SAMBUCUS NIGRA FRUITS AND THEIR PROCESSING SOLUTIONS: A REVIEW

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Abstract
Sambucus nigra has garnered attention in the food industry for its natural colouring and antioxidant properties, mainly due to its high content of anthocyanins, other polyphenols, and vitamins. These bioactive compounds not only contribute to the nutritional impact of food products, but also make it possible to replace synthetic additives that meet the current consumer demand for natural and functional foods. This study provides an overview of the bioactive compounds in *Sambucus nigra* (black elder) fruits, highlighting their potential health benefits and toxicity concerns. It examines the effect of different processing technologies on the phenolic and cyanogenic glycoside composition of elderberry, emphasizing the need to balance microbial decontamination with the sensory quality and nutritional value of food products. The study also reviews the mechanism of ultrasonic inactivation of microorganisms and the potential of fermentation to reduce toxic compounds in *Sambucus nigra* fruits, thus contributing to the improvement of food safety and nutritional quality, emphasizing the importance of considering the potential limitations of ultrasound and the use of combined methods to increase efficiency while minimizing the negative impact on both the product and the consumer. Additionally, the study discusses the significance of selecting appropriate processing methods to ensure the safety of elderberry products, given the presence of potentially harmful cyanogenic glycosides and lectins, which can be mitigated through heat treatment and fermentation.
Key words: Black elderberry, cyanogenic glycosides, fermentation, lectins, ultrasound treatment, polyphenols.

Introduction

Sambucus nigra L., commonly known as black elderberry, is a member of the *Adoxaceae* family and is native to the northern hemisphere, particularly in Europe, Northern America, and Western Asia. It is a large bush or a small tree that can reach a height of up to 10 meters, with small white flowers grouped in cymes. The flowering season typically occurs in June and July, usually in the plant's third or fourth year. Black elder is known for its preference for soils rich in nitrogen and calcium compounds. Although it has a particular fondness for alkaline environments, it can adapt to a wide range of soil types, thriving in conditions with pH from 4.2 to 8.0. The chemical composition of *Sambucus nigra* fruits is influenced by factors such as climate and agricultural practices (Bartak *et al*., 2020).

The scientific literature mentions different subspecies of elderberry, including *Sambucus nigra* subsp. *canadensis*, which is native to North America, and *Sambucus nigra* subsp. *nigra*, which is native to Europe. The European elderberry subspecies, *Sambucus nigra*, is known for its medicinal attributes (Thomas *et al*., 2015; Bartak *et al*., 2020; University of California Agriculture and Natural Resources 2024).

Elderberry is rich in anthocyanins, polyphenols, and vitamins, making it suitable for use as a natural colourant and antioxidant in the food industry. The high content of anthocyanins in elderberry makes it a promising natural colourant, contributing to the blue, purple, and red colour (Domínguez *et al*., 2021). Additionally, elderberry's bioactive compounds, including anthocyanins, contribute to its high antioxidant capacity, which can increase the shelf-life of food products. Numerous studies have confirmed the high antioxidant activity of elderberry and its abundance of important biologically active components, such as polyphenols, primarily anthocyanins, flavonols, and phenolic acids (Thomas *et al*., 2015; Bartak *et al*., 2020; Domínguez *et al*., 2021; Borodušķe *et al*., 2022; Ferreira *et al*., 2022). These compounds not only provide antioxidant properties but also offer potential as natural colourants for various food applications (Domínguez *et al*., 2020, 2021; Przybylska-Balcerek *et al*., 2021).

However, it should be considered that the content of biologically active substances valuable to human health can change considerably depending on the chosen processing technology. Selecting the most suitable processing method for *Sambucus nigra* fruits is crucial to ensure the safety of the final products for consumers. This is particularly important due to the presence of cyanogenic glycosides and lectins in the fruits, which pose health risks if not properly processed. The aim of the study was to review available scientific information on the composition of the *Sambucus nigra* fruits and the effect of different processing technologies on cyanogenic glycosides.

Materials and Methods

In total, 30 scientific publications or articles were selected and examined, of which 29 were published in the period from 2015 to 2023, and 1 article was published in 1997. The data was collected from various academic databases and literature sources, including Google Scholar, Taylor & Francis eBooks, Web of Science, PubMed, and Science Direct. The following key words and combinations of key words were used in the most relevant literature search: *Sambucus nigra*, black elderberry, cyanogenic glycosides, sambunigrin, ribosome inactivating protein, lectin, allergen, food processing technique, ultrasonic treatment. A total of 108,713 records were recognized by mentioned database searchings, of which 303 potential articles were examined more closely. In total, 30 full-text literature sources were selected and examined, as well as literature related to the field of study on nutritional value and compositional characteristics and expert opinions. The study employed a semi-systematic approach in its research methodology within the field of food science.

Results and Discussion

Nutritional and bioactive components, health benefits

Fruits of black elderberry play a vital role in strengthening the human body and improving immunity. Their fruit and flower extracts possess antiviral and anti-inflammatory properties (Borodušķe *et al*., 2022). Elderberry fruits contain such biologically active substances as protocatechuic acid, chlorogenic acid, rutin, quercetin-3-O-hexoside, quercetin and ursolic acid (Terzić *et al*., 2023). It is a valuable source of anthocyanins and other polyphenols, which are utilized in food industry as dyes and bioactive substances (Ferreira *et al*., 2019; Banach *et al*., 2021). *In vitro* tests confirm the significant antioxidant activity of berry extracts. It also has been found to be effective inhibitors of α-amylase and α-glucosidase, which can help in reducing blood glucose levels (Terzić *et al*., 2023). *Sambucus nigra* fruits have a high potential in reducing cellular oxidative stress and preventing inflammatory processes (Ferreira *et al*., 2022). Studies suggest that extracts from *Sambucus nigra* can serve as a source of bioactive compounds for the creation of new biologically active products, including pharmaceuticals and functional food ingredients (Ferreira-Santos *et al*., 2022; Terzić *et al*., 2023).

The major protein found in elderberry fruits is a lectin derived from a truncated type II ribosome-inactivating protein (RIP), known as *Sambucus nigra* agglutinin IVf or SNAIVf (Van Damme *et al*., 1997).

The fruits of the *Sambucus nigra* plant contain ribosome-inactivating proteins (RIPs), which are specialised proteins that target cells or substances that many pathogens bind to. These RIPs demonstrate greater therapeutic potential compared to those identified in other plant species. Additionally, black elderberries contain peptic polysaccharides, which have the ability to activate macrophages and other antiviral substances, such as phenolic compounds. These compounds have been shown to exhibit antiviral activity against influenza, human coronaviruses, and other viruses (Bartak *et al*., 2020).

Consumption of type II RIP in high concentrations has toxic effects. Although elderberry species have been reported to contain significant amounts of type II RIPs, *Sambucus nigra* species contain type II RIPs which are known as non-toxic. However, the amount of consumption should be considered (Torero *et al*., 2015; Aliç *et al*., 2021).

Toxicity concerns

It is important to note that the plant contains potentially toxic cyanogenic glycosides, particularly in the bark, leaves, seeds, and unripe fruits (Appenteng *et al*., 2021). The amount of these compounds depends on growing conditions (Mahboubi, 2021). Symptoms of cyanide poisoning from these glycosides can include nausea, vomiting, diarrhoea, and even coma (Appenteng *et al*., 2021).

Cyanogenic glycosides are glycosides from which hydrolytic enzymes produce hydrocyanic acid (De Vries, 2021). Cyanogenic glycosides are naturally occurring plant molecules (secondary plant metabolites of nitrogen) (Jeyakumar & Lawrence, 2022) consisting of a sugar moiety, an aglycone, and a β-hydroxynitrile. The saccharide group can consist of a monosaccharide, such as glucose, or a disaccharide, such as gentiobiose and vicyanose. Glycosidic bonds within saccharides can be hydrolyzed by glycosidases. Additionally, the nitrile in saccharides can be further degraded by lyases to form hydrogen cyanide and an aldehyde, ketone, or acid. For instance, the cyanogenic glycoside sambunigrin, found in black elderberries, is composed of the aglycon L-mandelonitrile and the sugar part D-glucose (De Vries, 2021).

The presence of cyanogenic glycosides in the fruits of *Sambucus nigra* is uncertain (Appenteng *et al*., 2021). However, Appenteg *et al*. (2021), in the study of cyanogenic glycosides in American elderberry, found that their concentration was generally low and at a level that does not pose a threat to consumers of fresh and processed American elderberry products. The authors also observed that the pattern of the results aligns with the fact that elderberry juice, regardless of being American elderberry or Europen elderberry, exhibited very low levels of cyanogenic glycosides (Appenteng *et al*., 2021). Rodríguez Madrera & Suárez Valles (2021) reported that the highest amounts of sambunigrin and prunasin were present in the older leaves of the plant of *Sambucus nigra*, which were 20.7 mg g^{-1} and 0.8 mg g^{-1} dry matter, respectively. On the other hand, the amount of sambunigrin in fruits was 0.3-0.4 mg g^{-1} dry matter, but the levels of amygdalin and prunasin were below the detection limit (Rodríguez Madrera & Suárez Valles, 2021).

In elderberry products, sambunigrin is the most common compound among cyanogenic glycosides (Senica *et al*., 2016). Sambunigrin is structurally similar to prunasin (Anjum *et al*., 2022).

Hydrocyanic acid is considered toxic to humans, animals and microorganisms. In humans, lethal doses range from 0.5 to 3.5 mg per kg of body weight (De Vries, 2021). Deaths in humans and animals have been reported from consumption of plants containing about 500 mg hydrogen cyanide per 100 g^{-1} seed. Several herbivores have been found to tolerate hydrogen cyanide. Animals can induce rapid detoxification of some cyanogenic glycosides by rhodanese (Anjum *et al*., 2022).

Another harmful compound found in *Sambucus nigra* are lectins, which are a type of protein that can bind to specific carbohydrate molecules. In the case of *Sambucus nigra*, lectins are found in various parts of the plant, including the bark and fruit.

Elderberry lectins, including Sam n1, are part of a broad family of allergens. The presence of these lectins in elderberries raises concerns regarding their role as allergens (Jimenez *et al*., 2017). In the context of food safety and allergen labelling, allergens like Sam n1 must be identified and assessed for their risk to consumers. The Sam n1 allergen shares a high degree of amino acid sequence homology with *Sambucus* lectins related to the Sam n1 allergen. This similarity raises concerns about the potential for cross-reactivity with other allergens and the overall safety of consuming elderberry products for individuals with allergies (Jimenez *et al*., 2013). The Codex Alimentarius, a collection of internationally recognized standards and guidelines related to food safety, has established a priority allergen list to guide the labelling of food products (FAO & WHO, 2022). While Sam n1 is not explicitly mentioned in the global priority allergen list, such allergens in food products necessitate consideration by food manufacturers and regulatory bodies to ensure consumer safety. Studies have shown that *Sambucus nigra* lectins are resistant to digestion unless they are heat-pre-treated. This suggests that the lectins can reach the small intestine mostly intact if not subjected to heat, which could have implications for their potential effects on the body (Jimenez *et al*., 2017). Heat treatment of elderberry lectins makes them more sensitive to pepsin attack, which is important for fruit preparations to retain healthy properties without the adverse effects of the lectins (Jimenez *et al*., 2017).

However, some positive properties of lectins are known, such as lectins found in *Sambucus nigra* fruits have protective effects against heavy metals and *Bacillus subtilis* (Aliç *et al*., 2021).

Processing methods and their effects on food products

Thermal processing is widely used in the food industry to ensure the safety of products by reducing or destroying microbial and enzyme activity (Aaliya *et al*., 2021).

The composition of elderberry phenolics and cyanogenic glycosides is significantly altered during processing. For instance, the levels of phenolics decreased from 958 mg kg^{-1} in unprocessed control fruits to 343 mg kg^{-1} in elderberry liqueur, 337 mg kg^{-1} in spread, 162 mg kg^{-1} in tea, and 114 mg kg^{-1} in elderberry juice. Moreover, higher temperatures (100–105 °C) not only reduced the content of beneficial compounds due to the decomposition of substances but also decreased the levels of harmful cyanogenic glycosides by 44% in elderberry juice, 80% in tea, and as much as 96% in elderberry liqueur and spread (Senica *et al*., 2016; Appenteng *et al*., 2021).

While it is highly effective for microbial decontamination, it is important to balance the intensity of heat treatment to maintain the sensory quality and nutritional value of the food products. High-temperature treatment accelerates degradation and diminishes the sensory and nutritional quality of products. Therefore, mild heat treatment (40–60 °C) is preferred to minimize quality loss. However, mild heat treatment alone is not sufficient for microbial decontamination and is often used in combination with other technologies such as pulsed electric field, high-intensity ultrasound, ultraviolet radiation, non-thermal plasma, electrolyzed water, organic acids, and essential oils. Combining various thermal processing methods with non-thermal techniques can create a synergistic effect against microorganisms, contributing to the optimization of bacterial decontamination in food products (Aaliya *et al*., 2021).

The study by Zhong *et al*. (2021) explored the effectiveness of ultrasonic pre-treatment (UPT) in reducing the levels of cyanogenic glycosides and hydrogen cyanide in cassava. The research found that a 10-minute UPT could considerably enhance the elimination of these compounds from cassava. Specifically, the study reported that under optimal conditions of 45 °C and 81 W for 10 minutes, UPT was able to remove 40% of hydrogen cyanide and 25% of cyanogenic glycosides from cassava juice.

Zhong *et al*. (2021) in the study proposed that the mechanisms of elimination for hydrogen cyanide and cyanogenic glycosides differ. Hydrogen cyanide was directly reduced by the ultrasound treatment, while the reduction in cyanogenic glycosides was indirectly achieved through the promotion of enzymatic hydrolysis. This enzymatic process is facilitated by β-glucosidase, an enzyme responsible for breaking down cyanogenic glycosides into less harmful components. The activity of β-glucosidase was found to increase by 18% following sonication, indicating that ultrasound not only helps in directly reducing harmful compounds, but also enhances the natural detoxification processes within the cassava (Zhong *et al*., 2021).

Ultrasound treatment not only reduces the content of cyanogenic glycosides, but also increases the phenolic content of the product. In the study conducted by Nascimento *et al*. (2021), the focus was on evaluating the effectiveness of ultrasound treatment as a method for extracting antioxidant phenols from the fruits of *Sambucus nigra* and *Punica granatum*. The research compared the efficiency of fermentation and ultrasound-assisted extraction techniques, concluding that ultrasound treatment is a significantly more effective method for phenol extraction.

Microorganisms are an important aspect regarding food safety. They can be beneficial in the production of food and contribute to its quality, but they can also cause spoilage and foodborne illnesses if not properly treated.

The mechanism of ultrasonic inactivation of microorganisms is a result of many complex physical processes based on fast-changing mechanical and extrusion of the intracellular matrix, ultimately killing the microorganisms. Inactivation of microorganisms by ultrasound depends on factors including ultrasonic power and wave amplitude, temperature, the volume of the sample, composition and physical properties of food, and type and characteristics of the microorganisms. However, there are limitations to the application of ultrasound in food processing, such as the problem of heat generation and its low efficiency in inactivating spores and yeasts. To increase effectiveness, combined techniques are used, such as the combination of ultrasonication with mild thermal treatment (Aaliya *et al*., 2021).

Nevertheless, it is important to consider the potential limitations of ultrasound. The formation of free radicals during cavitation could have adverse effects on both the product and the consumer. These effects include the inactivation of important enzymes, denaturation of proteins, and oxidation of fats (Ayodeji Adebo *et al*., 2020; Taha *et al*., 2022).

Fermentation can also be considered a suitable way to process *Sambucus nigra* fruit, considering the potential presence of cyanogenic glycosides. It is a non-thermal type of food processing, during which it is possible to significantly reduce the amount of toxic compounds, namely aflatoxins and cyanogens (Jeyakumar & Lawrence, 2022). The hydrolysis of cyanogenic glycosides during fermentation is attributed to the action of enzymes such as β-glucosidase, which leads to the reduction of cyanide content (Bolarinwa *et al*., 2016). Therefore, it can be concluded that fermentation has the potential to reduce cyanogenic glycosides in food products, thereby contributing to improved food safety and nutritional quality.

In addition, the fermentation process determines the changes in the taste and texture of the product. Also, fermentation contributes to the extension of the shelf life of the final product, and fermented products have a beneficial effect on health (Boukid *et al*., 2023).

Conclusions

1. *Sambucus nigra* fruits are recognized for its high content of anthocyanins, polyphenols, which contribute to its antioxidant properties and potential as a natural colourant in the food industry.
2. The bioactive compounds in elderberry, such as anthocyanins, flavonols, and phenolic acids, have been linked to various health benefits, including antiviral, anti-inflammatory, and anti-diabetic effects.
3. Elderberry extracts have shown potential in reducing cellular oxidative stress and preventing inflammatory processes, and they may be used in developing new pharmaceuticals and functional food ingredients.
4. *Sambucus nigra* contains potentially toxic cyanogenic glycosides, especially in the bark, leaves, seeds, and unripe fruits, which can lead to cyanide poisoning.
5. Heat treatment of elderberry lectins enhances their susceptibility to enzymatic digestion by pepsin, allowing for effective breakdown during digestion and reducing the risk of potential adverse effects associated with their intact form.
6. Thermal processing is commonly used to ensure product safety by reducing microbial and enzyme activity, but it can also reduce the content of beneficial compounds.
7. Ultrasonic treatment can reduce cyanogenic glycosides and increase phenolic content.
8. Ultrasonic inactivation of microorganisms is a complex process, but it has limitations, such as the potential formation of free radicals.
9. Combined methods, such as ultrasonication with mild thermal treatment, can increase efficiency while minimizing negative impacts.
10. Fermentation is a non-thermal processing method that can reduce the amount of toxic compounds in *Sambucus nigra* fruits and contribute to food safety and nutritional quality.
11. *Sambucus nigra* fruits are a valuable source of bioactive compounds with potential health benefits, but their processing must be carefully managed to balance safety concerns with the preservation of nutritional and sensory qualities. Ultrasonic treatment and fermentation are promising methods for improving the safety and quality of elderberry products.

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