 42]
Ocimene[b]	✓	1.55–9.32	[31, 32, 34, 41]
α-Phellandrene	✓	✓	[32, 34, 37, 41]
α-Pinene	✓	✓	[31, 37]
β-Pinene	✓	✓	[31, 37, 38]
α-Terpinene	✓	✓	[32, 34, 41]
γ-Terpinene	✓	✓	[31, 32, 34, 41]
Terpinolene	✓	✓	[31, 32, 37, 38]
Verbenene	—	✓	[37]
Oxygen-containing type			
Artemisia alcohol	—	✓	[37]
Borneol	—	✓	[37, 41]
Camphor	✓	✓	[37, 41, 42]
3-Caren-2-ol	—	✓	[37]
Carvacrol	✓	—	[42]
Carvone	✓	✓	[37, 42]
1,8-Cineole	✓	✓	[31, 34, 41, 42]
Citral	✓	✓	[31, 37, 41]
Citronellal	✓	—	[31, 37]
Citronellol	✓	✓	[34, 37, 42, 44]
Citronellyl formate	✓	—	[31]
p-Cymen-8-ol	—	✓	[37]
Dehydroxylinalool oxide	✓		[31]
Dihydromyrcenol	—	✓	[37]

Metabolites	Elderflowers[a]	Elderberries[a]	References
Fenchol	—	✓	[37]
Fenchone	✓	—	[31]
Geranial	✓	✓	[31, 41]
Geraniol[b]	✓	1.05–7.21	[31, 32, 37, 41]
Geranyl acetate	—	✓	[37]
Hydroxylinalool	✓	—	[32, 34]
Hotrienol[b]	✓	2.56–8.08	[31, 34, 37, 40–44]
Hydroxycitronellol	—	✓	[41]
Lilac aldehyde	✓	—	[31]
Lilac alcohol	✓	—	[31]
Limonene oxide	✓	—	[31]
Linalool[b]	✓	1.18–128.89	[31, 37, 40–43, 45]
E-Linalool oxide (furanic form)	✓	✓	[31, 37, 40, 42, 44]
Z-Linalool oxide (furanic form)	✓	✓	[31, 37, 40, 42, 44]
E-Linalool oxide (pyranic form)	✓	—	[31, 32, 34, 40, 42, 44]
Z-Linalool oxide (pyranic form)	✓	—	[31, 32, 34, 40, 42, 44]
Linalool methyl ether	✓	—	[31]
Menthol	✓	✓	[37, 41, 42]
Methyl citronellate	✓	—	[31]
Methyl geranate	✓	—	[31]
Myrcenol	✓	—	[31]
Myternol	✓	—	[31]
Nerol	✓	✓	[31, 32, 37, 41]
Nerolidol	✓	—	[42]
Nerol oxide[b]	✓	1.02–7.80	[31, 34, 41, 42, 45]
Pinocarvone	—	✓	[37]
E-Rose oxide	✓	✓	[31, 34, 37, 40–42, 45]
Z-Rose oxide[b]	✓	1.68–8.34	[31, 34, 37, 40–42, 45]
Tagetone	✓	—	[31]
α-Terpineol[b]	✓	70.85–2699.56	[31, 37, 41, 42, 45]
Terpinen-4-ol	✓	✓	[31, 32, 37, 41]
β-Terpinyl acetate	—	✓	[37]
α-Thujone	✓	—	[31, 42]
β-Thujone	✓	—	[31, 42]
Thymol	✓	—	[42]

Metabolites	Elderflowers[a]	Elderberries[a]	References
Verbenone	✓	—	[31]
Sesquiterpenic compounds			
Hydrocarbon type			
Aromadendrene	✓	✓	[31, 37]
α-Bergamotene	✓	—	[31]
β-Bourbonene	✓	✓	[31, 37]
Cadinene	✓	✓	[31, 37]
α-Calacorene	—	✓	[37]
Calamenene	✓	✓	[31, 37]
Calarene	—	✓	[43]
β-Caryophyllene	✓	✓	[31, 32, 34, 37]
α-Copaene	✓	✓	[31, 37, 42]
Cubebene	✓	✓	[31, 37]
β-Elemene	✓	✓	[31, 37]
α-Farnesene	✓	—	[31]
D-Germacrene	✓	—	[31]
α-Humulene	—	✓	[31, 38]
Longifolene	—	✓	[37]
α-Muurolene	—	✓	[37]
Oxygen-containing type			
β-Bourbonen-13-ol	—	✓	[37]
t-Cadinol	—	✓	[37]
Caryophyllene oxide	—	✓	[37]
Cubenol	—	✓	[37]
Globulol	—	✓	[37]
Epiglobulol	—	✓	[37]

[a]When available, quantitative information was reported;

[b]µg/kg of fresh berries; Marks "✓" correspond to nonquantified compounds or quantified but not expressed as berry or flower weight basis.

Table 1. Mono and sesquiterpenic compounds reported in *S. nigra* L. berries and flowers and related products, such as infusions, syrups, or juices.

of monoterpenic and sesquiterpenic compounds for the first time in these matrices [31, 37]. Representative total ion GC × GC chromatogram contour plots from fresh elderflowers and ripe elderberries are illustrated in **Figure 2**, highlighting the complexity of the natural matrices.
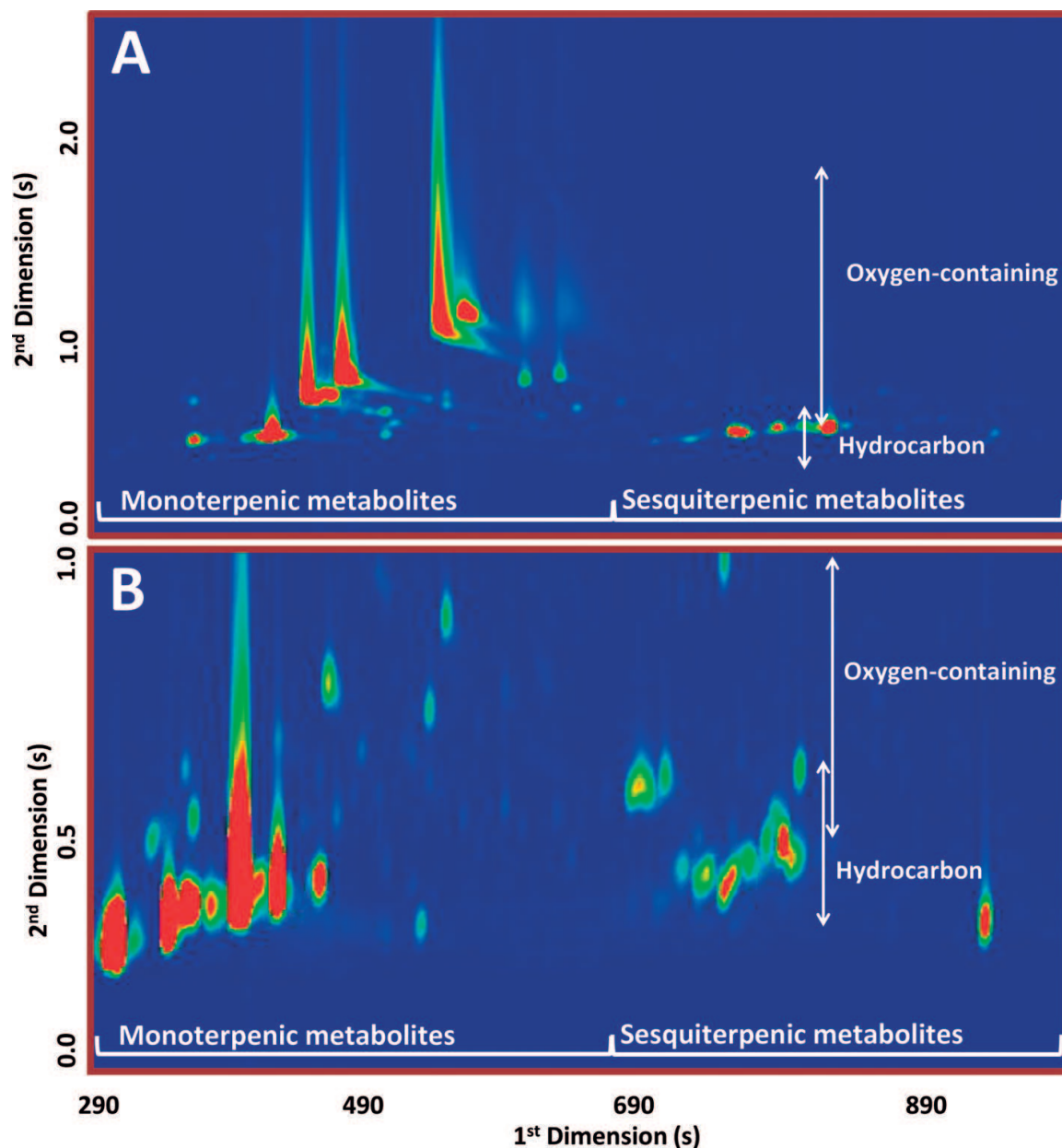
Figure 2. GC × GC–ToFMS chromatogram contour plots from fresh elderflowers (A) and fresh ripe elderberries (B). The chromatographic spaces corresponding to monoterpenic and sesquiterpenic compounds are highlighted.

As evidenced in **Table 1**, of the 64 volatile terpenic compounds reported from elderflowers, 40 are oxygen-containing structures. As shown in **Figure 2**, the peak intensities of the monoterpenic metabolites predominate, representing up to 99 and 77% of the overall elderflowers and elderberries terpenic content, respectively [31, 37]. Linalool oxide (in the pyranoid form) is a major component from fresh elderflowers, accounting for up to 87% (relative to the overall GC peak area) [31]. Other authors reported that hotrienol (14%, w/w), rose oxide (5%, w/w), linalool (4%, w/w), and linalool oxide (furanic forms, 3%, w/w) were the major monoterpenic metabolites from dried elderflowers [42] (chemical structures illustrated in **Figure 3**).

Regarding ripe elderberries, limonene and *p*-cymene are reported as the major monoterpenic components (**Figure 3**). Along with limonene (2.2–9.9 μg/kg of fresh berries), other authors reported as major components in fresh elderberries the monoterpenic compounds

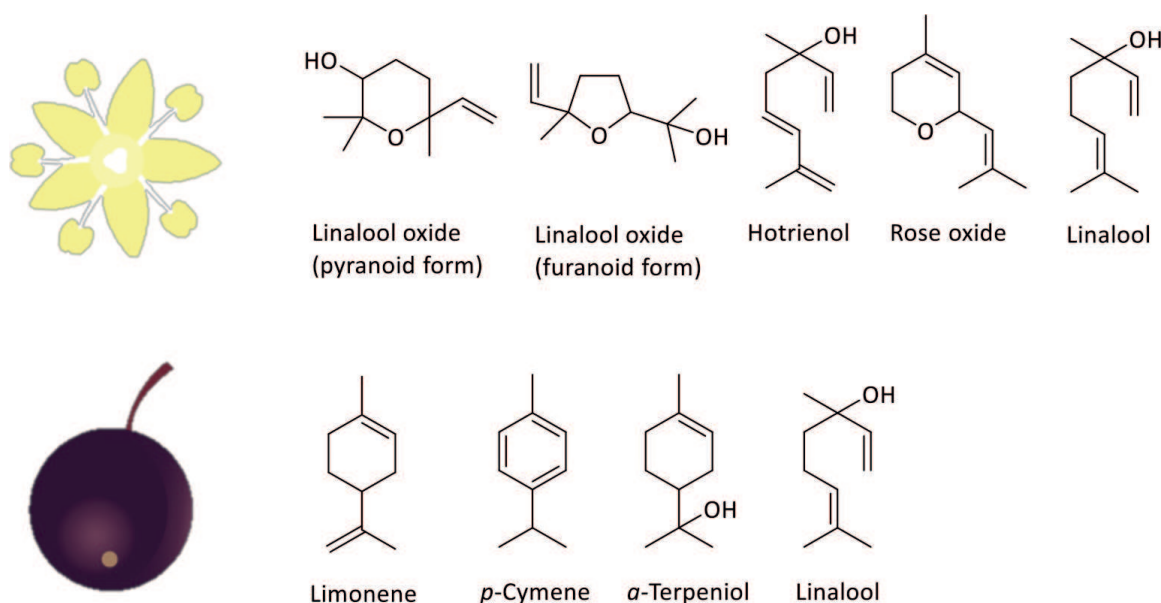**Figure 3.** Main monoterpenic components from *S. nigra* flowers and berries.

linalool (1.2–128.9 µg/kg of fresh berries) and α-terpineol (70.8–2699.5 µg/kg of fresh berries) (**Figure 3**) [41]. Monoterpenic compounds also prevailed in its juice, ranging from 8.9 to 77.2 ng/mL, limonene and linalool being the main monoterpenic components (**Figure 3**) [38]. Sesquiterpenic compounds are present in lower amounts, when compared to the monoterpenic ones, both in elderflowers and in elderberries. They represent up to 0.6% in elderflowers, with β-caryophyllene and α-farnesene as major sesquiterpenic components, while in elderberries, they account for up to 13% of the terpenic content, being β-caryophyllene and aromadendrene the major ones [31, 37]. No quantitative data for the resquiterpenic composition of fresh flowers and berries is available in literature.

4. Factors that modulate mono and sesquiterpenic profile

4.1. Preharvest impact

Crop quality could be defined as a set of agronomic/commercial, organoleptic, and nutritional qualities that are variable among (1) distinct species but also among different cultivars within the same species (genetic factors); (2) different climatic conditions, such as water availability and light exposition; and (3) different agronomic conditions, such as cultivation systems, fertilization, and harvesting date [46]. Altogether, these preharvest factors may have an impact on the final quality of the elderberry fruits and flowers; however, the information about these effects is scarce. The impact of preharvest factors is often focused on parameters with direct agronomic and commercial relevance, as plant yield, fruit size, sugar content and acidity (e.g., reviews on *S. nigra* plant [18, 23]), from which some nutritional quality parameters can be inferred. However, the comprehensive impact of these parameters on the chemical composition, specially in what concerns the target molecules with determining biological properties, still remains unknown. As relevant examples in the present appraisal, the impact of preharvest factors on *S. nigra* mono and sesquiterpenic compounds is still in the beginning and the

available literature is mainly focused on ripening and cultivar effects. However, considering that these components, as plant secondary metabolites, play an important role in plant growth and development, in the interaction with surrounding environment, such as temperature, water, radiation, chemicals, mechanical (as wind or soil movement), pathogen attacks, and nutrient deficiencies [10, 47, 48], as well as in their potential health benefits, the interest in the detailed understanding of the impact preharvest parameters on their profile is of obvious interest.

The production of terpenic metabolites depends on the physiological and developmental stage of the plant [10, 37]. Fruit ripening, in particular, is a crucial phenomenon that affects different physiological and biochemical processes, which are determinant to the development of nutritional and organoleptic characteristics [30]. The fruit organoleptic characteristics such as taste, color, and aroma are important quality and consumer acceptance-determining features [30, 49].

During ripening, major events occur, including cell expansion and softening, dismantling of the photosynthetic apparatus, and degradation of chlorophyll [11]. Elderberry ripening takes place from the 1 to 2-month period, starting with a green appearance and they ripen over a period of 6–8 weeks from July to September (depending on the geographic location). When elderberries become ripe, they have a characteristic deep purple color [23]. The accumulation of sugars (expressed as total soluble solids [TSS]) and decrease in acidity (pH and titratable acidity [TA]) have been routinely used by growers as a decision-making parameter to establish the harvesting moment and even the commercial price of the berries [18, 23, 37]. The ripe elderberries' pH ranges from 3.8 to 4.8; TA ranges from 0.48 to 1.43 g citric acid/100 g FW berries, while TSS ranges from 10.1 to 17.5°Brix [37, 38, 50, 51]. **Figure 4** illustrates the impact of ripening in those tree parameters on elderberries harvested in a Portuguese location (Tarouca, Távora and Varosa Valley), in the harvest season of 2013.
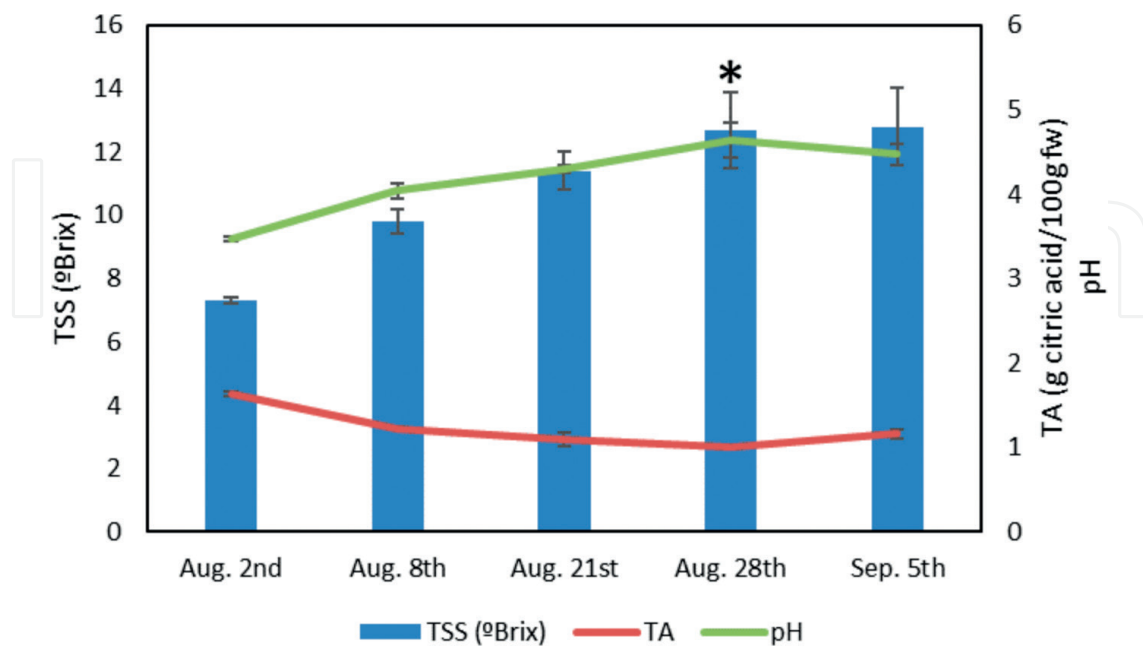

Figure 4. Total soluble solids (TSS), titratable acidity (TA), and pH from elderberries at five ripening stages [37], harvested in a Portuguese plantation (Tarouca, Távora and Varosa Valley), in the harvest season of 2013. The harvesting date assigned with * represents the ripe stage.

During the ripening process, several other phenomena occur, namely biosynthesis and degradation of a wide range of secondary metabolites that may have direct relevance in elderberry sensorial characteristics. A recent metabolomics-based study that exploited the effects of the developmental stages of different cultivars on the volatile terpenic components [37] demonstrated that the variability of monoterpenic compounds (β-pinene, 1,3,8-*p*-menthatriene, terpinolene, dihydromyrcenol, fenchol, α-terpineol, and citral) and of the sesquiterpene β-elemene was linked to elderberries ripening. Overall, monoterpenic and sesquiterpenic content exhibited a similar trend of variation through ripening, that is, gradually decreased over the ripening stages, which was mainly ruled by the major components, namely limonene, *p*-cymene, β-caryophyllene, and aromadendrene. These components were proposed as quality markers to follow-up the ripening process [37].

Plant cultivars generally differ in yield, organoleptic, and nutritional characteristics [23, 46], and their genetic background is a factor that influences quality traits [46]. In the particular case of elderberries, cultivars are classified based on their morphological characteristics and yield [52], as no definitive taxonomic DNA-based studies have been conducted in this species. Although, efforts have been made for their classification with molecular data. For instance, Portuguese *S. nigra* clones explored from local growers using different molecular characterization tools [52], and a genebank has been created for different *Sambucus* species and dozens of cultivars [53].

It is reported that elderberry yield ranges anywhere from 1 to over 30 kg per bush, depending on cultivar [54, 55]. This aspect, together with the fact that several cultivars are nowadays explored for the formulation of various products, where formula standardization is required, implying the comparison of cultivars' composition, can play a significant role in their application (e.g., [56]) and then become important decision tool for producers. The fact that mono and sesquiterpenic synthesis is encoded by a variety or cultivar-related genes implies that their levels can be cultivar-dependent, which, on the one hand, might be used to trace its varietal origin [57] and, on the other hand, can be used to better manage their final product and to maximize the commercial value of the crop. An exploratory study, suggested a possible cultivar effect over the mono and sesquiterpenic compounds profile from fresh elderflowers [31]; however, more consolidated data is still required to sustain the stated remarks, namely in what concerns the number of analyzed samples and different harvesting years.

The specific cultivar metabolite profile may imply differences at the sensorial level in *S. nigra*-based products, as shown for elderflower- and elderberry-based products obtained from different cultivars [32, 34, 56]. In a study that merges the results from the sensory evaluation and information on the aroma of the individual volatile compounds, the results highlighted that different elderberry cultivars had specific sensory characteristics (as fresh-fruity-sweet aroma) and, hence, volatile composition [38]. Differences in linalool and α-terpineol (ranging from 2.8 to 21.7 and from 213.6 to 2699.6 μg/kg, respectively) were reported for thawed ripe elderberries from different cultivars [41]. Likewise, the terpenic alcohols and oxides in elderflowers from different cultivars ranged from 0.8 to 3870 ng/mL for hotrienol; from 1.2 to 2320 ng/mL for *cis*-rose oxide; from 2.3 to 1840 ng/mL for linalool; and from 1.3 to 1100 ng/mL for linalool oxide (furanic form) [32, 34].

Despite the studies reported earlier, a more comprehensive understanding of the influence of preharvest parameters will require their analysis in an integrated approach, including,

among others, climate, agricultural practices, soil, and harvesting year to fully understand how these affect the biochemical mechanisms involved in the formation of mono and sesquiterpenic metabolites from elderflowers and berries and also to improve its valorization potential, particularly when related with health benefits and relevant sensorial characteristics. Also, the influence of climate change on the *S. nigra* plant response should be a noteworthy issue.

4.2. Postharvest impact

Postharvest management includes a set of postproduction practices comprising, among others, cleaning to eliminate undesirable elements and improve product appearance, sorting, cooling, control of variables such as temperature and relative humidity, and packing, ensuring that the product complies with the established quality standards for fresh and processed products [58, 59]. Postharvest practices may deeply affect the quality of a product in many aspects such as chemical and sensorial characteristics but also their potential health benefits, and ultimately, it may affect product's acceptability and marketability [30]. Therefore, reliable and objective quality-control tools to measure the impact of postharvest practices (ideally integrated with preharvesting conditions) over product quality and in the present appraisal on sensory quality are essential.

Elderflowers and elderberries go through different postharvest handling and storage conditions that precede processing, to prepare stable formulations for commercialization. **Figure 5** illustrates the main steps from harvesting for the storage of elderberries and elderflowers and the main chemical changes that may occur throughout these processes [31, 33, 42, 60–63].

S. nigra flowers or berries are typically collected during the morning and transported to processing facilities in specific plastic crates, avoiding damage caused by their own weight [5]. Flowers are often frozen or air-dried and then the stems are removed, while elderberries are sun dried or refrigerated, and the stems are removed and stored in silos at subzero
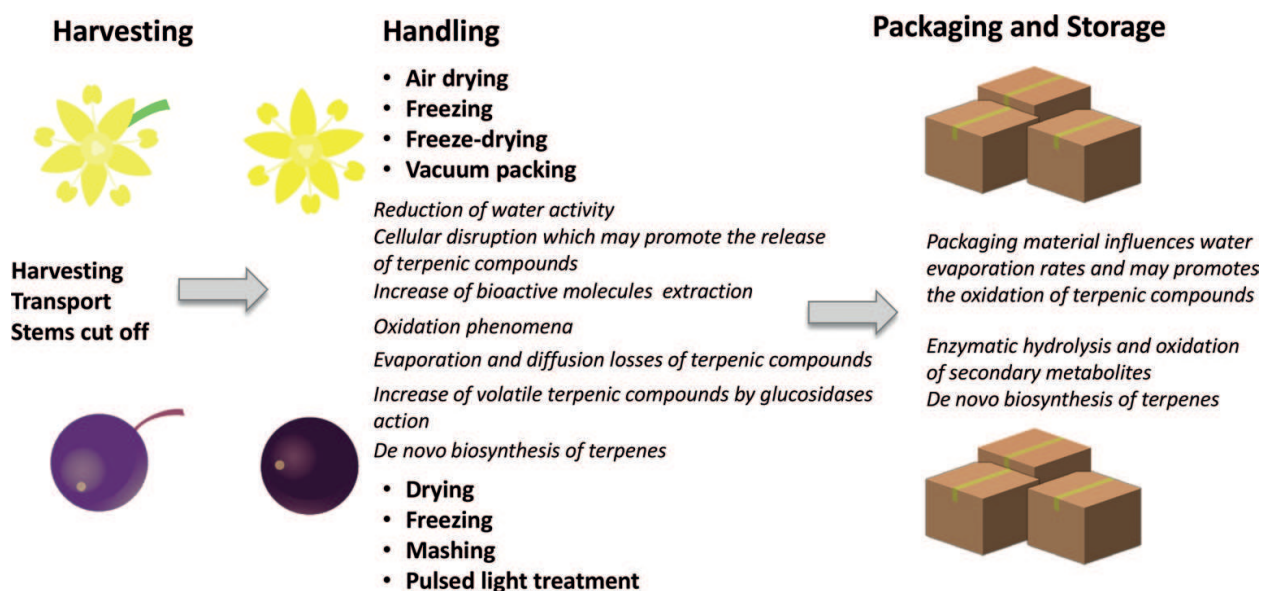

Harvesting

Harvesting
Transport
Stems cut off

Handling

- Air drying
- Freezing
- Freeze-drying
- Vacuum packing

Reduction of water activity
Cellular disruption which may promote the release of terpenic compounds
Increase of bioactive molecules extraction

Oxidation phenomena
Evaporation and diffusion losses of terpenic compounds
Increase of volatile terpenic compounds by glucosidases action
De novo biosynthesis of terpenes

- Drying
- Freezing
- Mashing
- Pulsed light treatment

Packaging and Storage

Packaging material influences water evaporation rates and may promotes the oxidation of terpenic compounds

Enzymatic hydrolysis and oxidation of secondary metabolites
De novo biosynthesis of terpenes

Figure 5. From harvesting to elderberry and elderflower storage. The main chemical changes that may occur through different steps are included [31, 33, 42, 60–63].

temperatures [5]. Elderberries can also be pulse-light treated and further crushed and mashed to produce concentrate juices [64–67]. These later steps promote the degradation of berry cell walls, contributing to the alteration of metabolites' profile, namely increasing the anthocyanin content of juices [63]. All these handling and storage processes may have an impact on the chemical composition of these matrices, as discussed as follows.

The knowledge of the impact of handling and storage conditions on the terpenic metabolites of *S. nigra* is still scarce for both, berries and flowers. However, more information is available regarding postharvest effects over the elderflowers' matrix. For instance, the impact of freezing, freeze-drying, air-drying, and vacuum packing over the volatile terpenic compounds was monitored for up to 1 year (**Figure 6**) [31].

After 1 year of storage, a decrease of the total terpenic content up to 47% for frozen elderflowers; up to 67 and 71% when vacuum packed and kept under light exposure and without light exposure, respectively; up to 82% for air-dryed elderflowers; and up to 85% for freeze-dried elderflowers (**Figure 6**) [31]. Under vacuum packing, there was no significant impact from light exposure. Linalool oxides were suggested as markers of the impact of the studied postharvest conditions over the volatile terpenic metabolites of elderflowers [31].

Drying methodologies, as air-drying or freeze-drying, often fail to completely preserve volatile aroma compounds [68], as reported in dried elderflowers, mainly due to diffusion and evaporation losses [31, 33, 69, 70]. Drying of elderberries or their products promotes a water activity reduction, contributing to the preservation of the samples against microbial contamination and also decreases the degradation of anthocyanins [60], by increasing their stability
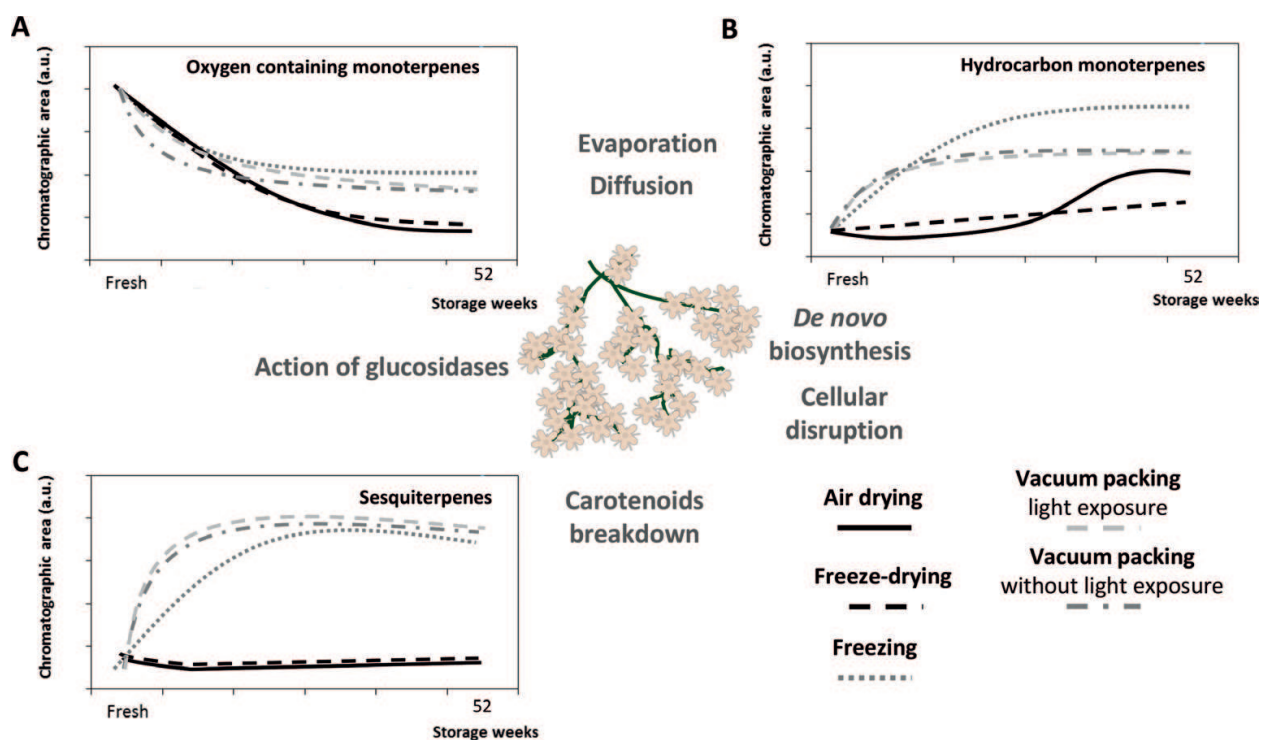

Figure 6. Variation trends of the abundance of oxygen-containing monoterpenes (A), hydrocarbon monoterpenes (B) and sesquiterpenes (C) toward the different handling and storage conditions for up to 1 year, based on the corresponding GC peak areas (au: Arbitrary units). Adapted with permission [31].

[60]. Other strategies have been used to preserve the elderberries' bioactive components or to enhance their nutritional value, as for instance, their processing with pulsed ultraviolet light to enhance the phenolic content [61]. However, no studies were performed so far on mono and sesquiterpenic fractions of elderberries.

Storage time also plays an important role in the mono and sesquiterpenic composition illustrated by the fact that 15 compounds, including rose oxides, hotrienol, linalool, α-terpineol, hydroxylinalool, and limonene, partially or completely vanished during storage of dried elderflowers [33]. Cellular disruption might explain the release of volatile compounds in certain postharvest conditions, namely freezing storage [31, 71, 72]. Likewise, the packaging material might affect their profile, being reported that elderflower's tea bags made of aluminum had the highest average concentrations of rose oxide, linalool oxide, nerol oxide, and hotrienol, when compared to paper and plastic bags [33].

Some components, such as hotrienol, were observed to increase during storage of elderflowers, which could be associated with the action of enzymes, such as glucosidases, that unbound the volatile components from glycosides present in the matrix [33]. Non-oxygen-containing structures, that is, monoterpenes and sesquiterpenes, also increased under certain postharvest conditions (**Figure 6**), again assuming that *de novo* biosynthesis of terpenes may play a key role in this phenomenon [31, 33].

The modifications in *S. nigra* terpenic profile upon different postharvest conditions have a significant impact on sensorial characteristics of its products, being linked to a dynamic and complex network of enzymatic and physicochemical phenomena. Understanding the postharvest impact is a step forward to manage and control the production of elderflower and elderberry formulations [31].

5. Concluding remarks

Plant secondary metabolites play key role in the plants' protection and communication processes. Beyond that, these components, and particularly, mono and sesquiterpenic compounds, are nowadays explored in industrial sectors due to their pleasant aroma characteristics and potential on the prevention and management of human diseases. The exploitation of *S. nigra* L. plant has gained increasing attention in the last decade. Literature highlights that this plant has been used, both, for the formulation of food products and in folk medicine. More recently, herbal supplements and nutraceuticals are also available. Given the diversity of biological activities reported for mono and sesquiterpenic components, as hepatoprotection [12], anti-inflammatory [13], analgesic [14], and antioxidant [15], among others [16], it allows to infer strong potential to foster its economic value. Actually, to go further in that direction, several challenges have to be overcome, namely (1) to in-depth know the mono and sesquiterpenic composition, (2) to study the impact of preharvest factors, and (3) to select the appropriate postharvest procedures to preserve bioactive molecules, as elderflower and elderberries seasonality requires handling and storage steps. Quality of crops, specifically elderberries and elderflowers, is a complex concept which could be defined by the yield efficiency, the organoleptic and the nutritional quality [46]. If,

for several years, the agronomic and organoleptic qualities were the main market drivers, nowadays, the importance of the nutritional value is strongly increasing, thanks to increased consumer awareness on the dietary health effects of plant consumption [46]. It is for this reason that understanding how *S. nigra* mono and sesquiterpenic metabolites respond to exposures of different biotic and abiotic stresses is of major importance. Understanding and managing the effects of preharvest and postharvest factors are critical for further economical exploitation of these natural products, namely to (1) provide the growers of robust decision-making tools, (2) fulfill the current standardization requirements for production of plant-based extracts and products, and (3) contribute to assure the high-quality *S. nigra*-based products.

Acknowledgements

Author details

Ângelo C. Salvador[1,2], Armando J. D. Silvestre[2] and Sílvia M. Rocha[1]*

*Address all correspondence to: smrocha@ua.pt

1 QOPNA, Department of Chemistry, University of Aveiro, Aveiro, Portugal

2 CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

References

[1] World Health Organization. WHO Traditional Medicine Strategy 2014-2023. Vol. 1. Geneva, Switzerland: WHO Press; 2013. pp. 1-78

[2] Committe on Herbal Medicinal Products. Assessment Report on *Sambucus nigra* L., Fructus. Vol. 44. London, UK: European Medicines Agency; 2013. pp. 1-26

[3] Committe on Herbal Medicinal Products. *Sambucus nigra* L., flos. London: European Medicines Agency Evaluation of Medicines for Human Use; 2008. pp. 1-24

[4] Salvador ÂC, Guilherme RJR, Silvestre AJD, Rocha SM. Sambucus nigra berries and flowers health benefits: From lab testing to human consumption. In: Mérillon J-M, Ramawat KG, editors. Bioact. Mol. Food. Cham: Springer International Publishing; 2018. pp. 1-35

[5] Salvador ÂC, Silvestre AJD, Rocha SM. *Sambucus nigra* L.: A potential source of health-promoting components. In: Atta-ur-Rahman F, editor. Front. Nat. Prod. Chem. Vol. 2. Sharjah, U.A.E: Bentham Science Publishers, Ltd.; 2016. pp. 343-392

[6] Smith RE, Tran K, Richards KM, Ryan S, Luo R, Salvador AC, et al. Elderberry juice composition and health benefits. In: Elder KE, editor. Fruit Juices Types, Nutr. Compos. Heal. Benefits—Nutr. Diet Res. Prog. New York: Nova Science Publishers, Inc; 2014. pp. 1-20

[7] Gershenzon J, Dudareva N. The function of terpene natural products in the natural world. Nature Chemical Biology. 2007;**3**:408-414

[8] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils—A review. Food and Chemical Toxicology. 2008;**46**:446-475

[9] Monson RK. Biology, Controls and Models of Tree Volatile Organic Compound Emissions. Dordrecht, The Nederlands: Springer Netherlands; 2013

[10] Ramakrishna A, Ravishankar GA. Influence of abiotic stress signals on secondary metabolites in plants Akula. Plant Signaling & Behavior. 2011;**6**:1720-1731

[11] Pandit SS, Kulkarni RS, Giri AP, Köllner TG, Degenhardt J, Gershenzon J, et al. Expression profiling of various genes during the fruit development and ripening of mango. Plant Physiology and Biochemistry. 2010;**48**:426-433

[12] Vinholes J, Rudnitskaya A, Gonçalves P, Martel F, Coimbra MA, Rocha SM. Hepatoprotection of sesquiterpenoids: A quantitative structure-activity relationship (QSAR) approach. Food Chemistry. 2014;**146**:78-84

[13] De Cássia R, Andrade LN, de Sousa DP. A review on anti-inflammatory activity of monoterpenes. Molecules. 2013;**18**:1227-1254

[14] Guimarães AG, Quintans JSS, Quintans-júnior LJ. Monoterpenes with analgesic activity—A systematic review. Phytotherapy Research. 2013;**15**:1-15

[15] Vinholes J, Gonçalves P, Martel F, Coimbra MA, Rocha SM. Assessment of the antioxidant and antiproliferative effects of sesquiterpenic compounds in *in vitro* Caco-2 cell models. Food Chemistry. 2014;**156**:204-211

[16] Murthy KNC, Jayaprakasha GK, Patil BS. Anti-inflammatory mediated applications of monoterpenes found in fruits. In: Trop. Subtrop. Fruits Flavors, Color. Heal. Benefits. Washington, DC: ACS Symposium Series; 2013. pp. 121-131

[17] Dawidowicz AL, Wianowska D, Gawdzik J, Smolarz DH. Optimization of ASE conditions for the HPLC determination of rutin and isoquercitrin in *Sambucus nigra* L. Journal of Liquid Chromatography & Related Technologies. 2003;**26**:2381-2397

[18] Atkinson MD, Atkinson E. *Sambucus nigra* L. Journal of Ecology. 2002;**90**:895-923

[19] Pieroni A, Giusti ME, Münz H, Lenzarini C, Turković G, Turković A. Ethnobotanical knowledge of the Istro-Romanians of Žejane in Croatia. Fitoterapia. 2003;**74**:710-719

[20] Kültür Ş. Medicinal plants used in Kırklareli Province (Turkey). Journal of Ethnopharmacology. 2007;**111**:341-364

[21] Passalacqua NG, Guarrera PM, De Fine G. Contribution to the knowledge of the folk plant medicine in Calabria region (Southern Italy). Fitoterapia. 2007;**78**:52-68

[22] Camejo-Rodrigues J, Ascensão L, Bonet MÀ, Vallès J. An ethnobotanical study of medicinal and aromatic plants in the natural park of "Serra de São Mamede" (Portugal). Journal of Ethnopharmacology. 2003;**89**:199-209

[23] Charlebois D, Byers PL, Finn CE, Thomas AL. Elderberry: Botany, horticulture, potential. In: Janick J, editor. Hortic. Rev. Vol. 37. Hoboken, New Jersey: John Wiley & Sons, Inc.; 2010. pp. 213-280

[24] Młynarczyka K, Walkowiak-Tomczaka D, Łysiakb G. Bioactive properties of *Sambucus nigra* L. as a functional ingredient for food and pharmaceutical industry. Journal of Functional Foods. 2018;**40**:377-390

[25] Smith T, Lynch M, Johnson J, Kawa K, Bauman H, Blumenthal M. Herbal dietary supplement sales in US increase 6.8% in 2014. HerbalGram. 2015:52-59

[26] Bermúdez-Soto MJ, Tomás-Barberán FA. Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. European Food Research and Technology. 2004;**219**:133-141

[27] Charlebois D, Richer C. Le sureau: exigences de la production, cultivars et potentiel de mise en marché. Agri-Réseau. 2005:1-8

[28] Cernusca M, Gold M, Godsey L. Elderberry Market Research - Report Based on Research Performed in 2009. USA: The Center for Agroforestry. University of Missouri; 2009

[29] Zafrilla P, Valero A, García-Viguera C. Stabilization of strawberry jam colour with natural colourants. Food Science and Technology International. 1998;**4**:99-105

[30] Paliyath G, Murr DP, Handa AK, Lurie S. Postharvest Biology and Technology of Fruits, Vegetables, and Flowers. Ames, Iowa, USA: Wiley-Blackwell; 2008

[31] Salvador ÂC, Silvestre AJ, Rocha SM. Unveiling elderflowers (*Sambucus nigra* L.) volatile terpenic and norisoprenoids profile: Effects of different postharvest conditions. Food Chemistry. 2017;**229**:276-285

[32] Jorgensen U, Hansen M, Christensen LP, Jensen K, Kaack K. Olfactory and quantitative analysis of aroma compounds in elder flower (*Sambucus nigra* L.) drink processed from five cultivars. Journal of Agricultural and Food Chemistry. 2000;**48**:2376-2383

[33] Kaack K, Christensen LP. Effect of packing materials and storage time on volatile compounds in tea processed from flowers of black elder (*Sambucus nigra* L.). European Food Research and Technology. 2008;**227**:1259-1273

[34] Kaack K, Christensen L, Hughes M, Eder R. Relationship between sensory quality and volatile compounds of elderflower (*Sambucus nigra* L.) extracts. European Food Research and Technology. 2006;**223**:57-70

[35] Dewick PM. Medicinal Natural Products: A Biosynthetic Approach. 3rd ed. Chichester, United Kingdom: John Wiley & Sons Ltd; 2009

[36] Faleiro M, Miguel M. Use of essential oils and their components against multidrug-resistant bacteria. In: Rai M, Kon K, editors. Fight. Multidrug Resist. With Herb. Extr. Essent. Oils their Components. London, UK: Academic Press; 2013. pp. 65-94

[37] Salvador ÂC, Rudnitskaya A, Silvestre AJD, Rocha SM. Metabolomic-based strategy for fingerprinting of *Sambucus nigra* L. berry volatile terpenoids and norisoprenoids: Influence of ripening and cultivar. Journal of Agricultural and Food Chemistry. 2016;**64**: 5428-5438

[38] Kaack K, Christensen LP, Hughes M, Eder R. The relationship between sensory quality and volatile compounds in raw juice processed from elderberries (*Sambucus nigra* L.). European Food Research and Technology. 2005;**221**:244-254

[39] Farré-Armengol G, Filella I, Llusià J, Peñuelas J. Pollination mode determines floral scent. Biochemical Systematics and Ecology. 2015;**61**:44-53

[40] Ağalar H, Demirci B, Demirci F, Kırımer N. The volatile compounds of the elderflowers extract and the essential oil. Records of Natural Products. 2017;**11**:491-496

[41] Vítová E, Divišová R, Sůkalová K, Matějíček A. Determination and quantification of volatile compounds in fruits of selected elderberry cultivars grown in Czech Republic. Journal of Food & Nutrition Research. 2013;**52**:1-11

[42] Toulemonde B, Richard HMJ. Volatile constituents of dry elder (*Sambucus nigra* L.) flowers. Journal of Agricultural and Food Chemistry. 1983;**31**:365-370

[43] Ağalar HG, Demirci B, Başer K. The volatile compounds of elderberries (*Sambucus nigra* L.). Natural Volatiles & Essential Oils. 2014;**1**:51-54

[44] Eberhardt R, Pfannhauser W. Analyse flüchtiger Inhaltsstoffe des Holunders. Microchimica Acta. 1985;**85**:55-67

[45] Jensen K, Christensen LP, Hansen M, Jørgensen U, Kaack K. Olfactory and quantitative analysis of volatiles in elderberry (*Sambucus nigra* L.) juice processed from seven cultivars. Journal of the Science of Food and Agriculture. 2001;**81**:237-244

[46] Di Vittori L, Mazzoni L, Battino M, Mezzetti B. Pre-harvest factors influencing the quality of berries. Scientia Horticulturae. 2018;**233**:310-322

[47] Seigler DS. Plant Secondary Metabolism. New York, USA: Springer Science+Business Media, LLC; 1998

[48] Mahajan S, Tuteja N. Cold, salinity and drought stresses: An overview. Archives of Biochemistry and Biophysics. 2005;**444**:139-158

[49] Shi Y, Jiang L, Zhang L, Kang R, Yu Z. Dynamic changes in proteins during apple (Malus x domestica) fruit ripening and storage. Horticulture Research. 2014;**1**:6

[50] Thomas AL, Perkins-veazie P, Byers PL, Finn CE, Lee J. A comparison of fruit characteristics among diverse elderberry genotypes grown in Missouri and Oregon. Journal of Berry Research. 2013;**3**:159-168

[51] Lee J, Finn CE. Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. Journal of the Science of Food and Agriculture. 2007;**87**:2665-2675

[52] Lima-Brito J, Castro L, Coutinho J, Morais F, Gomes L, Guedes-Pinto H, et al. Genetic variability in *Sambucus nigra* L. clones: A preliminary molecular approach. Journal of Genetics. 2013;**90**:47-52

[53] Bushakra JM, Bassil N, Finn CE, Hummer KE. *Sambucus* genetic resources at the U.S. National clonal germplasm repository. Acta Horticulturae. 2015;(1061):135-146

[54] Finn CE, Thomas AL, Byers PL, Serce S. Evaluation of American (*Sambucus canadensis*) and European (*S. nigra*) elderberry genotypes grown in diverse environments and implications for cultivar development. Hortscience. 2008;**43**:1385-1391

[55] Wazbinska J, Puczel U, Senderowska J. Yield in elderberry cultivars grown on two different soils in 1997-2003. Journal of Fruit and Ornamental Plant Research. 2004;**12**:175-181

[56] Kaack K. Aroma composition and sensory quality of fruit juices processed from cultivars of elderberry (*Sambucus nigra* L.). European Food Research and Technology. 2008;**227**:45-56

[57] Rocha SM, Coelho E, Zrostlíková J, Delgadillo I, Coimbra MA. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry of monoterpenoids as a powerful tool for grape origin traceability. Journal of Chromatography A. 2007;**1161**:292-299

[58] Arah I, Arah IK, Amaglo H, Kumah EK, Ofori H. Preharvest and postharvest factors affecting the quality and shelf life of harvested tomatoes: A mini review preharvest and postharvest factors affecting the quality and shelf life of harvested tomatoes : A mini review. International Journal of Agronomy. 2016;**2015**:478041

[59] El-ramady HR, Domokos-szabolcsy É, Abdalla NA, Taha HS, Fári M. Postharvest management of fruits and vegetables storage. In: Lichtfouse E, editor. Sustain. Agric. Rev. Vol. 15. Switzerland: Springer International Publishing; 2013. pp. 65-152

[60] Flink JM. Anthocyanin colourants from elderberry (*Sambucus nigra* L.). 2. Process considerations for production of a freeze dried product. Journal of Food Technology. 1985;**20**:713-723

[61] Murugesan R, Orsat V, Lefsrud M. Effect of pulsed ultraviolet light on the total phenol content of elderberry (*Sambucus nigra*) fruit. Food and Nutrition Sciences. 2012;**3**:774-783

[62] Kaack K, Christensen LP. Phenolic acids and flavonoids in tea processed from flowers of black elder (*Sambucus nigra* L.) stored in different packing materials. European Journal of Horticultural Science. 2010;**75**:214-220

[63] Galić A, Dragović-Uzelac V, Levaj B, Kovačević D, Pliestić S, Arnautović S. The polyphenols stability, enzyme activity and Physico-chemical parameters during producing wild elderberry concentrated juice. Agriculturae Conspectus Scientificus. 2009;**74**:181-186

[64] Kaack K. Processing of aroma extracts from elder flower (*Sambucus nigra* L.). European Food Research and Technology. 2008;**227**:375-390

[65] Plieninger AK. Process for preparing a natural extract. EP2186424 A1, 2008

[66] Valovic I, Nuhlicek V. Method for producing a beverage syrup and a refreshing beverage prepared from said syrup. EP1507462 B1, 2002

[67] Kaack K, Austed T. Interaction of vitamin C and flavonoids in elderberry (*Sambucus nigra* L.) during juice processing. Plant Foods for Human Nutrition. 1998;**52**:187-198

[68] Abascal K, Ganora L, Yarnell E. The effect of freeze-drying and its implications for botanical medicine: A review. Phytotherapy Research. 2005;**19**:655-660

[69] Venskutonis R, Poll L, Larsen M. Influence of drying and irradiation on the composition of volatile compounds of thyme (*Thymus vulgaris* L.). Flavour and Fragrance Journal. 1996;**11**:123-128

[70] Venskutonis P. Effect of drying on the volatile constituents of thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.). Food Chemistry. 1997;**59**:219-227

[71] de Ancos B, Ibañez E, Reglero G, Cano MP. Frozen storage effects on anthocyanins and volatile compounds of raspberry fruit. Journal of Agricultural and Food Chemistry. 2000;**48**:873-879

[72] Siegmund B, Innerhofer G, Pabi N, Fedl D, Leitner E. The processing of elder flowers (*Sambucus nigra* L.) as a major factor of influence for the sensory quality of elder flower products. In: Current Topics Flavor Chemistry & Biology—Proceedings of the 10th Wartbg. Symp.; 2013. pp. 176-82