
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

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Abstract

Plant secondary metabolites are synthesized for their protection and regulation purposes. 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' valuation. This chapter will cover aspects linked to elder plant uses, mono and sesquiterpenic composition, and the influence of preharvest and postharvest effects over these metabolites. 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 C₅ isoprene units, with over 35,000 known structures [35]. The volatile and semi-volatile 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

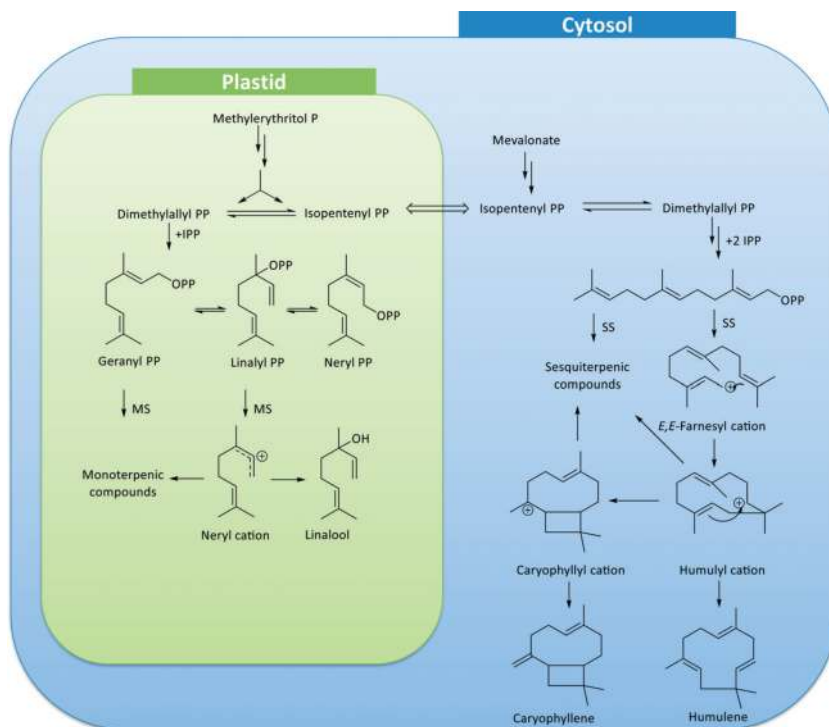


Figure 1. Simplified mono and sesquiterpenic compounds biosynthetic pathways, illustrated with the routes for linalool, caryophyllene and humulene found in *S. nigra*. MS, monoterpene synthases; SS, sesquiterpene synthases; PP, pyrophosphate.

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			
<i>Hydrocarbon type</i>			
Camphene	—	✓	[37]
3-Carene	✓	✓	[31, 38, 39]
Cosmene	✓	—	[31, 40]
<i>o</i> -Cymene	—	✓	[41]
<i>p</i> -Cymene	✓	—	[31, 32, 37]
2,6-Dimethyl-2,6-octadiene	✓	—	[31]
<i>D</i> -Limonene ^b	✓	2.24–9.92	[31, 32, 37, 38, 41–43]
1,3,8- <i>p</i> -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]
<i>Oxygen-containing type</i>			
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]
<i>p</i> -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]
<i>E</i> -Linalool oxide (furanic form)	✓	✓	[31, 37, 40, 42, 44]
<i>Z</i> -Linalool oxide (furanic form)	✓	✓	[31, 37, 40, 42, 44]
<i>E</i> -Linalool oxide (pyranic form)	✓	—	[31, 32, 34, 40, 42, 44]
<i>Z</i> -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]
<i>E</i> -Rose oxide	✓	✓	[31, 34, 37, 40–42, 45]
<i>Z</i> -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			
<i>Hydrocarbon type</i>			
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-Murolene	—	✓	[37]
<i>Oxygen-containing type</i>			
β-Bourbonen-13-ol	—	✓	[37]
t-Cadinol	—	✓	[37]
Caryophyllene oxide	—	✓	[37]
Cubenol	—	✓	[37]
Globulol	—	✓	[37]
Epiglobulol	—	✓	[37]

^aWhen available, quantitative information was reported;

^bμg/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.

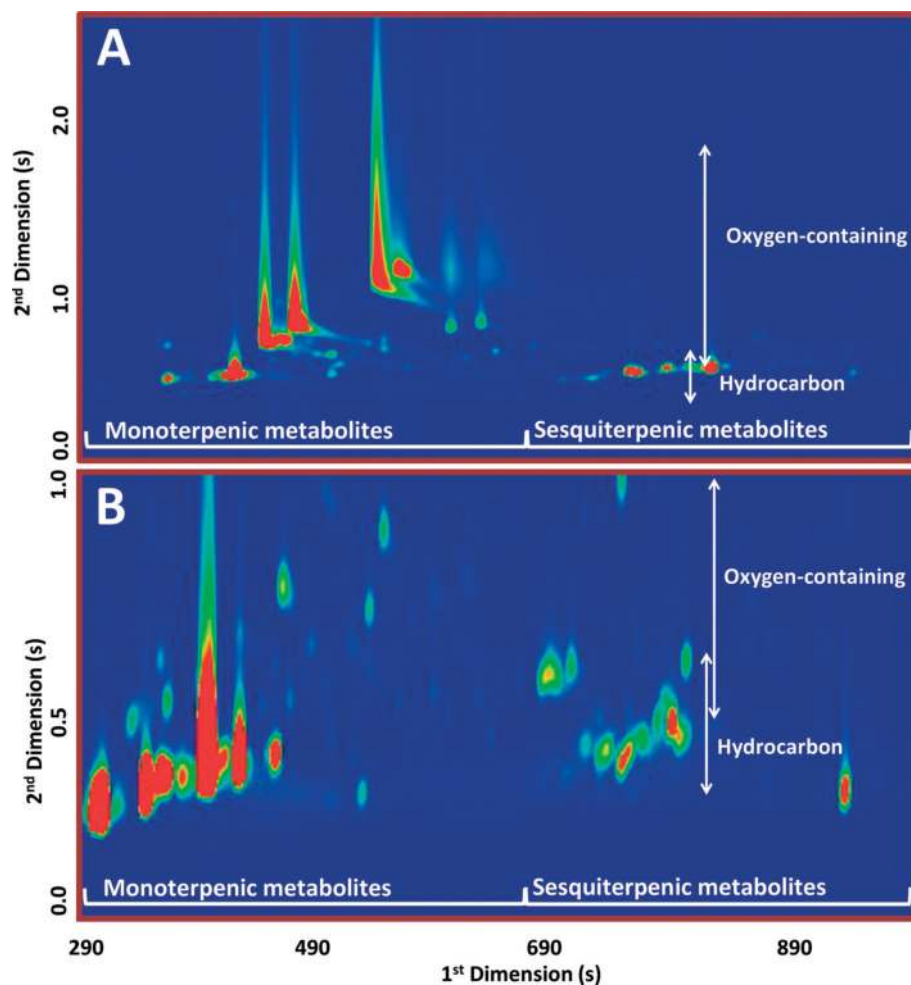


Figure 2. GC \times GC–ToFMS chromatogram contour plots from fresh elderflowers (A) and fresh ripe elderberries (B). The chromatographic spaces corresponding to monoterpenic and sesquiterpenic compounds are highlighted.

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 $\mu\text{g}/\text{kg}$ of fresh berries), other authors reported as major components in fresh elderberries the monoterpenic compounds

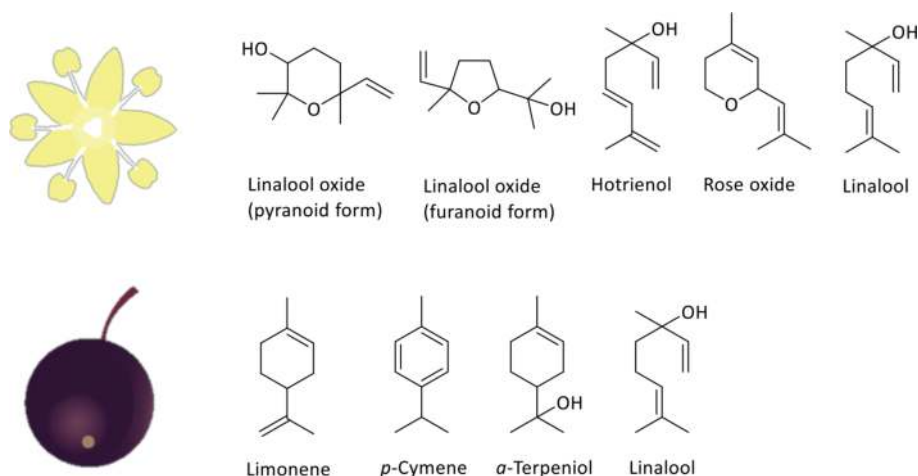


Figure 3. Main monoterpene components from *S. nigra* flowers and berries.

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

4. Factors that modulate mono and sesquiterpene 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 sesquiterpene 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.

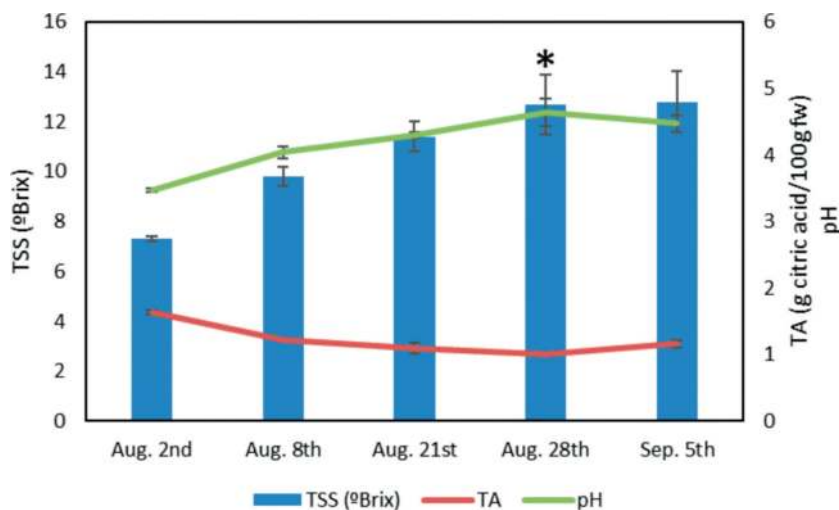


Figure 4. Total soluble solids (TSS), titratable acidity (TA), and pH from elderberries at five ripening stages [37], harvested in a Portuguese plantation (Tarouca, Távora and Varosa Valley), in the harvest season of 2013. The harvesting date assigned with * represents the ripe stage.

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 monoterpene 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, monoterpene and sesquiterpene 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 sesquiterpene 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 sesquiterpene 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 $\mu\text{g}/\text{kg}$, 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

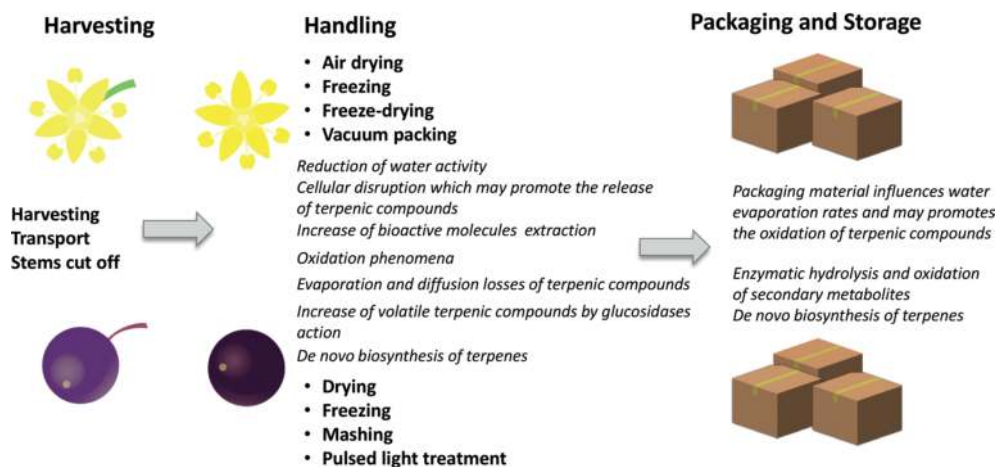


Figure 5. From harvesting to elderberry and elderflower storage. The main chemical changes that may occur through different steps are included [31, 33, 42, 60–63].

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-dried 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 post-harvest 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

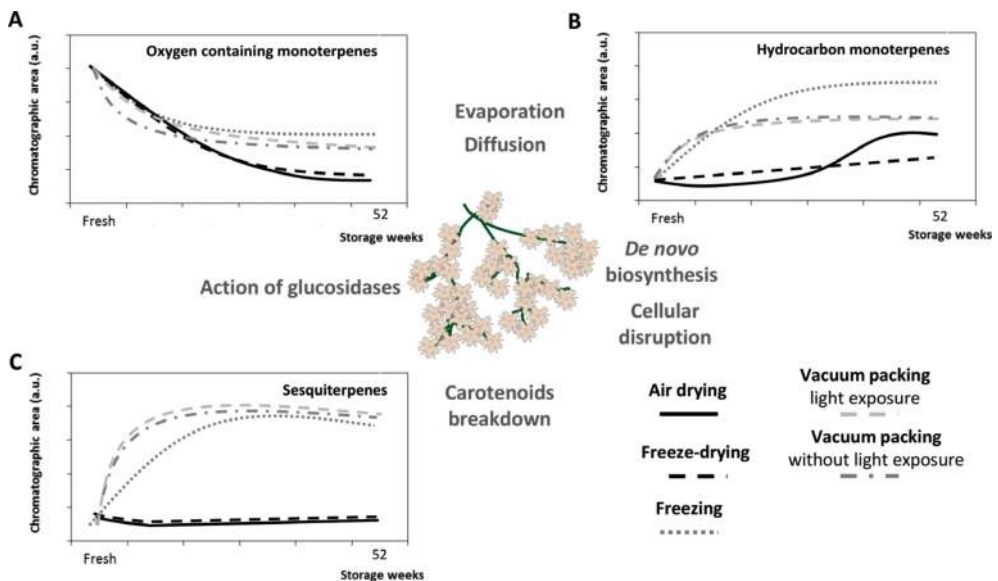


Figure 6. Variation trends of the abundance of oxygen-containing monoterpenes (A), hydrocarbon monoterpenes (B) and sesquiterpenes (C) toward the different handling and storage conditions for up to 1 year, based on the corresponding GC peak areas (au: Arbitrary units). Adapted with permission [31].

[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.

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Author details

Ângelo C. Salvador^{1,2}, Armando J. D. Silvestre² 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

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