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Effects of High Hydrostatic Pressure on Fungal Spores and Plant Bioactive Compounds

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Definition: Fungi, and their spores, are responsible for the spoilage of several foods and plants and are susceptible to contamination with mycotoxins, which have associated health hazards. In this context, proper methodologies for inactivating such fungi and controlling mycotoxin production are critical. High-pressure processing (HPP) has recently become popular as a nonthermal alternative to conventional thermal pasteurization processes. Even though HPP can effectively eliminate some fungal spores, some species, such as those from the genera *Byssochlamys*, *Talaromyces*, and *Aspergillus*, are quite resistant to this treatment. Additionally, high pressure can also be used as a cold extraction technique for bioactive compounds from medicinal plants and other matrices (termed high pressure-assisted extraction, HPE). With this method, safe use for food, cosmetic, and pharmaceutical applications is guaranteed. This method simultaneously works (depending on the applied pressure level) as an extraction technique and induces the pasteurization effect on the extracts. This encyclopedia entry aims to highlight the effects of nonthermal HPP on fungal spores, the prevalence of mycotoxins in plant materials and how high pressure can be used as an extraction technique to produce high-value cold pasteurized extracts with biological activity.

Keywords: fungi; spores; high-pressure processing; mycotoxins; high-pressure extraction

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1. Introduction

The consumption of natural products, especially those derived from medicinal plants and herbs, is increasing worldwide, as plants have been used since ancient times to prevent or treat maladies in both humans and other animals. These medicinal plants are rich in several compounds with biological activities that are beneficial for consumers, such as anti-inflammatory, analgesic, antioxidant, and antimicrobial activities [1,2]. These biologically active chemicals are derived from the secondary metabolism of plants or those produced when the plant is subjected to a stress source that triggers different metabolic pathways that will counteract the stress source with different compounds.

As these plants are usually grown in open fields, they are susceptible to several sources of contamination, such as insects, rodents, birds, and others, which can transmit plant diseases and damage crop health and development [3].

Spores from heat-resistant fungi (HRF) cause food spoilage and food-borne illness in pasteurized fruit juices, pulps, and concentrates, representing considerable economic losses for the food industry and a threat to food safety. In this sense, several strategies have been developed to ensure fungal spore destruction by means of chemical additives or by improving the traceability of the raw materials.

In addition to the animal and plant kingdoms, fungi include a broad range of eukaryotic microorganisms. The fungus kingdom includes yeasts, molds, mushrooms etc., with some having applications in the food, cosmetic and pharmaceutical industries [4]. Interestingly, fungi and human health regulation are concurrently linked, as high-fat diets are associated with changes in gut fungi, which can play a key role on the development of obesity and gut inflammation, even though fungi involved in this process represent less than 1% of the human gastrointestinal tract [5].

However, certain fungi present a risk to the industry since they can be a source of contamination for meals and value-added products, such as bioactive-rich plants [6]. For example, dried tea leaves and medicinal plants may contain different types of microorganisms and their toxins, especially those from fungi, and contaminate food products and extracts originated from contaminated plants, consequently raising, food safety issues [7]. Even though the presence of mycotoxins (in very low concentrations) in dried plants is normal, the presence of several mycotoxins in the same plant materials is considered a major concern, as it is reported to have a synergistic effect in toxicity between mycotoxins when compared to the individual mycotoxins, i.e., the toxicity effect of two mycotoxins in plant materials is higher than the sum of the individual toxicity levels of each individual mycotoxin [8]. In legal terms, Europe and Malaysia typically have the most restrictive limitations on the maximum concentration of mycotoxins in foods and products, followed by the United States, which requires extremely stringent agricultural practices to ensure such low levels [9].

This encyclopedia article aims to provide a concise summary of fungal spores in plants and how they can impact the quality and availability of bioactive compounds extracted from such plant materials. This points to the methodological application of high-pressure processing (HPP) simultaneously as a nonthermal pasteurization methodology to destroy vegetative fungi and some fungal spores, as well as an extraction methodology of bioactives from medicinal plants.

2. Fungal Spore Structures (Special Focus on Ascospores)

There are two possible routes for spore generation in fungi, the first of which is sexual reproduction (called sexual morph), in which thermally resistant ascospores are formed largely in Ascomycota. Inside the ascus, and these mature inside another structure termed ascoma. Upon maturation, the ascospores are released from the asci as dormant ascospores, and they must be activated before germination, which often entails high temperatures, high hydrostatic pressures, chemicals, and other techniques. The second route is termed anamorph, which refers to the asexual growth of fungal spores in which conidiophores are produced within a structure known as conidia. Ascospores are more heat and pressure resistant than asexual spores [10,11]. As a result, the focus of this entry will be on ascospores, with conidiophores referenced and explored briefly.

High quantities of trehalose and mannitol (a protective agent against reactive oxygen species) and low water content in the cytoplasm are assumed to be responsible for certain ascospores' exceptional resilience to high temperatures and pressures, freezing, drying, and other circumstances [12]. Indeed, the presence of these protective agents will decrease the water availability and increase the viscosity of the cytoplasm, thus increasing ascospore resistance [13].

Typically, ascospore germination begins with an activation trigger, which could be initiated by chemicals, elevated temperatures (quick heat treatments), high pressures, or other factors, which will ultimately result in trehalose breakdown and glucose release into the external medium. Due to the opening of the outer cell wall, the protoplast (which is enclosed by the inner cell wall) is immediately ejected to the external media, a process known as prosilition. Subsequently, water absorption causes the released protoplast to enlarge, resulting in the production of a germ tube. As a result, mycelia, which will help in the infection and development of further germinated spores, are produced [13,14].

These structures are common in industrial environments because of the air dispersion ability of ascospores, resulting in food spoiling and, as a result, economic losses. Despite a few exceptional cases, these heat-resistant molds may thrive in complex conditions where bacterial spores are typically prevented from developing, such as low pH and low oxygen pressure environments [11].

Spoilage-associated fungi can be categorized as either xerophilic fungi (able to develop at water activities below 0.85) or thermal-resistant fungi (able to withstand pasteurization treatments) [15]. Indeed, several fungi can develop at very low relative humidity levels, as is the case for *Aspergillus halophilicus*, which is among the most xerophilic *Aspergillus* species and is able to grow at a relatively low humidity level of 68 percent [16].

3. Prevalence of Fungi and Their Spores on Medicinal Herbs

Plants and other herbs are generally commercialized as either fresh or dry. Especially when dry, the majority of microorganisms are unable to develop in plants, although, some fungi find optimal conditions for development, as there is no competition with bacteria [17]. Indeed, fungi that can develop at low water activity values (usually below 0.85) are termed xerophilic fungi [18,19]. Some of these fungi, are even able to develop under refrigeration conditions (classified as phychrophilic/phychrotolerant) and under vacuum and modified atmosphere packaging (anaerobic fungi), which can ultimately lead to herb and plant materials spoilage [15]. In other cases, despite the high water activity, some plants present low pH, which will compromise the development of most bacteria, although some fungi find optimal development conditions at such pH levels [20].

According to [21], several factors may contribute to introducing either bacterial or fungal contamination in the fresh produce of medicinal herbs, such as improper cultivation practices, harvesting and handling, and poor sanitizing conditions of both the plant materials and operators.

Fusarium spp. Are responsible for most of the fungi-related spoilage of dry plants, and their development is accompanied by mycotoxin production, such as zearalenone, fumonisins, moniliformin, and trichothecenes [22]. Other genera, such as *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Alternaria*, *Absidia*, *Cladosporium*, and *Trichoderma*, are recognized as pathogens of medicinal plants, and some of them can produce mycotoxins (for example, *Aspergillus flavus* is a known producer of aflatoxin and ochratoxin), which raises awareness for proper plant handling, processing, and storage [7]. Figure 1 shows the principal mycotoxins, and Table 1 provides examples of the prevalence of mycotoxins in medicinal plants and spices.

Table 1. Literature regarding the prevalence of fungi in medicinal herbs and spices.

Plant (Part of the Plant)	Microorganism	Mycotoxin	Reference
Chamomile	-	Aflatoxin	[23]
		Citrinin Fumonisin	[24]
Cinamomum zeylanicum blume	*Aspergillus* spp. *Penicilium* spp.	Aflatoxin B1, B2 ang G2	[25]
Roasted coffee	*Aspergillus* spp. *Penicilium* spp.	15-acetyldeoxynivalenol, Deoxynivalenol, Neosolaniol, Fumonisin B1 and Ochratoxin A	[26,27]
Ginseng root	-	Aflatoxin B1 and B2 Ochratoxin	[28]

Table 1. *Cont.*

Plant (Part of the Plant)	Microorganism	Mycotoxin	Reference
Glycyrrhiza glabra (root)	*Aspergillus flavus*	Aflatoxin B1 and B2	[29]
	Fusarium oxysporum	Zearalenone Fumonisin B1	
	-	Citrinin	[30]
		T-2 and HT-2	[23]
Milk thistle	-	Aflatoxin T-2 HT-2 Fumonisin	[31]
Mint	-	Aflatoxin Citrinin	[24]
		Fumonisin	[32]
Tribulus terrestris (seeds)	*Aspergillus ochraceus*	Ochratoxin A	[29]

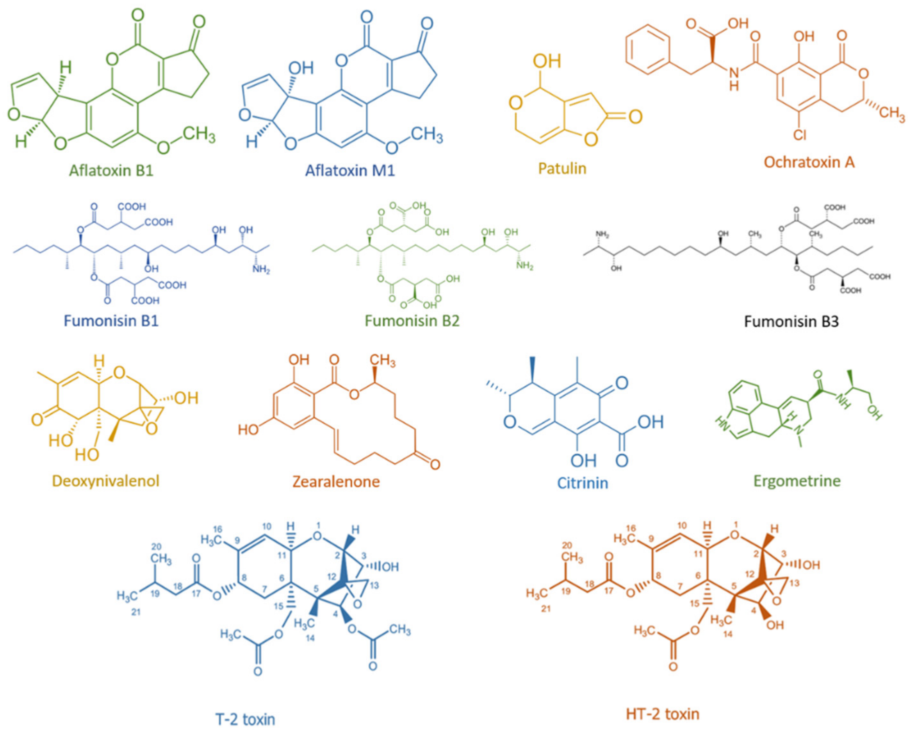

Figure 1. Chemical structures of the major mycotoxins produced by fungi frequently found in food products, ingredients, medicinal herbs, and plants.

Some of the aforementioned mycotoxins are proven to be mutagenic, carcinogenic, teratogenic, neurotoxic, and nephrotoxic agents, and can even present immunosuppressive activity. As such, a strict control of toxins (and their primary source, namely, fungi) needs to be effectively applied to avoid serious hazards to consumers, health and economic losses [33]. This is truly an issue for medicinal plants and herbs, as the high levels of mycotoxins in such plants make the process of obtaining bioactive compounds from such sources plainly untenable [34].

Powdery mildew is a disease seen in some plants. (Figure 2), which is caused by fungi of the order *Erysiphales*. Infection by this group is characterized by the appearance of white spots on the plant leaves.

Figure 2. Example of plant leaves infected with powdery mildew. On the left, a powdered-like substance over the leaves, revealing extensive contamination. On the right, a magnification of the plant leaves, reveals a network of mycorrhizae. Retrieved from the Wikipedia entry "Powdery Mildew" (2022) [35].

In addition, powdery mildew is one of the most prevalent parasite diseases that cause significant economic losses in greenhouse and field crops. The main cultures affected by this disease are barley, wine, beets, cucumbers, and eggplants. Lettuce, melons, peas, peppers, tomatoes, etc. [36].

Powdery mildew is spread by air through spores (Figure 3) that germinate up to 48 h upon being placed on the plants' leaves and the infection manifests after 7 to 10 days afterwards, which is also accompanied by spore production to continue the life cycle of the mold. Truly, this fungus can cover practically all the plant surface, including the fruits and buds [37].

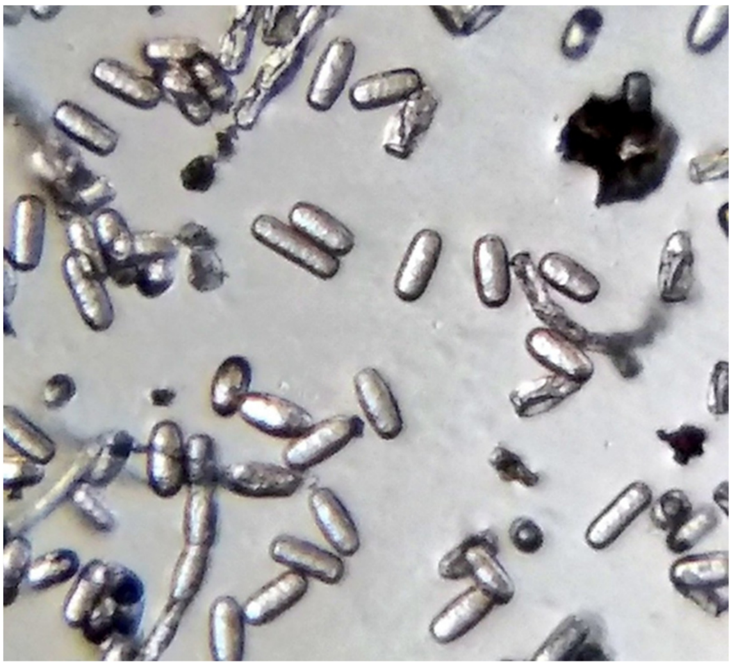

Figure 3. An illustration of powdery mildew spores that were isolated from cucumber leaves that was retrieved from the "Powdery Mildew" Wikipedia entry (2022) [35].

Some plants are unable to produce phytoalexins, an alkaloid with antibacterial characteristics produced by other plants, which can be used to prevent or halt the growth of fungi, especially when the infectious agent is introduced in a new environment from which it is not common (for example, when it comes from foreign geographies) [20].

Notwithstanding, fungi (and their spores) are not necessarily prejudiced for plant materials. Indeed, fungal-plant consortia (symbiotic relationship termed mycorrhizae) is very important for the adaptation and development of several plants [38].

4. Possible Limitations Caused by Fungi and Their Toxins to the Use of Bioactive Compounds from Food Materials

Medicinal plants are widely used as natural sources of pharmaceutical bioactive compounds obtained by extraction and the detection of mycotoxins in these plants is common [39]. However, these mycotoxins may be present in the final extracts/products if proper and strict quality control is not performed. This can ultimately result in poisoning and, in extreme cases, death, in addition to economic losses. [20].

Nevertheless, some biobased approaches to either control the production of mycotoxins or even their elimination are being widely evaluated. One such approach involves the use of plant extracts and their phytochemicals synergistically with mycotoxin adsorbents [40]. Fungi in fresh produce mitigation techniques mostly rely on the use of chemical sanitizers such as chlorine in the form of hypochlorite salts, chlorine dioxide, alcohols, copper sulphate, organic acids, hydrogen peroxide, peracetic acid and ozone [41]. However, the aforementioned chemical-based methodologies are efficient in reducing/eliminating fungi and their spores. They will interact with plant tissues and chemically react with a number of bioactive chemicals, reducing their availability. Mycotoxins can be significantly decreased below acceptable limits via enzymatic, chemical, and physical methods (Figure 4).

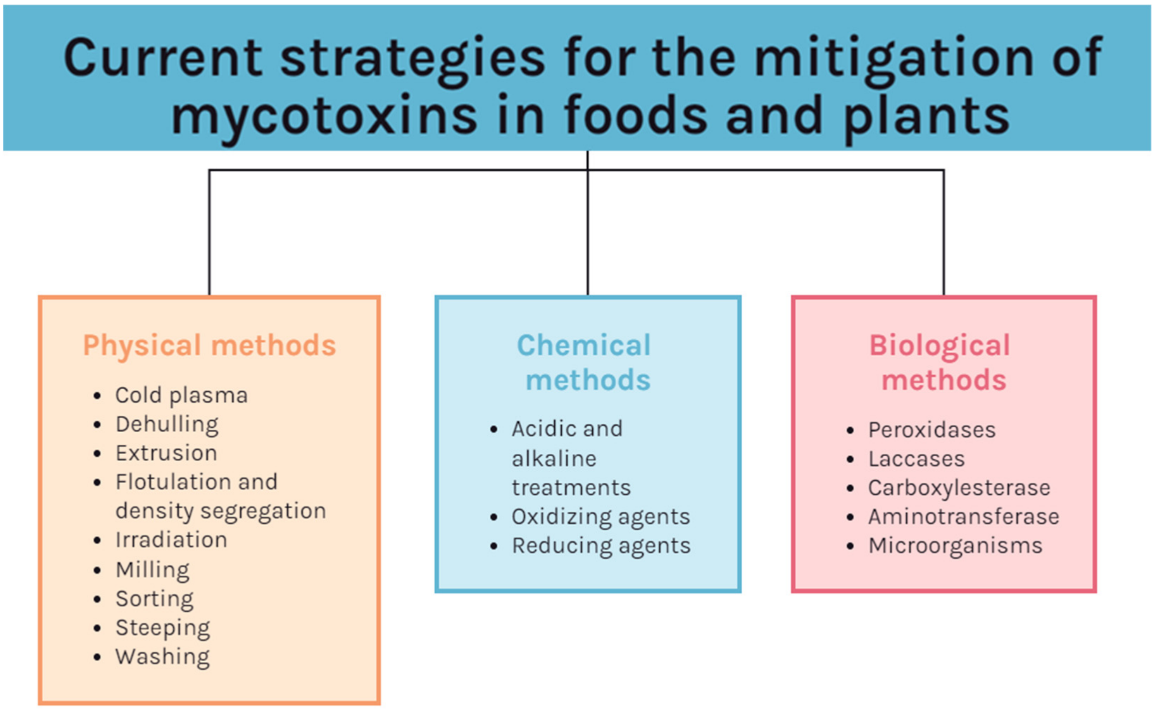

Figure 4. Current prevention strategies for mycotoxins in foods and plants. Adapted from information available in [42,43].

Roasting techniques (such as roasted coffee beans) can physically eliminate the majority of mycotoxins found in green coffee [44]. Heat treatments appear to be the most effective way to reduce mycotoxins in dietary ingredients; nevertheless, in many cases, applying heat is difficult or undesirable.

5. Effects of Nonthermal HPP on Ascospores—Impact on Ultrastructure

HPP is a nonthermal processing technology that makes use of elevated hydrostatic pressures (commercially up to 600 MPa) for a few minutes to inactivate vegetative pathogenic and spoilage-related microorganisms. This effect on microorganisms is related to cell membrane rupture, inactivation of essential enzymes for microbial metabolism, and so forth [45].

As stated previously, it is widely reported that HPP has the ability to crack membranes, leading to the formation of pores that will cause cell leakage. In regard to fungal spores, HPP also results in cracks on the rigid membrane of ascospores. Some of these ascospores have crown-like ornaments that will be destroyed by HPP. Interestingly, HPP can also cause ascospore dissociation, i.e., ascospores can aggregate due to the electrostatic attractivity, which can be destroyed by hydrostatic pressure. In addition, the application of pressure on ascospore suspensions can lead to an apparent increase in the ascospore load after the treatment. This is due to the release of ascospores that were entrapped inside the asci rather than microbial development [46–48].

6. Potential of HPP to Eliminate Toxin-Forming Fungi in Plants and Herbs

HPP is widely known for its nonthermal pasteurization-inducing effect in foods by means of microorganism inactivation through the formation of pores/cracks on the membranes. High pressure-assisted extraction, a technique derived from HPP, is an interesting technique to consider in this context (HPE). This technique makes use of elevated hydrostatic pressures to accelerate mass transfer processes, promoting the impregnation of solid matrices (such as plants) with a desirable solvent (water, ethanol, etc.), and the solubilization of bioactive compounds in the solvent, thus increasing the extraction yields [49]. Considering the polarity of the compounds to be extracted, different combinations of solvents can be made to maximize the extraction yields and provide a certain level of selectivity toward the compounds to be extracted [49].

An interesting fact about this technique is that the range of pressures used (up to 600 MPa), along with the extraction times (up to 15 min), can undeniably induce the pasteurization effect on these plant matrices, allowing the elimination of most fungi. As such, HPE could be a very pertinent technique to prepare plant-based natural extracts, as it could induce the pasteurization effect during the extraction process, while simultaneously extracting bioactive compounds, in a single step. In addition to the food industry, this technique has great potential for use in the cosmetic, pharmaceutical, and biotechnological industries, to develop safe, high value-added products, based on bioactives extracted from plant-based materials, in which fungi and their toxins are a concern.

As this methodology can increase extraction yields, it is expected that some of the biological activities in extracts obtained by this methodology increase as well, namely those regarding antimicrobial potential against fungi. Thus, the release of these chemicals from plant-based materials would work synergistically with the elevated pressures to eliminate fungal spores [50].

7. Impact of HPP and HPE on Bioactive Compounds

Several authors have stated the potential of nonthermal HPP to retain bioactive compounds in fruit and vegetable juices when compared to the conventional heat-based pasteurization techniques in plant-based foods. Patras and colleagues [51], for example, showed that HPP (600 MPa, 15 min, 10–30 °C) increased the retention of ascorbic acid and anthocyanins in strawberry purée when compared to thermal processing (70 °C, 2 min) (which caused a 21 and 9.7% decrease of the ascorbic acid and anthocyanin content compared to unprocessed samples), which consequently affected the color of thermally-treated samples. Additionally, the same authors reported an increase in the antioxidant activity for HPP-treated strawberry puree, especially those processed at 600 MPa (wherein a 67% increase was observed), with the authors attributing this increase to a higher extractability of antioxidant compounds from the puree.

High pressure-assisted extraction (also known as HPE as stated above) can actually improve the extraction yields (and bioavailability) of the extraction components when utilized as an extraction technique, as demonstrated by Corrales and colleagues [52], who reported higher anthocyanin content for grape byproducts' extracts obtained by HPE (600 MPa, 70 °C, 1 h) when compared to the conventional thermal process (70 °C for 1 h at atmospheric pressure). At such a pressure level, temperature, and holding time, most fungal spores would be inactivated [12], allowing the provision of safe(r) extracts.

Moreira and colleagues [53] optimized the extraction of phenolic components, flavonoids, and pigments from stinging nettle leaves in relation to medicinal herbs and observed that the optimal conditions for extraction yield, total phenolics and antioxidant activity were 200 MPa for 10.2–15.6 min at room temperature using distilled water as a solvent. Additionally, it was reported that the extraction yield with HPE increased 50%, and the total phenolic content and antioxidant activity also increased approximately 84.4 and 77.7% when compared to the control samples (kept at atmospheric pressure).

Compared to other extractions, HPE can provide higher extraction yields than other extraction techniques, such as ultrasound-assisted extraction, as observed by Briones-Labarca and others [54], who reported higher extraction yields from papaya seeds using HPE (550 MPa for 15 min) than with ultrasound (130 W, 42 kHz, 15 min) (6.49 and 4.75%, respectively).

For a successful application of HPE, to maximize extraction yields, a careful optimization process must be performed. The final result will be influenced by the pressure level, holding time, solvent (typically mixtures of water and ethanol, or others), mass/solvent ratio, use of fresh or dried biomass, and previous homogenization of the mixture. Indeed, using a response surface methodology with experimental design may be a good choice for the optimization of extraction processes. Regarding temperature, "balancing" must be considered to account for the consequent adiabatic heating, which is advantageous for quick heating and cooling of the extraction media. Although it may depend on the extraction solvent used, the container's thickness, the pressurization fluid, and where the biomass and solvent are placed under pressure [49,55].

8. Conclusions

Fungi (and particularly fungal spores) play a vital role in the quality and functionality of plant materials with regard to the extraction of compounds with biological activity. Furthermore, fungi and their spores can develop within such plant components and have a negative impact on the availability of bioactive chemicals and the safety of their application in many industries. High pressure processing cannot only be used to extract bioactive compounds from plant materials (as a nonthermal extraction method), but also assist the safety associated with the extracts acquired by their pasteurization, and therefore vegetative fungi removal and reduction of mycotoxin-related problems. However, the complex interaction between fungi and plants can be advantageous for the growth of a plant's structural components and the production of compounds with biological activity, such as alkaloids, which have exquisite biological activities.

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