Chapter 21
Mycosterols

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Abstract Sterols are amphipathic lipids that play essential roles in the physiology of eukaryotic organisms in general. The fungal sterols are collectively known as mycosterols and they exert numerous physiological functions. For humans, the interest on this class of compounds relies heavily on the fact that they can promote health benefits. For this reason, fungal extracts rich in sterols of various forms are valuable and promising ingredients. One of the best-known benefits of mycosterols is their inhibitory actions on cholesterol absorption and biosynthesis, but there are several interesting regulatory and modulatory phenomena that mycosterols can affect and that might eventually be of therapeutic interest. Within this domain, the practical application of mycosterols or mycosterol-enriched fungal extracts presents several challenges. The latter include isolation of novel bioactive mycosterols from still underexploited fungi species, the optimization of existing methodologies for production and recovery, extensive study of their applications and, finally, substantial clinical trials for attesting their health benefits and safety.

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21.1 Introduction

Sterols are amphipathic lipids that play essential roles in the physiology of eukaryotic organisms in general. In animals, for example, the most common form cholesterol is an integral part of the cellular membrane, where it exerts a key role in determining its fluidity and plasticity. Sterols are also important in plants and fungi. In the latter they are collectively designated mycosterols and are also involved in many physiological functions. The by far most abundant form of this class of compounds in fungi is ergosterol, but there are many other derivates exerting a great number of physiological functions. Besides the importance for the fungi themselves, on the other hand, many mycosterols have been described to possess pharmacological and therapeutic effects in mammals, a feature that makes investigations on their respect a highly interesting field. These properties will be analyzed in the present chapter after a short overview on their biosynthetic routes.

21.2 Sterol Biosynthesis

A key intermediate of the biosynthetic pathway of sterols in general is squalene as this compound already contains the carbon backbone from which all other compounds of the class are derived. The carbon backbone of squalene comes ultimately from acetyl-CoA. In a long series of reactions 3 acetyl-CoA molecules are firstly condensed into β-hydroxy-β-methylglutaryl CoA (HMGCoA). In the sequence, the six carbons of the β-hydroxy-β-methylglutaryl moiety are deprived of one carbon unit giving origin to the more proximal precursor of squalene synthesis, which is the 5-carbon isoprenoid isopentenyl pyrophosphate. The latter, through condensation reactions, gives origin to the 15-carbon molecule farnesyl-pyrophosphate. Condensation of two farnesyl-pyrophosphate molecules, finally, forms the 30-carbon molecule squalene. To arrive at this point, thus, 12 acetyl-CoA molecules are required. Figure 21.1 provides a summary of these transformations. The condensation reaction that forms squalene is catalyzed by squalene synthase and the subsequent epoxidation that produces 2,3-oxidosqualene is catalyzed by an epoxidase. Lanosterol is the first cyclic intermediate in the production of mycosterols and contains 30 carbon atoms. Singularly, this is also the precursor in the cholesterol biosynthesis in animals (Weete et al. 2010). The fungal sterol pathway, that involves at least 20 enzymes, is analogous to that one operating in animal cholesterol biosynthesis, usually known as the acetate mevalonate pathway (Dhingra and Cramer 2017). The formation of mycosterols initiates by the methylation of lanosterol at C-24, which is followed by a series of demethylations at C-4 and C-14 and double bond rearrangements that (in most cases) produce the C28 sterols that are frequently found in most fungi (Fig. 21.2; Weete et al. 2010). The diverse pathways culminating with the formation of ergosterol vary in accordance with the sequence by which the double bonds are transformed (Song and Nes 2007). In some taxa, a second methylation that originates

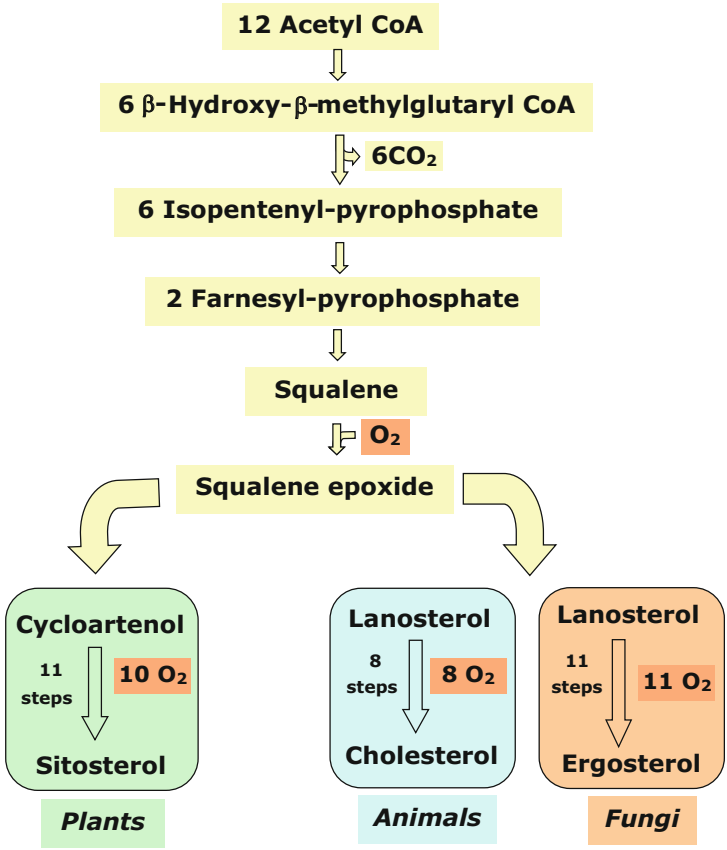

Fig. 21.1 Simplified biosynthetic pathways of sterols

a 24-ethylidene, which is later reduced to 24-ethyl, produces C29 sterols (Fig. 21.2; Weete et al. 2010). For additional information on ergosterol biosynthesis see these references (Alcazar-Fuoli et al. 2008; Abe and Hiraki 2009; Weete et al. 2010; Dupont et al. 2012).

21.3 Main Mycosterols

Ergosterol (ergosta-5,7,22-trien-3β-ol), a type of natural steroid alcohol, has been considered the main sterol of hyphal membranes, followed by derivatives such as ergosta-5,8,22-trien-3-ol, ergosta-7,22-dien-3-ol, ergosta-5,7-dien-3-ol, and ergosta-7-en-3-ol (fungisterol) (Gil-Ramirez et al. 2013). Other products of sterol biosynthesis, such as cholesterol, 24-methyl cholesterol, 24-ethyl cholesterol and brassicasterol, are some examples of the main sterols present in fungi (Weete et al.

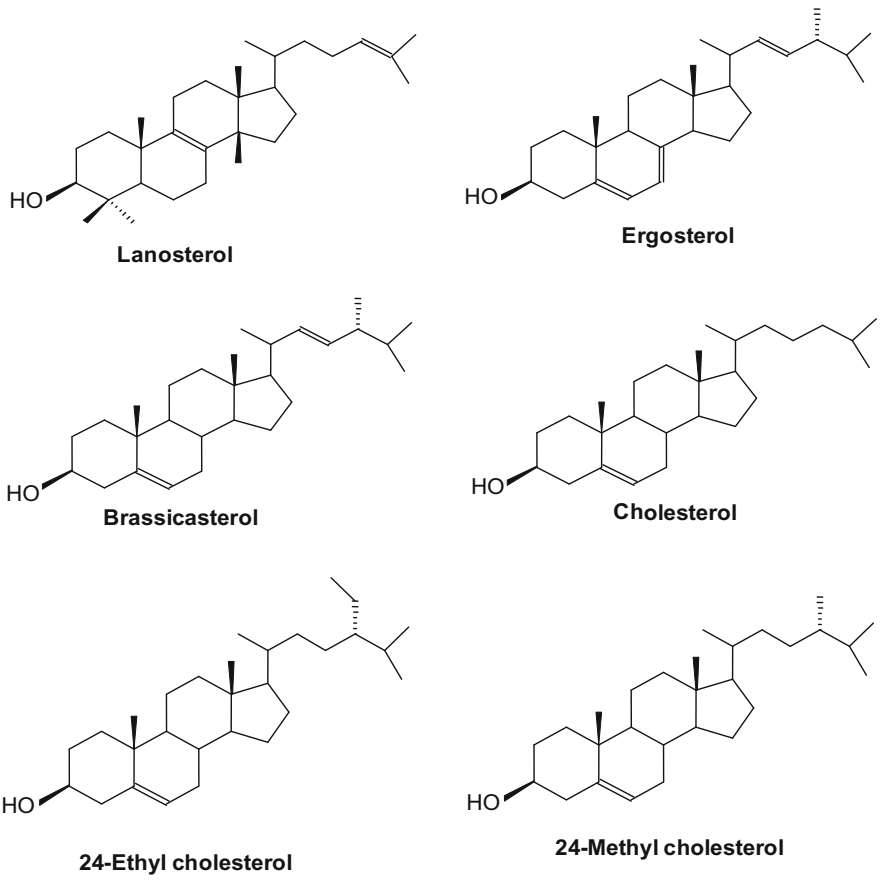

Fig. 21.2 Structure of sterols found in fungi

2010; Fig. 21.2). For most fungi, ergosterol may represent up to 80% of their sterol content (w/w), and it plays an essential role in membrane function, regulating its fluidity, plasma membrane biogenesis and permeability (Abe and Hiraki 2009). Proper cellular ergosterol levels are important for maintaining normal cellular functions that include environmental stress response, cellular detoxification, nutrient transport, and host-pathogen interactions (Bhattacharya 2021). It is completely or almost absent in animal, plant, and bacterial cells (Gomez-Lopez et al. 2011). The ergosterol content is amply related to structural and growing fungal features (Barreira et al. 2014), for example, maturation, hyphal formation, and sporulation (Villares et al. 2014), and can vary according to the fungal species (Phillips et al. 2011). For all the above-cited reasons, ergosterol and its biosynthetic pathways are crucial for fungal growth, so much that both are under consideration for the development of azole antifungals (Alcazar-Fuoli et al. 2008).

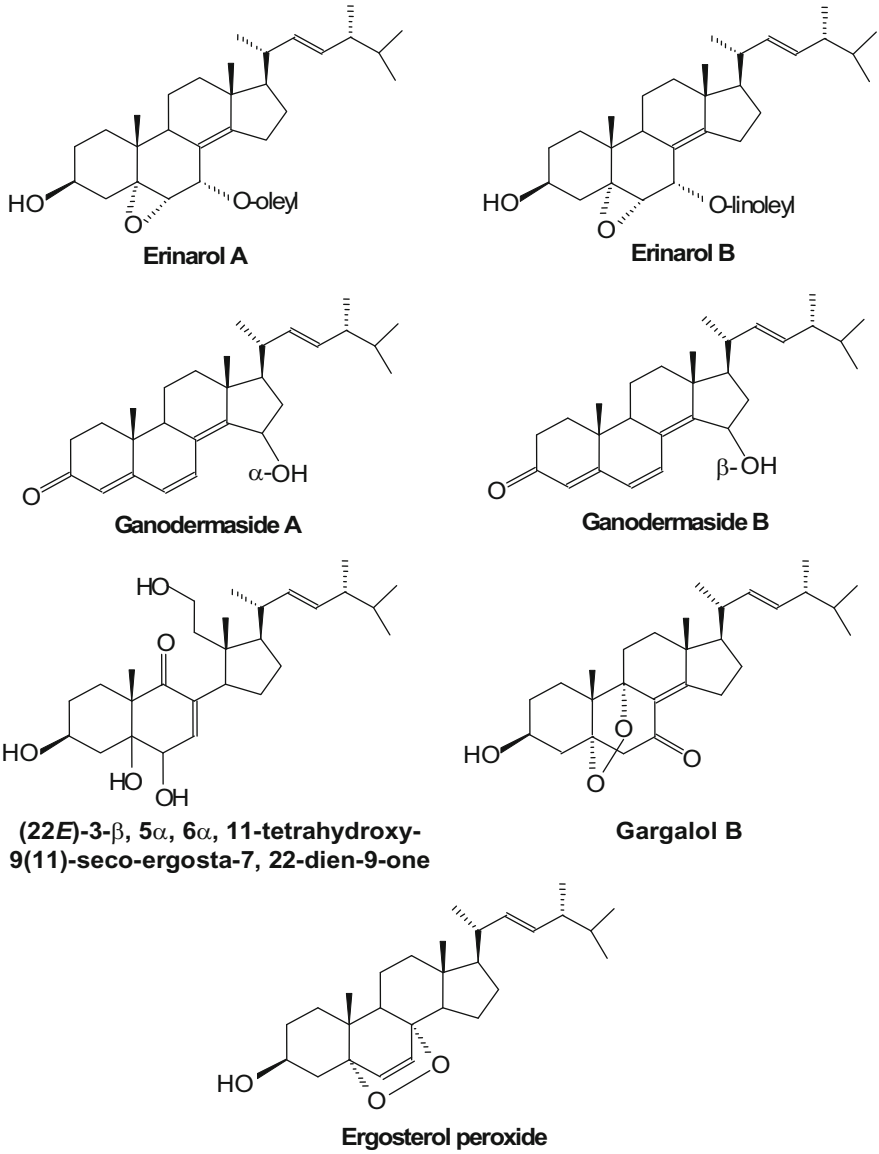

Fig. 21.3 Structure of some ergosterol derivatives from common edible/medicinal mushrooms (Chen et al. 2017, modified)

In the last few years, various new mycosterols have been isolated in both basidiomata (fruiting bodies) and mycelial masses of different fungi and their structures elucidated (Fig. 21.3). Several of them are ergosterol derivatives and present important biological properties (Chen et al. 2012; Shimizu et al. 2016). One of the most studied is ergosterol peroxide, which is an ergostanoid, namely

ergosta-6,22-dien-3-ol, with a peroxyl group between positions 5 and 8 (the 3beta, 5alpha, 8alpha, 22E stereoisomer; see Fig. 21.3). Isolated from *Ganoderma lucidum* and *Cordyceps* sp. (Chen et al. 2017) it exhibits antimycobacterial, trypanocidal, and antineoplastic activities. It has also a role as metabolite, antineoplastic agent, antimycobacterial drug, and trypanocidal drug.

Depending on the mushroom species, the ergosterol concentration may vary from 0.2 to 10 mg/g. In general, the most cultured and consumed species worldwide are precisely those ones containing the highest amounts. Probably the main reason for this phenomenon is that mushroom farmers cultivate them under strict observation of the ideal nutritive requirements (using specific and enriched substrates), and harvest the basidiomata at their optimal developmental stage, while the mushrooms that grow in nature rarely benefit from optimal environmental conditions (Gil-Ramírez et al. 2014; Gil-Ramirez and Soler-Rivas 2014). Hence, the genera *Agaricus* and *Pleurotus* not only concentrate the largest number of studies on bioactive mycoesterols, but also on sterol composition and recovery methodologies (Barreira et al. 2014; Gil-Ramirez et al. 2013; Phillips et al. 2011; Villares et al. 2014).

21.4 Bioactive Properties of Mycosterols

Numerous studies have indicated that fungal extracts rich in ergosterol and ergosterol derivatives can reduce cholesterol absorption (Corrêa et al. 2017; Gil-Ramírez et al. 2014; Yeh et al. 2014) as well as inhibit its biosynthesis in the human body (Chen et al. 2012; Caz et al. 2016). Other health benefits described for this type of extracts are antitumoral and antiproliferative (Kang et al. 2015; Nowak et al. 2016; Torres et al. 2017), anti-inflammatory (Li et al. 2015a, b) and anti-microbial (Sinanoglou et al. 2015) effects. Moreover, being a precursor of vitamin D2, ergosterol (and its derivatives) might enhance bone metabolism, immunity, and mood (Feeney et al. 2014; Xu et al. 2020).

21.4.1 Agaricus bisporus Mycosterols

Agaricus bisporus L., the most consumed mushroom in the world, contains a fraction of mycosterols composed mainly by ergosterol (∼90%) (Barreira et al. 2014). Ergosterol can be easily converted into vitamin D via irradiation, a useful property in industrial applications such as dietary supplements and food fortifiers (Heleno et al. 2016a). Gil-Ramirez et al. (2013) efficiently recovered sterol-enriched fractions (ergosterol and other minor sterols) from *A. bisporus* basidiomata and corresponding by-products via pressurized liquid extraction and supercritical fluid extraction techniques.

Heleno et al. (2016a) studied the use of ultrasound-assisted extraction to recover mycosterols from *A. bisporus* basidiomata applying response surface methodology,

and found that this methodology is powerfully efficient in terms of ergosterol extraction yield and extract purity, also allowing a dramatic reduction of extraction time in comparison with the traditional Soxhlet method. In a later work, Heleno et al. (2016b) proved the feasibility of using *A. bisporus* by-products as a valuable source of ergosterol and microwave-assisted extraction as a suitable technique for its extraction. The authors successfully optimized the extraction process by applying response surface methodology and reported a recovery yield of more than 550 mg of ergosterol/100 g of mushroom (on dry basis) at the best-optimized conditions, using ethanol as extractor solvent. However, other species of the genus *Agaricus* still remain as underutilized sources of ergosterol and could be exploited to obtain this molecule of interest. Mokochinski et al. (2015) studied the production of ergosterol by *A. brasiliensis* in mycelium phase cultures using different agro-industrial by-products as substrates. According to the authors both solid-state fermentation and submerged fermentation were efficient in generating mycelia biomass. The combination of malt substrate and submerged fermentation was the one that generated the highest yields in terms of biomass and ergosterol. Ergosterol and β-sitosterol were the major sterol compounds identified in their samples.

21.4.2 *Lentinula edodes* Mycosterols

Extracts of the second most extensively cultivated mushroom worldwide, *Letinula edodes* (Berk.) Pegler, displayed plasma cholesterol-lowering activity in hypercholesterolemic mice fed with lard (Caz et al. 2016). The ongoing expansion of mushroom industries has generated enormous spent mushroom substrates with the potential for the production of valuable chemicals. To valorize this waste, spent shiitake substrate was submitted to ultrasound-assisted extraction (Wang et al. 2018). Under optimized conditions, the extract contained ergosterol, ergosta-7,22-dienol, and β-sitosterol as the main sterols. The extract showed a comparable antitumor effect against three cancer cell lines.

21.4.3 *Pleurotus spp.* Mycosterols

Schneider et al. (2011) investigated the cholesterol-lowering properties of *Pleurotus ostreatus* in a randomized placebo-controlled intervention. The authors suggested that the significant improvements in human blood parameters, including triglyceride levels, total cholesterol concentration, and oxidized low-density lipoprotein levels, are related to the presence of linoleic acid, ergosterol, and ergosterol derivatives of *P. ostreatus* fruiting bodies.

Numerous studies have attributed functional properties to both basidiomata and mycelia of *Pleurotus* spp., some of them precisely defining the compounds, among them sterols, involved in these bioactivities (Corrêa et al. 2016). Some examples are:

(1) an ergosterol peroxide isolated from the dried fruiting bodies of *Pleurotus ostreatus* (Jacq.) P. Kumm showed potent amoebicidal activity against the intestinal parasite *Entamoeba histolytica*, but no toxicity against human colon cells (Meza-Menchaca et al. 2015); (2) a novel 5,6-seco-ergostane-type steroid from the basidioma of *Pleurotus eryngii* (DC.) Quél., showed anti-inflammatory potential in lipopoly-saccharide-induced mouse macrophages along with slight cytotoxicity (Kikuchi et al. 2015); (3) six ergostane-type steroids were isolated from *P. eryngii* basidioma, and presented inhibitory effects on nitric oxide production (Kikuchi et al. 2016).

21.4.4 Ganoderma lucidum Mycosterols

Polysaccharides and triterpenoids are considered the most important bioactives of the medicinal mushroom *Ganoderma lucidum* (Lu et al. 2020; Liang et al. 2019. However, the biological properties of their mycosterols have received more attention in the last years (Chen et al. 2017; Xu et al. 2021; Weng et al. 2010; see some chemical structures of *G. lucidum* mycosterols in Fig. 21.3). Recently, ergosterol and ergosterol peroxide from *G. lucidum* were innovatively isolated by means of a single-step procedure in an aqueous two-phase system. Interestingly, ergosterol and ergosterol peroxide were obtained with much higher yields compared to the traditional saponification method, and exhibited promising anti-inflammatory properties (Xu et al. 2021).

21.4.5 Hericium erinaceum Mycosterols

Fruiting bodies of *Hericium erinaceum* (Hericiaceae) are a traditional herbal medicine widely used in China, Korea, and Japan. *H. erinaceum* is also a well-known edible mushroom, known as the Lion's Mane Mushroom (Li et al. 2015a, b). Several ergostane-type sterol fatty acid esters, including erinarol A and erinarol B (see Fig. 21.3), were isolated from the dried fruiting bodies of *H. erinaceum* and presented anti-inflammatory and peroxisome proliferator-activated receptors (PPARs) transactivational effects (Li et al. 2014).

21.5 Potential of Mycosterols in Foods

To the best of our knowledge, until the present moment, no functional food capable of inhibiting cholesterol synthesis has been introduced into the market. In this sense, edible mushroom extracts might be explored as sources of biomolecules that could not only impair cholesterol absorption (such as ergosterol and/or derivatives, soluble

polysaccharides, β-glucans and chitins), but also inhibit its biosynthesis (e.g., natural statins).

In a pioneer study on the cholesterol-lowering activity of mycochemicals added to food matrices, Caz et al. (2016) investigated the hypocholesterolemic effects of lard, functionalized by the addition of a *L. edodes* ergosterol-enriched extract (0.44%) at the proportion of 12%, used as animal feed. The ergosterol-added lard intake significantly reduced plasma cholesterol, LDL-cholesterol, and HDL-cholesterol levels of hypercholesterolemic mice, being the hypocholesterolemic response not related to transcriptional changes (post-transcriptional mechanisms might be involved).

Heleno et al. (2017) studied, for the first time, the incorporation of ergosterol obtained from *A. bisporus* into dairy beverages at concentrations mimicking commercial phytosterol-added yogurts. The ergosterol-enriched yogurt was assessed for nutritional and bioactive properties and compared with controls (no additives or phytosterol-added yogurts), at two storage times (right after product manufacture and after seven days at 4 °C). The ergosterol-enriched yogurt showed similar antioxidant properties as the PS-added yogurt. It had, however, superior cytotoxicity against tumor cells, being that the ergosterol-enriched yogurt sample was the strongest in both bioactivities. Although nutritional parameters were identical for all samples, ergosterol protected ergosterol-enriched yogurt from oxidation throughout the 7-day storage period.

More recently, Corrêa et al. (2018) used commercially discarded *Agaricus blazei* fruiting bodies for obtaining an extract rich in ergosterol as a fortifier ingredient for yogurts. When added to the latter it significantly enhanced their antioxidant properties. Thus, A. *blazei* fruiting bodies that do not conform to the commercial requirements of the market and are normally discarded could be exploited for obtaining a natural high-added value food additive, following the circular bioeconomy concept.

Lovastatin (mevinolin), a secondary metabolite from fungal growth, integrates the class of statin drugs, which diminish cholesterol biosynthesis in the liver by competitively inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoA reductase) (Chen et al. 2012). Thus, lovastatin can reduce LDL-cholesterol and triglyceride levels while it increases HDL-cholesterol, consequently lowering cardiovascular disease risk (Ng and Ng 2014). Atli and Yamac (2012) identified six isolates of Turkey basidiomycete mushrooms as good lovastatin producers. The best results were found for *Omphalotus olearius* (DC.) Sing. (5.8 mg/L) and *Pleurotus ostreatus* (4 mg/L). Chen et al. (2012) investigated the contents of lovastatin in both fruiting bodies and mycelia of 29 fungi species, including edible and medicinal mushrooms. Among the assessed basidiomata, *P. ostreatus* (606 mg/kg) and *A. bisporus* (565 mg/kg) showed the most expressive contents of lovastatin, while among mycelia, *Cordyceps sinensis* (Berk) Sacc. (1365 mg/kg) and *Antrodia salmonea* TT Chang & WN Chou (1032 mg/kg) had the highest concentrations.

Several authors have reported other fungal compounds with HMGCoA reductase inhibitory activity. For instance, Gil-Ramirez et al. (2013) suggested that β-glucans are the compounds involved in the HMGCoA reductase inhibitory effects displayed by *A. bisporus* fractions obtained via pressurized solvent technologies. The tested

A. bisporus extracts, which were also able to lower cholesterol levels in hypercholesterolaemic rats (probably by inhibiting cholesterol absorption), are great examples of promising ingredients for cholesterol-lowering functional foodstuff formulations.

Ergosterol plays also a substantial role in the human body as a precursor of vitamin D_2 (ergocalciferol), which is generated in response to ultraviolet radiation (sunlight) on sterols present in the skin (Mokochinski et al. 2015). Thus, the intake of food products enriched with fungal ergosterol might contribute to address vitamin D deficiency issues, and consequently offer to consumers several vitamin D-related health benefits such as improvements in immunity, bone metabolism, muscle function and cognition, and mood outcomes (Feeney et al. 2014).

21.6 Challenges for Obtaining Mycosterols on Large Scale

Almost all the fungal ergosterol fractions (and other bioactive mycosterols) are obtained from fruiting bodies. Although the by-products of basidioma's commercial production represent a sustainable and quite interesting source of ergosterol (Heleno et al. 2016a, b), producing fruiting bodies for sterol obtainment purposes is not viable. Large-scale mushroom production is an effortful and time-consuming process (can take several months), what demands huge volumes of substrate and consequently wide spaces, high energy costs to ensure the right temperature and humidity for cultivation (especially in tropical countries) and skilled labor (Corrêa et al. 2016). On the other hand, vegetative phase cultivation techniques are dramatically faster and more functional for the obtainment of high value-added molecules like mycosterols, as they require much smaller spaces, and, more important, when automatized, enable the accurate control of cultivation parameters (Inácio et al. 2015). This strict control of temperature, humidity, pH, and aeration provided by bioreactors can be extremely useful in optimizing the production of specific biomolecules.

While submerged fermentation has been considered the most effective technique to produce fungal mycelia and their bioactive compounds after shorter fermentation times (in up to a week), minimum space and superior control of process parameters, solid-state fermentation reproduces the natural environment of fungi development, demands low capital investment and minimizes contamination due to the little amount of water that is used, thus figuring out as the ideal technique for large-scale production (Mokochinski et al. 2015). However, the resemblances of the bioactive compound profiles in basidiomata and mycelia need to be previously confirmed (Corrêa et al. 2016). In an inedited comparative study on the ectomycorrhizal symbiont *Suillus bellinii* (Inzenga) Watling, Souilem et al. (2017) found that its mycelium, independently of the culture conditions, presented higher contents in ergosterol (8.9–12.4 mg/g extract) than its corresponding basidioma sample (6.5 mg/g extract).

21.7 Conclusion and Perspectives

Mycosterol-enriched fungi extracts are potential sources of biomolecules that could not only impair cholesterol absorption but also inhibit its biosynthesis and to promote other significant human health benefits. This makes them valuable and promising ingredients for the development of sterol-enriched food products. The isolation of novel bioactive mycosterols (especially ergosterol derivatives) from still underexploited fungi species, the optimization of the existing methodologies for production and recovery of these target mycochemicals (mainly automatized liquid fermentation technologies), the extensive study of their application in food product formulations (including products' bioaccessibility, efficacy, stability, as well as nutritional and sensorial evaluation), and finally further clinical trials for attesting their health benefits and safety, are challenges that science should seek to overcome in the coming years.

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References

Abe F, Hiraki T (2009) Mechanistic role of ergosterol in membrane rigidity and cycloheximide resistance in *Saccharomyces cerevisiae*. Biochim Biophys Acta (BBA) – Biomembr 1788:743–752

Alcazar-Fuoli L, Mellado E, Garcia-Effron G, Lopez JF, Grinalt JO, Cuenca-Estrella JM (2008) Ergosterol biosynthesis pathway in *Aspergillus fumigatus*. Steroids 73:339–347

Atli B, Yamac M (2012) Screening of medicinal higher basidiomycetes mushrooms from Turkey for lovastatin production. Int J Med Mushrooms 14:149–159

Barreira JC, Oliveira MBP, Ferreira IC (2014) Development of a novel methodology for the analysis of ergosterol in mushrooms. Food Anal Methods 7:217–223

Bhattacharya S (2021) Ergosterol synthesis. Encyclopedia of Mycology 1:230–238

Caz V, Gil-Ramirez A, Santamaria M, Soler-Rivas C, Martin-Hernandez R, Marín FR, Reglero G, Largo C (2016) Plasma cholesterol-lowering activity of lard functionalized with mushroom extracts is independent of Niemann–Pick C1-like 1 protein and ABC sterol transporter gene expression in hypercholesterolemic mice. J Agric Food Chem 64:1686–1694

Chen SY, Ho KJ, Hsieh YJ, Wang LT, Mau JL, J.L. (2012) Contents of lovastatin, γ-aminobutyric acid and ergothioneine in mushroom fruiting bodies and mycelia. LWT–Food Sci Technol 47:274–278

Chen S, Yong T, Zhang Y, Su J, Jiao C, Xie Y (2017) Anti-tumor and anti-angiogenic ergosterols from *Ganoderma lucidum*. Front Chem 5:article 85

Corrêa RCG, Brugnari T, Bracht A, Peralta RM, Ferreira ICF (2016) Biotechnological, nutritional and therapeutic uses of *Pleurotus* spp. (oyster mushroom) related with its chemical composition: a review on the past decade findings. Trends Food Sci Technol 50:103–117

Corrêa RC, Peralta RM, Bracht A, Ferreira ICFR (2017) The emerging use of mycosterols in food industry along with the current trend of extended use of bioactive phytosterols. Trends Food Sci Technol 67:19–35

Corrêa RCG, Barros L, Fernandes A, Sokovic M, Bracht A, Peralta RM, Ferreira ICFR (2018) A natural food ingredient based on ergosterol: optimization of the extraction from *Agaricus blazei*, evaluation of bioactive properties and incorporation in yogurts. Food Funct 9:1465–1474

Dhingra S, Cramer RA (2017) Regulation of sterol biosynthesis in the human fungal pathogen *Aspergillus fumigatus*: opportunities for therapeutic development. Front Microbiol 8:article 92

Dupont S, Lemetais G, Ferreira T, Cayot P, Gervais P, Beney L (2012) Ergosterol biosynthesis: a fungal pathway for life on land? Evolution 66:2961–2968

Feeney MJ, Miller AM, Roupas P (2014) Mushrooms—biologically distinct and nutritionally unique: exploring a "third food kingdom". Nutr Today 49:301–307

Gil-Ramirez A, Soler-Rivas C (2014) The use of edible mushroom extracts as bioactive ingredients to design novel functional foods with hypocholesterolemic activities. Chapter 2. In: Pesti G (ed) Mushrooms: cultivation, antioxidant properties and health benefits. Nova Science Publishers, New York, pp 43–73

Gil-Ramirez A, Clavijo C, Palanisamy M, Ruiz-Rodriguez A, Navarro-Rubio M, Pérez M, Marin FR, Reglero G, Soler-Rivas C (2013) Study on the 3-hydroxy-3-methyl-glutaryl CoA reductase inhibitory properties of *Agaricus bisporus* and extraction of bioactive fractions using pressurized solvent technologies. J Sci Food Agric 93:2789–2796

Gil-Ramírez A, Ruiz-Rodríguez A, Marín FR, Reglero G, Soler-Rivas C (2014) Effect of ergosterol-enriched extracts obtained from *Agaricus bisporus* on cholesterol absorption using an in vitro digestion model. J Funct Foods 11:589–597

Gomez-Lopez A, Buibrago MJ, Rodriguez-Tudela JL, Cuenca-Estrella M (2011) In vitro antifungal susceptibility pattern and ergosterol content in clinical yeast strains. Rev Iberoam Micol 28:100–103

Heleno SA, Diz P, Prieto MA, Barros L, Rodrigues MF, Barreiro MF, Ferreira ICF (2016a) Optimization of ultrasound-assisted extraction to obtain mycosterols from *Agaricus bisporus* L. by response surface methodology and comparison with conventional Soxhlet extraction. Food Chem 197:1054–1063

Heleno SA, Prieto MA, Barros L, Rodrigues A, Barreiro MF, Ferreira ICF (2016b) Optimization of microwave-assisted extraction of ergosterol from *Agaricus bisporus* L. by-products using response surface methodology. Food and Bioprod Process 100:25–35

Heleno SA, Rudke AR, Calhelha RC, Carocho M, Barros L, Gonçalves OH, Barreiro MF, Ferreira ICFR (2017) Development of dairy beverages functionalized with pure ergosterol and mycosterol extracts: an alternative to phytosterol-based beverages. Food Funct 8:103–110

Inácio FD, Ferreira RO, Araújo CAVD, Brugnari T, Castoldi R, Peralta RM, Souza CGM (2015) Proteases of wood rot fungi with emphasis on the genus *Pleurotus*. BioMed Research International 2015:Article 290161

Kang JH, Jang JE, Mishra SK, Lee HJ, Nho CW, Shin D, Jin M, Kim MK, Choi C, Oh SH (2015) Ergosterol peroxide from Chaga mushroom (*Inonotus obliquus*) exhibits anti-cancer activity by down-regulation of the β-catenin pathway in colorectal cancer. J Ethnopharmacol 173:303–312

Kikuchi T, Masumoto Y, In Y, Tomoo K, Yamada T, Tanaka R (2015) Eringiacetal A, 5,6-seco-(5S,6R,7R,9S)- 5,6:5,7:6,9-Triepoxyergosta-8(14),22-diene-3β,7β-diol, an unusual ergostane sterol from the fruiting bodies of *Pleurotus eryngii*. Eur J Org Chem:4645–4649

Kikuchi T, Maekawa Y, Tomio A, Masumoto Y, Yamamoto T, In Y, Yamada T, Tanaka R (2016) Six new ergostane-type steroids from king trumpet mushroom (*Pleurotus eryngii*) and their inhibitory effects on nitric oxide production. Steroids 115:9–17

Li W, Zhou W, Song SB, Shim SH, Kim YH (2014) Sterol fatty acid esters from the mushroom *Hericium erinaceum* and their PPAR transactivational effects. J Nat Prod 77:2611–2618

Li X, Wu Q, Zie Y, Ding Y, Du WW, Sdiri M, Yang BB (2015a) Ergosterol purified from medicinal mushroom *Amauroderma rude* inhibits cancer growth in vitro and in vivo by up-regulating multiple tumor suppressors. Oncotarget 6:17832–17846

Li W, Zhou W, Cha JY, Kwon SU, Baek KH, Shim SH, Lee YM, Kim YH (2015b) Sterols from *Hericium erinaceum* and their inhibition of TNF-α and NO production in lipopolysaccharide-induced RAW 264.7 cells. Phytochemistry 115:231–238

Liang C, Tian D, Liu Y, Li H, Zhu J, Li M, Xin M, Xia J (2019) Review of the molecular mechanisms of *Ganoderma lucidum* triterpenoids: ganoderic acids A, C2, D, F, DM, X and Y. Eur J Med Chem 174:130–141

Lu J, He R, Sun P, Zhang F, Linhardt RJ, Anqiang Z (2020) Molecular mechanisms of bioactive polysaccharides from *Ganoderma lucidum* (Lingzhi), a review. Int J Biol Macromol 150:765–774

Meza-Menchaca T, Suárez-Medellín J, Ángel-Piña D, Trigos A (2015) The amoebicidal effect of ergosterol peroxide isolated from *Pleurotus ostreatus*. Phytother Res 29:1982–1986

Mokochinski JB, Sovrani V, Dalla Santa HS, Felsner ML, Sawaya ACHF, González-Borrero PP, Bataglion GA, Eberli MN, Torres YR (2015) Biomass and sterol production from vegetal substrate fermentation using *Agaricus brasiliensi*s. J Food Qual 38:221–229

Ng TB, Ng CCW (2014) Antihyperlipidemic effects of mushroom extracts and compounds, Chapter 9. In: Pesti G (ed) Mushrooms: cultivation, antioxidant, properties and health benefits. Nova Science, New York, pp 189–197

Nowak R, Drozd M, Mendyk E, Lemieszek M, Krakowiak O, Kisiel WR, W., Szewczyk, K. (2016) A new method for the isolation of ergosterol and peroxyergosterol as active compounds of *Hygrophoropsis aurantiaca* and in vitro antiproliferative activity of isolated ergosterol peroxide. Molecules 2016(21):946

Phillips KM, Ruggio DM, Horst RL, Minor B, Simon RR, Feeney MJ, W.C., Haytowitz, D.B. (2011) Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. J Agric Food Chem 59:7841–7853

Schneider I, Kressel G, Meyer A, Krings U, Berger RG, Hahn A (2011) Lipid lowering effects of oyster mushroom (*Pleurotus ostreatus*) in humans. J Funct Foods 3:17–24

Shimizu T, Kawai J, Ouchi K, Kikuchi H, Osima Y, Hidemi R (2016) Agarol, an ergosterol derivative from *Agaricus blazei*, induces caspase-independent apoptosis in human cancer cells. Int J Oncol 48:1670–1678

Sinanoglou VJ, Zoumpoulakis P, Heropoulos G, Proestos C, Cric A, Petrovic J, Glamoclija J, Sokovic M (2015) Lipid and fatty acid profile of the edible fungus *Laetiporus sulphurous.* Antifungal and antibacterial properties. J Food Sci Technol 52:3264–3272

Song Z, Nes WD (2007) Sterol biosynthesis inhibitors: potential for transition state analogs and mechanism-based inactivators targeted at sterol methyltransferase. Lipids 42:15–33

Souilem F, Fernandes A, Calhelha RC, Barreira JC, Barros L, Skhiri F, Martins A, Ferreira ICFR (2017) Wild mushrooms and their mycelia as sources of bioactive compounds: antioxidant, anti-inflammatory and cytotoxic properties. Food Chem 230:40–48

Torres S, Cajas D, Palfner G, Astuya A, Aballay A, Pérez C, Hernández V, Becerra J (2017) Steroidal composition and cytotoxic activity from fruiting body of *Cortinarius xiphidipus*. Nat Prod Res 31:473–476

Villares A, Mateo-Vivaracho L, Garcia-Lafuente A, Guillamón E (2014) Storage temperature and UV-irradiation influence on the ergosterol content in edible mushrooms. Food Chem 147:252–256

Wang Q, Cheng J, Wang L, Yan S, Wang R, Zhang H, Shao H, Yang X (2018) Valorization of spent shiitake substrate for recovery of antitumor fungal sterols by ultrasound-assisted extraction. J Food Biochem 42:e12602

Weete JD, Abril M, Blackwell M (2010) Phylogenetic distribution of fungal sterols. PLoS One 5(5): e10899

Weng Y, Xiang L, Matsuura A, Zhang Y, Huang Q, Qi J (2010) Ganodermasides A and B, two novel anti-aging ergosterols from spores of a medicinal mushroom *Ganoderma lucidum* on yeast via *UTH1* gene. Bioorg Med Chem 18:999–1002

Xu Z, Meenu M, Xu B (2020) Effects of UV-C treatment and ultrafine-grinding on the biotransformation of ergosterol to vitamin D_2, physiochemical properties, and antioxidant properties of shiitake and Jew's ear. Food Chem 309:125738

Xu J, Xiao CM, Xu, H.S>, Yan, S.X., Chen, Z.M., Wang, H.Z., Zheng, B.S., Mao, B.Z., Wu, X.Q. (2021) Anti-inflammatory effects of *Ganoderma lucidum* sterols via attenuation of the p38 MAPK and NF-κB pathways in LPS-induced RAW 264.7 macrophages. Food Chem Toxicol 150:112073

Yeh MY, Ko WC, Lin LY (2014) Hypolipidemic and antioxidant activity of enoki mushrooms (*Flammulina velutipes*). Biomed Res Int 2014:352385