







Review

Roles of Medicinal Mushrooms as Natural Food Dyes and Dye-Sensitised Solar Cells (DSSC): Synergy of Zero Hunger and Affordable Energy for Sustainable Development

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Abstract: In 2015, approximately 195 countries agreed with the United Nations that by 2030, they would work to make the world a better place. There would be synergies in accomplishing the 17 Sustainable Development Goals (SDGs). Synergy using a single sustainable resource is critical to assist developing nations in achieving the SDGs as cost-effectively and efficiently possible. To use fungal dye resources, we proposed a combination of the zero hunger and affordable energy goals. Dyes are widely used in high-tech sectors, including food and energy. Natural dyes are more environment-friendly than synthetic dyes and may have medicinal benefits. Fungi are a natural source of dye that can be substituted for plants. For example, medicinal mushrooms offer a wide range of safe organic dyes that may be produced instantly, inexpensively, and in large quantities. Meanwhile, medicinal mushroom dyes may provide a less expensive choice for photovoltaic (PV) technology due to their non-toxic and environmentally friendly qualities. This agenda thoroughly explains the significance of pigments from medicinal mushrooms in culinary and solar PV applications. If executed effectively, such a large, unwieldy and ambitious agenda may lead the world towards inclusive and sustainable development.

Keywords: medicinal mushroom; fungal dyes; food colourants; clean energy; sustainability

1. Introduction

Climate change has been aggravated due to global warming. The global temperature increment of 1.5 °C above pre-industrial levels, emissions of greenhouse gases worldwide [1], and biodiversity loss [2] serve as a wake-up call for urgent action to enhance the global response to the climate change threat as well as to achieve sustainable development and poverty alleviation [3]. In 2015, the United Nations (UN) set the 2030 agenda for

sustainable development to achieve the 17 Sustainable Development Goals (SDGs) [4]. The SDGs build on the Millennium Developmental Goals (MDGs) for the year 2015 and extend the thematic and geographic scope [5,6], and includes all countries from the Global South to the Global North. Additionally, the SDGs unambiguously resolve ecological sustainability issues [7] most cost-effectively and competently.

The growing awareness about the harmful impacts of synthetic dyes on both consumers and the environment has heightened the interest in natural dye replacements [8]. Therefore, adopting the usage of natural dyes [9] could offer cheap energy with 'no hunger'. Therefore, natural dye industries have emerged and have become an ever-growing market [8,10]. However, relying on natural pigments derived from plants and animals confront a number of hurdles, including raw material availability, instability, and sensitivity to many external conditions [8,11].

Pigments made from micro-organisms, on the other hand, have a number of advantages, including supply sustainability, high yield, cost-effectiveness, consistency, labour cost, and ease of downstream processing. Among microbes, fungi have produced a myriad of pigments since prehistoric times, which are mostly beneficial to human welfare in various facets, especially the in cosmetic, textile, and food sectors, unlike some of which proved to be detrimental. Filamentous fungi, ascomycetes, and basidiomycetes fungi (mushrooms), and lichens (symbiotic association of a fungus with a green alga and cyanobacterium) are known to produce a variety of water soluble pigments with different chemical structures and colours, such as quinines, flavins, phenazines, azaphilones, and melanins [8,10], that have wide ecological applications [12]. On the other hand, filamentous fungi sourced from marine (marine sediment inhabitant fungi, invertebrate inhabitant fungi, and halophytes), mangrove (mangrove sediment habitat fungi and mangrove endophytes), and terrestrial ecosystems (terrestrial sediments and soil inhabitant fungi, endophytes from geothermal soil, endophytes from terrestrial plants, lichenic endophytes, and symbionts), can produce pigments in a large scale and be easily grown in the laboratory [8]. Four genera of filamentous fungi, viz. *Aspergillus*, *Penicillium*, *Paecilomyces*, and *Monascus* [13] are well-studied fungal pigment producers. For instance, the ascomycetes fungi *Monascus* sp. produces ankaflavin and canthaxanthine, while *Penicillium oxlicum* var. *armeniaca* secretes red™ pigment, which is commercialised [8]. Similarly, several species of *Aspergillus*, such as *A. sulphureus*, *A. ochraceus*, *A. glaucus*, and *A. varicolor* synthesise Viopurpin (Compound: Naphtoquinones; Colour: Purple), Viomellein (Compound: Quinone; Colour: Reddish brown), Catenarin (Compound: Hydroxyanthraquinone; Colour: Red), Variicolorquinone A (Compound: Quinone; Colour: Yellow), respectively [8,14,15]. Several other fungal genera, such as *Fusarium*, *Alternaria*, *Euraniium*, *Trichoderma*, and *Trypethelium*, also produce these pigments. Biosynthetically, these pigments are polyketides derivatives and are chemically grouped into carotenoids, melanins, polyketides, and azaphilones [16].

Basidiomycetous fungus (mushroom) species produce a variety of colours that have been widely employed in the textile industry for dyeing. In a symposium organised by the International Fungi and Fibre Federation in Canada in 2016, varied species of *Cortinarius* viz. *C. sanguineus*, *C. cinnamomeus*, and *C. malicorius* could efficiently dye fibres with their pigments at pH range of 3.5–7 in the presence of (SnCl₂) [17]. Several other mushroom pigments, such as carotenoid and the red pigment lilacinone are produced by *Cordyceps militaris* [18] and *Lactarius lilacinus* [19], respectively, that are used commercially. Similarly, melanin pigment, which has a high molecular weight and is composed of complex heterogeneous polymers of phenolic and/or indolic monomers, comes in three forms: eumelanin and allomelanin, which give a black colour, and phe-nomelanin, which gives a yellow-reddish colour and is found in a wide variety of mushrooms, such as *Auricularia auricular* [20], *Pleurotus cystidiosus* [21], *Armillaria cepistipes* [22], *Agaricus bisporus* [23,24], and *Lentinula edodes* [25].

These dyes have been recently used to build solar cells known as dye-sensitised solar cells (DSSC), which are used in the development of photovoltaic (PV) technology and operate well under low irradiation conditions [26–28]. Therefore, this review paper

summarises natural dyes extracted from mushrooms and their utility in the food industries and energy sectors. It also discusses the safety, production, limitation, and future strategy of medicinal mushroom dyes in DSSC.

2. Medicinal Mushroom Dyes

Dyes have always been essential in various fields of human activity, dating back to prehistoric times [29]. In addition to pigments extracted from numerous plants, mushrooms were also a common material for dyeing fabrics due to the wide range of vivid colours [30]. However, during the development of human society, the need for pigments grew steadily, and their purpose was very diverse. Dyes obtained from natural sources were no longer sufficient to meet all needs, which in addition to costly extraction and instability led to the development of synthetic dyes during the 1800s. Nevertheless, modern society is trying to return to natural sources of pigments, which is of particular interest in the food industry, as synthetic dyes lead to many harmful health effects, including the carcinogenic [31].

Mushrooms are a vibrant but still insufficiently researched natural source of dyes. Their pigmentation can vary depending on age, and some species are subject to discoloration when the tissue is damaged. Chromophores contain valuable organic compounds that play multiple roles in the life of mushroom, such as protection from harmful UV radiation, antimicrobial action, and attracting insects to spread their spores [32]. Melanin is a pigment that protects us from the effects of the environment [33]. Flavins as cofactors of enzymes [29] or carotenoids necessary to prevent the harmful effects of photooxidation [12] are just some of the pigments that directly affect a number of biological activities.

The pigments present in mushrooms differ from those that are dominant in plants, hence they do not contain any chlorophylls and anthocyanins. In contrast, some species contain betalains and terpenoids, among which carotenoids are common [32]. According to Lin and Xu [10], four main pathways of biosynthesis of the most important mushroom pigments have been described to date:

1. Polyketide synthetic pathway; *Antrodia cinnamomea* [34]—allowing the formation of melanin, flavins, ankaflavin, quinones, and azaphilones. Aromatic ketides or fatty acids are formed via this pathway. Cyclisation reactions and partial reductions lead to stabilisation of the growing chain in the synthesis of aromatic ketides. In the case of fatty acid synthesis, the carbonyl groups of the chain are reduced prior to binding to the next C2 group. This process produces tetra-, hepta-, octa-, and higher number of aromatic ketides as well as fatty acid-derived compounds;
2. Shikimate pathway; *Agaricus bisporus* [35]—the key intermediates of shikimic and chorismic acids are used to manufacture the important amino acids tyrosine, tryptophan, and phenylalanine. Various important building components of many colours found in fungi, such as benzoic, arylpyruvic, and cinnamic acids, are produced from tyrosine and phenylalanine precursors. Tyrosine is crucial in the phylum Basidiomycota because it is a precursor of betalaine, a pigment found solely in the genera *Hygrocybe* and *Amanita*;
3. Terpenoid synthetic pathway; *Hypsizygus marmoreus* [36]—significant for the formation of carotenoids belonging to terpenoids. The condensation routes of C5 isoprene units and terpenoid synthesis are triggered during carotenoids production. A common precursor to terpenoid formation is isopentenyl pyrophosphate, which is formed by mevalonate pathway;
4. Nitrogen-containing metabolite pathway; *Agaricus bisporus* [37]—ensure the formation of various fungal pigments.

The *Agrocybe cylindracea* fruiting body contains two indole pigments with pronounced free radical scavenging potential as well as distinct inhibitory activity on lipid peroxidation. *Chalciporus piperatus* produces chalciporone, a type of 2H-azepine alkaloid pigment, which has a protective role against insects and other predators. *Pycnoporus cinnabarinus* is a rich source of cinnabarinic acid, a red pigment that acts antibacterially against the

genus *Streptococcus* and is derived from precursors 3-hydroxyanthranilic acid that undergo oxidative dimerisation.

In recent decades, the biological properties of therapeutic mushrooms have been widely studied [38,39]. Although the specific biological effects of pigments remain understudied, research confirms the potential use of these important compounds in formulating health-promoting drugs that could be used to treat many disorders, such as cardiovascular disease, Alzheimer's disease, and infectious diseases [10]. Various pigments derived from some medicinal mushrooms are presented in Table 1.

Table 1. Pigment-producing medicinal mushrooms and their respective pigments.

| Mushroom Species | Family | Key Uses | Bioactive Pigment | Ref. |
|------------------------------------|----------------------|-------------------|---|------|
| <i>Agaricus bisporus</i> | Agaricaceae | Bruising-tolerant | γ -glutaminyl-4-hydroxybenzene | [24] |
| <i>Agrocybe cylindracea</i> | Strophariaceae | Immunity | 6-hydroxy-1H-indole-3-carboxaldehyde, | [10] |
| <i>Albatrellus confluens</i> | Albatrellaceae | Immunity | 6-hydroxy-1H-indole-3-acetamide | [40] |
| <i>Albatrellus flettii</i> | Albatrellaceae | Anti-cancer | albatrellin | [41] |
| <i>Auricularia auricula</i> | Auriculariaceae | Anti-cancer | grifolin, neogrifolin, confluentin | [42] |
| <i>Boletus pseudocalopus</i> | Boletaceae | Antibiofilm | melanin | [43] |
| <i>Cantharellus cibarius</i> | Cantharellaceae | Antioxidant | pheomelanin | [44] |
| <i>Ceriporus squamosus</i> | Polyporaceae | Anti-cancer | grifolin derivatives 1–3 | [45] |
| <i>Chalciporus piperatus</i> | Boletaceae | Antihypertensive | lycopene and β -carotene | [46] |
| <i>Cordyceps farinosa</i> | Cordycipitaceae | Blood regulation | melanin | [10] |
| <i>Fomes fomentarius</i> | Polyporaceae | Bio deterrent | chalciporone | [47] |
| <i>Inonotus hispidus</i> | Hymenochaetaceae | Protecion | anthraquinone derivative | [46] |
| <i>Inonotus obliquus</i> | Hymenochaetaceae | Anti-tumour | melanin | [46] |
| <i>Lactarius spp.</i> | Russulaceae | Antiviral | melanin, inotodiol | [48] |
| <i>Lactarius subvellereus</i> | Russulaceae | Antioxidant | melanin, azulenes | [49] |
| <i>Laetiporus sulphureus</i> | Fomitopsidaceae | Antimicrobial | subvellerolactone B, subvellerolactone D, subvellerolactone E, laetiporic acid A, 2-dehydro-3-deoxylaetiporic acid A, | [50] |
| <i>Lentinus brumalis</i> | Polyporaceae | Anti-cancer | laetiporic acid A, 2-dehydro-3-deoxylaetiporic acid A, laetiporic acids B, laetiporic acids C | [51] |
| <i>Ophiocordyceps unilateralis</i> | Ophiocordycipitaceae | Antioxidant | melanin | [46] |
| <i>Pleurotus citrinopileatus</i> | Pleurotaceae | Not available | Erythrostrominone, 3,5,8-TMON, deoxyerythrostrominone, deoxyerythrostrominol, 4-O-methyl, erythrostrominone, epierythrostrominol, naphthoquinones | [49] |
| <i>Pleurotus cornucopiae</i> | Pleurotaceae | Antimalarial | eumelanin, phaeomelanin | [52] |
| <i>Pleurotus djamor</i> | Pleurotaceae | Antioxidant | eumelanin, phaeomelanin | [52] |
| <i>Pycnoporus cinnabarinus</i> | Polyporaceae | Antioxidant | eumelanin, phaeomelanin | [52] |
| <i>Termitomyces albuminosus</i> | Agaricaceae | Antibacterial | cinnabarinic acid | [10] |
| <i>Trametes versicolor</i> | Polyporaceae | Antioxidant | melanin | [53] |
| <i>Tuber melanosporum</i> | Tuberaceae | Breast cancer | melanin | [46] |
| | | Anti-fatigue | melanin | [54] |

Carotenoids are a diverse set of pigments found throughout nature; not only are they a crucial component of plants that aid in photosynthesis, but their presence has also been proven in over 200 species of fungi [55]. Fungi are hypothesised to be protected by these pigments from UV radiation and oxidative damage. Carotenoids have an aliphatic polyene chain, which is made up of eight isoprene units and contains conjugated double bonds that absorb light, giving them their characteristic yellow, orange or red colour [56]. They benefit human health by acting as precursors to vitamin A as well as slowing down and preventing aging-related changes, such as retinal degeneration and cataracts. They also lower the risk of coronary heart disease and certain types of tumours. Their ability to scavenge free radicals and act as antioxidants has positive impacts on health [10]. The presence of two carotenoid pigments in significant amounts, lycopene and β -carotene,

as well as their contribution to the antioxidant potential of the methanolic mushroom extract *Cantharellus cibarius* (which has a distinct yellow-orange colour) was confirmed by Kozarski et al. [45].

Melanins are also very common pigments in fungi [57]. These are in fact polyphenolic and/or polyindole heterogeneous polymers that usually give a brown, black or dark green colour. The black extremophilous fungus *Cryomyces antarcticus*, which was discovered on the outside walls of the International Space Station and was initially isolated from the Antarctic desert, is an excellent example. The wide distribution of melanised fungi indicates that pigmentation allows them to survive in very hostile conditions prevailing in cold and hot deserts as well as in areas contaminated with ionising radiation and toxic metals [58]. As excellent scavengers of reactive nitrogen species as well as reactive oxygen species, melanins protect cells from the damaging effects of free radicals [10]. The pathway of melanin synthesis in mushrooms involves the conversion of benzoquinone from a γ -glutaminy-4-hydroxybenzene (GHB) precursor by tyrosinase. Weijn et al. [24] discovered the presence of GHB as the most abundant phenolic component in the fruiting body and spores of *Agaricus bisporus*. One of the highly melanised medicinal mushrooms, *Inonotus obliquus*, also known as Chaga, contains bioactive phenolic components, such as melanin as well as triterpenoids (e.g., inotodiol) [59]. As Lin and Xu [10] stated, research to date indicate a significant contribution of these pigments to health improvement, among other things due to their hypoglycemic ability and antiproliferative effect on some cancer cell lines. The high content of melanin has also been confirmed in the medicinal mushroom *Auricularia auricula*, which has a proven potential in the treatment of various health disorders. This pigment may affect the antioxidant capacity as well as inhibit *quorum sensing*, causing the prevention of biofilm formation [43]. The presence of several pigments in one genus has also been proven, and the final colour of the mushroom depends on the quantities and relative ratios of individual pigments. The colours of the caps *Pleurotus citrinopileatus*, *P. cornucopiae*, and *P. djamor* are yellow, gray, and pink, respectively. In all three species, the confirmed presence of melanin consist of eumelanin and pheomelanin, the interrelationships of which result in a corresponding colour [52].

There are many other types of pigments that are an integral part of medicinal mushrooms and affect their beneficial effects on health. Due to the abundance and variety of pigments, medicinal mushrooms are also potentially important sources of food colourants. Mushroom pigments could provide visual appeal to food and contribute to improving health due to their beneficial effect on many body functions.

There are key chemical structures found in pigment producing medicinal mushrooms, such as azulenes from *Lactarius indigo* blue mushroom (Figure 1a,b) as well as other common *Lactarius deliciosus* and *Ganoderma theaeecolum*. These azulene derivatives are identified as guaiane sesquiterpenes, such as 7-(1-hydroxyethyl)-4-methyl-1-azulenecarboxaldehyde, 7-isopropenyl-4-methyl-1-azulenecarboxylic acid, 4-methyl-7-(1-methylethenyl)azulene-1-carbaldehyde, and 7-(1-hydroxy-1-methylethyl)-4-methylazulene-1-carbaldehyde. Meanwhile, anthraquinonens (Figure 1c,d) are found in the red coloured *Cortinarius sanguineus* [60], such as Emodin, dermocybin-1- β -D-glucopyranoside, Icterinoidin B₁, Austrocolorin A₁, and Hypericin (Table 2).

Melanine derivatives are commonly found in *Xylaria polymorpha* (Figure 2a) and *Auricularia auricula* (Figure 2b), such as GHB-melanin (green), Eumelanins (pink), Pyomelanins (red), and Allomelanins (blue). The antecedents for the four types of fungal melanin are simplified in Figure 2. GHB-melanins are produced in the body by a sequence of events involving tyrosinase. The L-Dopa pathway, which uses tyrosine as a precursor, produces eumelanins. Pyomelanins, such as tyrosine, have a tyrosine precursor but are eventually made from HGA. 1,8-DHN is the source of allomelanins [61].

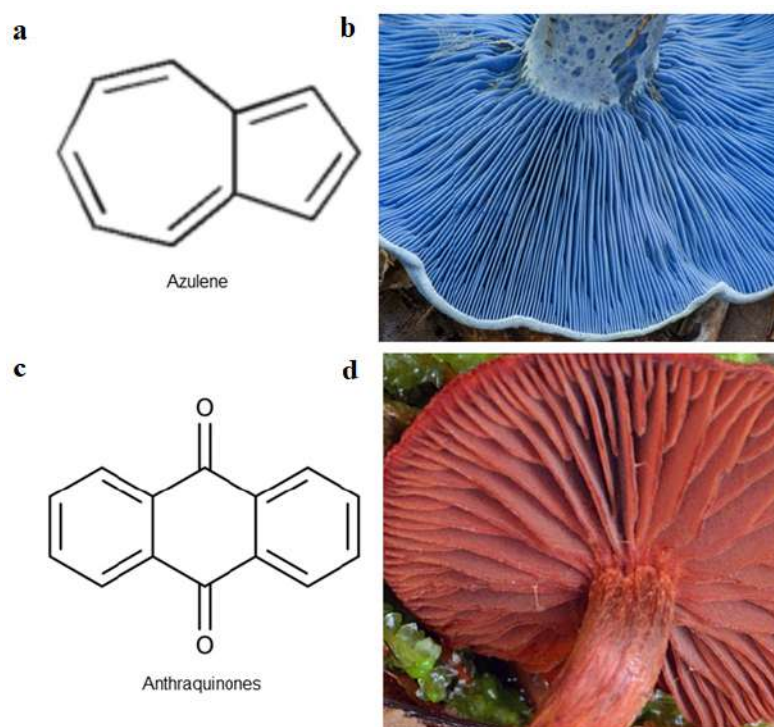


Figure 1. Chemical structures found in pigment-producing medicinal mushrooms. (a) chemical structure of azulene; (b) *Lactarius indigo* containing azulenes; (c) chemical skeleton of anthraquinones; (d) *Cortinarius sanguineus* containing anthraquinone dye derivatives.

Table 2. Anthraquinone dyes derivatives from the common *Cortinarius sanguineus*.

| Compounds Name | Molecular Weight [g/mol] and Formula | Chemical Structure | Species |
|----------------------------------|--------------------------------------|--------------------|--|
| Emodin | 270.0528 $C_{15}O_5H_{10}$ | | <i>Cortinarius sanguineus</i> , <i>C. semisanguineus</i> |
| Dermocybin-1-β-D-glucopyranoside | 478.1111 $C_{22}O_{12}H_{22}$ | | <i>C. vitiosus</i> , <i>C. sanguineus</i> , <i>C. semisanguineus</i> |
| Icterinoidin B ₁ | 528.1056 $C_{29}H_{20}O_{10}$ | | <i>C. icterinoides</i> |
| Austrocolorin A ₁ | 602.2152 $C_{34}H_{34}O_{10}$ | | <i>Dermocybe</i> sp. WAT 26641 |
| Hypericin | 504.0845 $C_{30}H_{16}O_8$ | | <i>C. austrovenetus</i> |

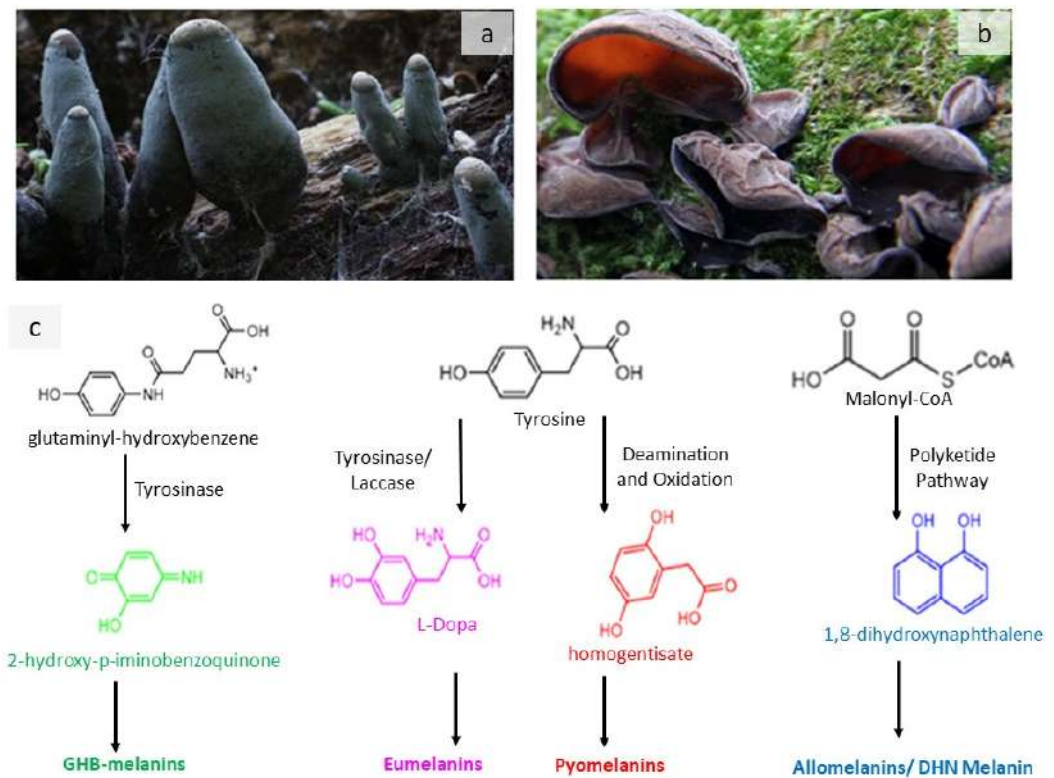


Figure 2. Simplified depiction of the four basic types of fungal melanin precursors. (a) *Xylaria polymorpha* containing melanins; (b) *Auricularia auricula* containing melanins; (c) Biosynthesis scheme. The figure is improvised based on a previous work [61].

3. Medicinal Mushroom Pigments in Food Production

Apart from being the raw material for nutraceuticals, medicinal mushrooms are increasingly explored as functional foods, whether alone or as an additive [62]. Because of its antioxidant, anti-inflammatory, anti-obesity, hepatoprotective, antibacterial, and antidiabetic action, mushroom components are believed to provide health advantages [38,63]. However, introducing any new compound into the already established food product can affect its physicochemical and sensorial characteristics, such as cohesiveness, chewiness, volume, texture, smell, taste, aroma, and colour. Each has its significance, which contributes to the overall acceptancy, and colour is among the most important [64]. Thus, studies dealing with novel food products with mushrooms have also examined their influence on colour. Some of the several categories of foods in which mushrooms have been used as functional ingredients and for the colour are summarised in Figure 3.

Bread and pasta are staple and popular foods worldwide, hence it is no wonder that much of the contemporary research effort is going into this sector. Mushrooms, mainly the commercial species, such as white button, oyster, black ear mushroom, and shiitake, were added as a supplement in bread making in concentrations ranging from 2.5% to 20%. The addition of mushroom flour (dried and powdered fruit bodies or mycelium) caused a decrease in crumb and crust lightness, which was attributed to the flour colour and Maillard reaction as well as caramelisation. These loaves of bread were generally less acceptable among panelists, and the recommendation was to use up to 5% of mushroom powder in white bread recipes. However, in a study by Sulieman et al. [65], the darker crumb of mushroom supplemented bread was marked as positive since the new formulation was compared with the gluten-free type of bread, which is usually lighter. Ulzjargal et al. [66] experimented with some less common mushroom species as additives in bread production, such as *Anthrodia camphorata*, *Hericium erinaceus*, and *Phellinus linteus*. They concluded that colour changes in final products were the result of pigments present in mushroom mycelium as well as oxidation and caramelisation during thermal treatment.

In the case of noodles, the addition of mushroom powder caused a decrease in lightness and yellowness, while the redness factor (obtained by instrumental colour assessment) increased. *Ganoderma lucidum* caused the most significant colour change in noodles [67]. Colour was not the most significant sensory attribute in the case of noodles, but the addition of mushroom powder affected other characteristics, such as texture and mouthfeel, hence the adequate concentration of mushroom supplement should not exceed 5% [68]. The same was concluded in the case of muffins, sponge cake and cookies enriched with mushrooms. The introduction of this component affected the decrease in product's lightness [69–71].

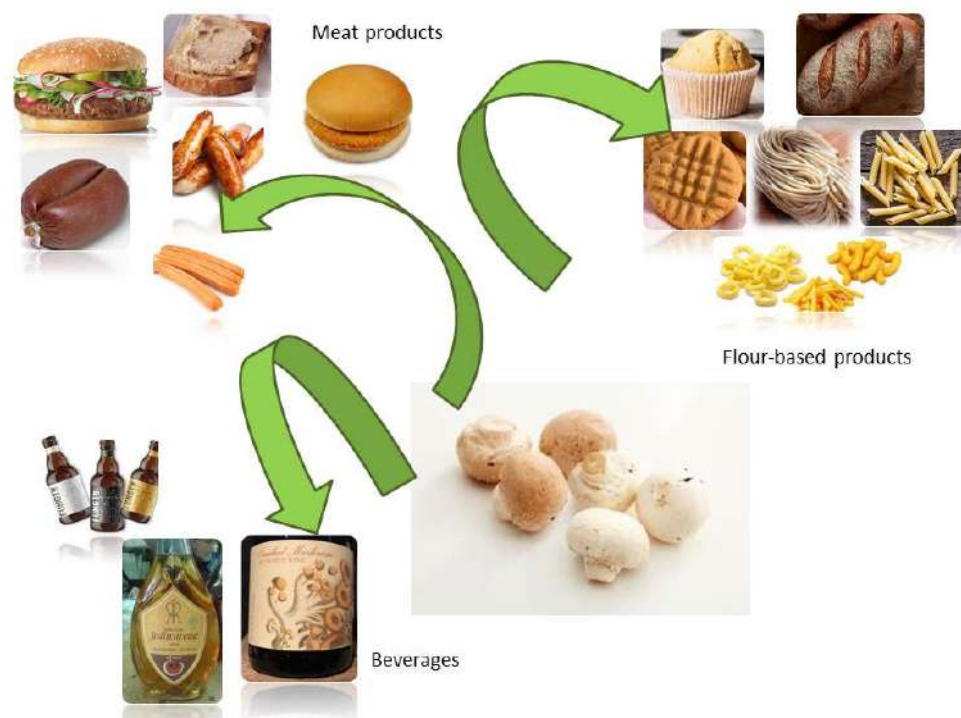


Figure 3. Mushroom-associated food products.

Meat products are the second large group of foods in which mushrooms have been used as a source of flavour or preservative, especially due to their antioxidant properties [45]. Gencelep [72] explored the effect of *Agaricus bisporus* powder on colour properties of a Turkish traditional meat product, sucuk. All instrumental colour parameters (lightness, redness, and yellowness) were affected, meaning that the addition of a white button mushroom caused the darker colour of the product's surface and cross-section. Pérez Montes et al. [73] reviewed research on meat products recently supplemented with different mushrooms. In the case of burgers, patties, sausages, frankfurters, nuggets, and salami, the addition of mushrooms decreased the lightness. However, during a several-month storage period, mushroom components preserved or retarded colour change due to antioxidant activity. Wan-Mohtar et al. [74] prepared a chicken patty with *Pleurotus sapidus* stems, which affected the lightness of this product. The authors speculated that the colour of the patty originated from the colour of the mushroom powder supported by polysaccharide caramelisation during cooking. Likewise, Cerón-Guevara et al. [75] developed liver pâté supplemented with *A. bisporus* and *Pleurotus ostreatus*. In this case, the dark colour originating with the button mushroom addition affected lower colour scores. These pâtés differed greatly from the control because colour is among the most important sensory characteristic appreciated by consumers of this type of product. Stephan et al. [76] added *P. sapidus* mycelium to a vegan boiled sausage analog system. The mycelium was cultivated on apple pomace and isomaltose molasses. The product cultivated on molasses appeared very similar to meat products with a slightly more pronounced yellowish to an orange tone.

However, the researchers proposed the adjustment of raw material colour by using clean label dye.

Several research groups have prepared extruded snacks using mushroom powder. The conclusion is that the higher the percentage of this additive, the darker the final product. Still, the colour contributed to the Maillard reaction and to caramelisation during cooking [77]. Pecic et al. [78] reported the potential use of *G. lucidum* in spirit fabrication when the addition of 40 g/L of this mushroom (fruit body) into grain brandy affected the colour of the brandy after seven days, similar to that of an 11 year old plum brandy in sessile oak and an 18 year old in mulberry cask. The colour was strongly correlated with total phenolic compounds. Higher concentrations of mushroom added to brandy increased the yellow and red colour. In another study, Reishi mushrooms significantly influenced the colour of Shiraz wine when added prior to or after fermentation [79]. This study's panellists thought the wines containing 4 g/L of mushroom (added after fermentation) were darker in colour than that of the control wine. Beer made with mushroom extracts was also investigated, and while visual appearance and colour influenced other sensory aspects, this attribute has not been investigated [80]. Encapsulated *G. lucidum* extract added to beer, on the other hand, generated a more prominent and darker hue, making it more appealing to customers [81].

Except for beverages, medicinal mushrooms were never intended as a colourant. On the contrary, this healthy ingredient was included to improve storage quality to retard oxidative changes, or for its nutritional and physiological benefits. Modern consumers are ever more aware of artificial dye issues and demand the use of colourants of natural origin [82]. These new pigments derived from plants, micro-organisms or animals are expected to be safe, healthy, and functional. Surprisingly, none of the mushroom pigments has reached the market yet because they have not been examined as a food colourant. The only exception is melanin from *Auricularia auricula*. Wu et al. [83] characterised and examined its physicochemical and stability in the presence of salt, sugar, light, and high temperature. Melanin from this commercially cultivated medicinal mushroom may be a healthy colour for the food industry due to its antioxidative, antibiofilm, quorum sensing, and radical scavenging effect. However, it is still in the research phase. The three pigments laetiporic acid A, B, and C, isolated from *Laetiporus sulphureus* by Davoli et al. [51] are oxygen and light-stable during several months. Considering that this mushroom species is also edible, there is a potential use of its pigments as food colourants with GRAS status. Besides melanin and laetiporic acids, mushrooms produce black, brown, red, orange, yellow, violet, green, and blue pigments [32] and Lagashetti et al. [84]. Such a wide array of compounds, especially blue which are rare in nature [64], have not been widely studied.

Altogether, novel food products with a darker colour have received lower sensory scores with a lower acceptancy among panelists, which were all compared with a lighter colour control, such as functional bread compared with commercial white bread. The sensory scores for bread supplemented with mushroom flour were naturally low since mushroom powder, rich in melanin, proteins, amino acids, and polysaccharides, is originally darker and undergoes the Maillard reaction and caramelisation during thermal processing. Moreover, studies were performed mainly with commercial species, such as white button or oyster mushroom, which contain melanin. It would be wise to compare functional bread with bread prepared with different coloured wheat, composite flours or wholemeal flours. Adulteration with flour of a lesser value, or wheat flour used in wholemeal bread is another problem encountered in bread production. In Serbia, the bread is expected to be of dark colour, which is considered to be a healthier option, and is usually adulterated with white bread flour while the dark colour is obtained by adding a discrete amount of cocoa powder. This can be eliminated by using mushroom-derived pigments or powders which will also provide taste and functional characteristics to the final product. Furthermore, mushroom species rich in orange, yellow, and red pigments might be functional colourants used to develop novel bread types (Figure 4).



Figure 4. Yellow-dye mushrooms used in bread making.

4. Isolation of Mushroom Dyes

Mushroom dyes have been extracted from the fruiting body using a variety of techniques, with the cap being the most common. When it comes to mushrooms, cap colour is an essential commercial characteristic, and it requires pre-treatment in order to obtain crude dyes. Considering minor differences, the four basic colour kinds of oyster mushroom caps are yellow, white, black, and pink. The economic benefits of oyster mushroom cultivation will be increased by the production in a range of colours to appeal to varied consumer tastes. However, the mechanism responsible for oyster mushroom colour development remains unknown, making molecular breeding for cap colour-type cultivar improvement problematic. Pigments are the fundamental components of colour production [85], thus determining the pigment content of mushrooms is necessary.

Melanin is collected in the cell walls of mushrooms, which is likely cross-linked to polysaccharides [86], whereas polysaccharides are required for fungi to survive and reproduce. Polysaccharides and pigments can work together to offer critical channels and linkages for the transmission of chemical information [87]. Acids-bases or enzymes can be used to eliminate natural melanin [88], yet melanin identification is difficult. Integrative physicochemical approaches, such as solubility and chemical reaction tests [89], Fourier transform infrared spectroscopy, ultraviolet–visible spectroscopy, and nuclear magnetic resonance spectroscopy are widely used.

A range of solvents, including water, alkali solution, aqueous acid, and organic reagents (such as ethanol and acetone), are currently used to extract pigments from mushrooms (Table 3). The colours of the oyster mushroom fruiting bodies could only be extracted with a basic solution and precipitated with acid [52]. *P. cornucopiae*, *P. citrinopileatus*, and *P. djamor* have black caps, whilst *P. djamor* has yellow and pink caps. Meanwhile, the brown *Cordyceps militaris* [18] and red *Lactarius lilacinus* [19] fruiting bodies from two different mushrooms were obtained through alcoholic extraction. The fruiting body of *Lentinula edodes* was dispersed in a mixture of NaOH and weak acid [25], resulting in a brown colouration of the solution. The mycelium biomass from *Pleurotus cystidiosus* and *Armillaria cepistipes* used alkaline and high heat extraction, respectively, to generate a black dye from the mycelium biomass. These extractions demonstrate that mushroom dyes are cost-effective, consistent, and easy to produce in large quantities using straightforward procedures.

Table 3. Recent dye extraction technique of mushroom dyes.

| Species | Biomass Source | Technique Details | Dye Colour | Reference |
|----------------------------------|----------------|--|------------|-----------|
| <i>Cordyceps militaris</i> | Fruiting body | 0.50 g of sample mixed with 10 mL 80% ethanol | Brown | [18] |
| <i>Lactarius lilacinus</i> | Fruiting body | 500 g of frozen mushroom mash with MeOH (2 × 500 mL) for 30 min | Red | [19] |
| <i>Lentinula edodes</i> | Fruiting body | In 15 mL of 1 M NaOH, 1 g of sample is dispersed. The mixture is cooked for 3 h in water and then strained. The filtrate is acidified with 1 M HCl to a pH of 1.7 and incubated at 25 °C overnight | Brown | [25] |
| <i>Pleurotus cystidiosus</i> | Mycelium | Autoclaved at 120 °C for 20 min after a 1 M NaOH wash. With concentrated HCl, the alkaline pigment centrifuge-supernatant extract is acidified to pH 2 | Black | [21] |
| <i>Armillaria cepistipes</i> | Mycelium | A 0.45 m nitrocellulose membrane is used to filter the pH 6 sample. The filtrate is sterilised by autoclaving at 121 °C and 1 bar for 20 min. Lyophilisation is used to obtain “raw melanin” | Black | [22] |
| <i>Pleurotus cornucopiae</i> | Fruiting body | 1:30 of (mushroom mash/1.5 mol/L NaOH solution) | Black | [52] |
| <i>Pleurotus citrinopileatus</i> | Fruiting body | 1:30 of (mushroom mash/1.5 mol/L NaOH solution) | Yellow | [52] |
| <i>Pleurotus djamor</i> | Fruiting body | 1:30 of (mushroom mash/1.5 mol/L NaOH solution) | Pink | [52] |

5. Natural Colourants in the Dye-Sensitized Solar Cell (DSSC)

Clean energy generation is one of the most serious challenges facing the world, due to population growth and the associated increase in energy demand. DSSCs are new techniques producing clean, endless energy. Diverse issues related to dye-based solar cells have been classified and discussed. Further Grätzel [90] reported DSSC energy conversion based on functional ruthenium compounds, which were both expensive and hazardous when discovered. Furthermore, organic dyes are more difficult to synthesise and have lower efficacy levels when compared to ruthenium complexes, which is another disadvantage. Natural dyes and their synthetic derivatives have potential as efficient photosensitisers for dual-source solar cells, as evidenced by the need to thoroughly study additional dyes. Since 1970, the study of wide band gap semiconductor photoelectrodes with dyes that absorb visible light and have a large band gap has sparked a lot of interest. The photo-excited dye injects excited electrons into the semiconductor conduction band can conduct electricity. Photosensitisers or dyes are critical components of the DSSC because they are excited when photons are absorbed and the photo-excited dye injects electrons into the semiconductor conduction band to conduct electricity (usually TiO₂).

According to the International Energy Agency (IEA), third-generation solar cell technology, which includes DSSCs, polymer solar cells, heterojunction cells, quantum dot solar cells, and hot carrier cells, is focusing on boosting efficiency while cutting production costs [28]. The most prevalent natural colours are flavonoid pigments including chlorophyll, anthocyanin, carotenoid, and betalains, which are derived from various portions of plants. Natural dyes generated from fruits and plants, which include a variety of pigments that are commonly available and easy to extract, have emerged as viable substitutes for synthetic dyes in educational applications. Natural vegetable sensitiser, in contrast to synthetic sensitiser, are widely available, easy to extract, inexpensive, non-toxic, and environmentally benign. Since its discovery by Grätzel and colleagues in 1991, the DSSC has received a lot of attention since it can be sensitised with synthetic and natural dyes and performs well under low irradiance [27]. Their work includes the relationship between photosensitisers and DSSC in greater detail.

The link between photosensitisers and DSSC is depicted in further detail in Figure 5 [91]. Light is transformed to electrical energy in photovoltaic systems when photons excite the electrons of a photoelectric material from its valence band to its conduction band, and the electrons are then collected and transmitted to an external circuit band [92]. The working electrode (photoanode), the electrolyte, and the counter electrode (cathode) are the three

primary components in the DSSCs, which are all sandwiched together [93], as shown in Figure 5.

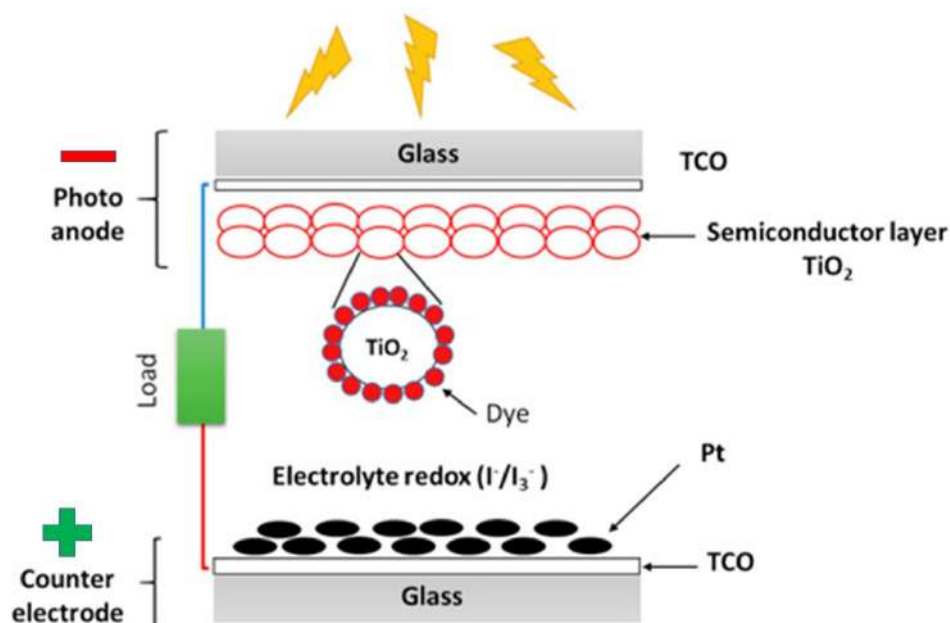


Figure 5. Diagram of a dye-sensitized solar cell (DSSC) which improvised from [91].

The working electrode (photoanode) is typically made up of a conductive transparent substrate, such as fluorine-doped SnO₂ glass (FTO-glass), covered by a thin mesoporous layer of semiconductor nanoparticles, such as TiO₂, ZnO, and SnO, sensitised by a dye that extends the semiconductor’s absorption spectra from the ultraviolet to the visible region [94]. Assume that the light is directed at a working electrode. In that case, two important processes occur: first, the photochemical process in which the dye absorbs light energy and transfers an electron from the High Occupied Molecular Orbital (HOMO) to the Low Unoccupied Molecular Orbital (LUMO), and second, the electron transfer from the dye’s LUMO to the TiO₂ conduction band, which leaves a positive charge on the oxidised dye. The electrolyte is a solution or gel that contains a redox pair capable of reducing the oxidised dye to its base state, such as iodide/triiodide. The positive electrode required to complete the circuit is the cathode. To complete the circuit, a conductive substrate on which a catalyst material, such as metals or carbon-based compounds, is coated [91,95]. The performance of DSSCs is influenced by a number of factors, including dye absorption qualities, TiO₂ mesoporous structure, TCO transparency and conductivity, electrolyte redox characteristics, and cathode catalytic activity [96]. Figure 6 shows the many types of sensitisers utilised in DSSCs.

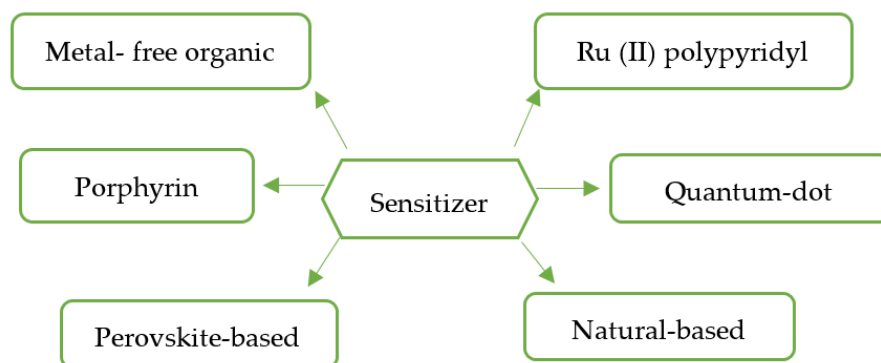


Figure 6. Different type of photosensitisers.

Natural colourants have regained popularity in a wide range of industries, including textiles and electronics, due to their high antiviral, antioxidant, antibacterial, and antifungal qualities. Perfumes, food and flavouring, and medications all employ them. DSSCs, which are gaining popularity due to their high economic worth, are an example of naturally occurring dyes with a wide range of possible applications in the field of energy production [97]. The DSSC energy conversion performance of natural colourants is mostly determined by the properties of these pigments, influenced by three factors: extraction parameters, photo electrode thickness, and annealing temperature. Natural dyes are used as sensitizers in the DSSC, where they play a vital role. This has prompted a number of studies to improve dye efficiency and develop new families of dye sensitizers. Natural dyes absorb photons from the sun, causing electron excitation [98]. Sensitization experiments have been carried out to improve light absorption and DSSC performance by combining natural colourants and dyes to absorb different solar magnetic radiation spectrum regions. Consequently, the system has low manufacturing costs, flexibility, light weight, ease of processing, colour variety, and translucent properties [99].

Natural colourants, such as those obtained from discarded vegetables, are available as various DSSC photosensitizers. Without the use of expensive and time-consuming purification techniques, this extract was made from the fresh leaves and bark of eggplant twigs. Calogero et al. [100] discovered that peeling the leaves and bark of the eggplant twigs to remove the green portions is easier than peeling the eggplant skin to remove the small, thin, green components, resulting in a 1% DSSC conversion efficiency. Ayalew and Ayele [101] reported natural dye extraction from flowers and leaves as a light-harvesting substance. Because it captures solar energy and converts it to electrical energy via a semiconducting photoanode, the dye is crucial in the assembly of the DSSC. Natural dyes from *Acanthus sennii* Chiov and the flow and leaf of *Euphorbia cotinifolia* were isolated using a simple extraction procedure. The optical properties of the extracted dyes were characterized using UV–VIS absorption spectroscopy, and the dyes with the best optical properties were employed as a light-harvesting material. As shown by Kim et al. [102], the absorption peak of the extracted dyes is substantially identical to that of chlorophyll, which absorbs in the red (600–750 nm) and blue (400–500 nm) sections of the absorption spectrum. In contrast, Iqbal et al. [103] discovered an absorption peak in the green region from 500 to 600 nm that was identical to that of anthocyanin reported by Chang and Lo [104].

The use of electron-rich heterocyclic rings as spacers in sensitizers has been described, lowering the bandgap and improving absorption characteristics in the near-infrared range [105]. Researchers are interested in azo colourants, coumarin derivatives, hydrazones, and other heterocycles because of their easily tunable qualities. Azo colourants are used in optoelectronic devices, such as optical switches, non-linear optic devices, and second harmonic generators, in addition to textiles [106]. Because of their optical and electrical qualities, as well as their superior thermal and chemical stability, azo dyes are more viable options for DSSC applications. A few theoretical and experimental research on azo dyes in DSSC applications with low PCE have been published. Toor et al. [107] looked at 49 commercial mordant dyes with efficiencies ranging from 0.025% to 0.032%. Meanwhile, Golshan et al. [108] collected natural pigments from the Persian Gulf and tested them as sensitizers in natural DSSCs created using the doctor blade technology with energy conversion.

Natural colourants have been extracted from the skin of *Canarium odontophyllum* (COP), a plant that contains active photo-energy conversion molecules, such as pelargonidin, cyanidin, and maritimein [109]. The exotic fruit COP can be found on the Indonesian island of Borneo. When ethanol is extracted from the black skin of the fruit, it produces a purple tint. Column chromatography was used to separate the components of this purple dye, and their UV–Vis absorption properties were used to identify them. COP's purple dye contains optically active components, such as cyanidin, pelargonidin, and maritimein, which is an aurone, while anthocyanidins cyanidin and pelargonidin are also anthocyanidins. These dyes are used as sensitizers in the production of DSSC. Using UV–

Vis and cyclic voltammetry measurements, the DSSC's HOMO and LUMO energy levels were estimated and information on the performance of COP constituents as sensitizers, as well as their projected energy HOMO–LUMO levels were provided with a comparison to density-functional theory and time-dependent density functional theory (DFT–TDDFT) results. Finally, utilising a mix of experimental and calculated data, the anchoring groups onto the TiO₂ surface were determined.

6. Medicinal Mushroom Dye as an Active Photo-Energy Conversion Molecules in DSSCs

To achieve SDG 7, “Affordable and Clean Energy,” it is necessary to use sustainable resources in a way that balances economic and social needs [3]. When the capacities of a natural source or sink are exceeded to a level that threatens societal well-being and ecological consequences, resource use can produce sustainability difficulties. Continuous economic success during industrialisation was predicated on increased fossil energy intake, resulting in a major drop in the renewable energy usage and fast increasing fossil-fuel-related CO₂ emissions [110]. In the early stages of industrialisation, non-renewable energy and non-sustainable resources impeded the advancement of the SDGs. As a result, using medicinal mushroom fungal pigments in energy generation is essential for reaching SDG 7 in terms of sustainable resources. Goal 7 aims for a cumulative investment in wind, biomass, waste, biofuel, and solar energy of more than 270 billion dollars [111].

The DSSC, a low-cost method for converting solar light into electrical energy, is recently gaining popularity. Sensitizer dyes have received much attention due to their importance in catching sunlight and converting it to electricity [112]. To date, fungal pigments obtained from mushrooms have yielded a substantial variety of natural sensitizer colours. Orna-Navar et al. [26] discovered that fungus pigments have a photoconversion efficiency of 0.26–2.3%, second only to algae and microalgae. While not the first species used in a DSSC, fungi do produce secondary metabolites, such as melanin and carotenoids. Mushroom pigments are also temperature stable, work in a wide pH range, are environmentally friendly, and can be produced in large quantities utilising bioreactor fermentation [26,113–115]. Table 4 shows a current list of therapeutic mushroom dyes in DSSC and other possible species for future use.

Table 4. Medicinal mushroom dyes in dye-sensitised solar cells (DSSC) and other potential species.

| Species | Melanin Extract | DSSC Status | References |
|---|--------------------------|--|------------|
| <i>Agaricus bisporus</i> | (White-brown) Riboflavin | TiO ₂ sintered at 500 °C for 30 min | [116] |
| <i>Cortinarius</i> sp. | Brown | TiO ₂ sintered at 500 °C for 30 min | [117] |
| <i>Pleurotus cornucopiae</i> CCMSSC 00406 | Black | Untested | [52] |
| <i>Pleurotus citrinopileatus</i> CCMSSC 04208 | Yellow | Untested | [52] |
| <i>Pleurotus djamor</i> CCMSSC 00450 | Pink | Untested | [52] |
| <i>Lentinula edodes</i> | Brown | Untested | [25] |

To date, only two species of medicinal mushroom dyes have been tested in DSSCs, the Button mushroom *Agaricus bisporus* [116] and pale-brown *Cortinarius* sp. [117], both of which are successful in the field. These two species contain significant amounts of electron-absorbing dyes extracted from the fruiting body and act as sensitizers in the DSSC system. Poland's *Cortinarius* methanolic extracts, such as *C. croceus*, *C. semisanguines* and the most successful *C. sanguineus*, have established themselves as the most successful species, producing a voltage of 0.54 V, a current density of 1.79 mA cm^{−2} and an efficiency of 0.64%. In addition, four potential mushroom species with intense dye characteristics have been identified, capable of producing black, yellow, pink, and brown pigments, which are suitable for use with DSSC technology. Natural dyes derived from mushroom sources have an advantage over other types of emerging photovoltaic technology because they are abundant and cost-effective, and their extraction and preparation methods are simple

to scale; they also pose no health risk and are not considered persistent compounds in the environment.

Oyster mushrooms are produced worldwide, particularly in developing countries, due to their ease of growing and high biological efficiency [118]. *Pleurotus eryngii*, *P. citrinopileatus*, *P. djamor*, *P. ostreatus*, *P. cornucopiae*, *P. citrinopileatus*, *P. pulmonarius*, and *P. cystidiosus* are just a few of the species that have been cultivated and generated significant economic benefits [119,120]. Polysaccharides, terpenoids, and flavonoids, which have anti-inflammatory, anticancer, antiviral, antioxidant, and immunity-boosting properties, are abundant in oyster mushrooms [121–123]. The colour of the cap, white, black, yellow, and pink, on oyster mushrooms is a major selling element. The economic benefits of oyster mushroom production will be boosted by the availability of varied coloured oyster mushrooms that appeal to client tastes. However, the process behind oyster mushroom colour generation is unknown, limiting molecular breeding application to develop cap colour-type cultivars. Because pigments are the building blocks of colour, the pigment composition of oyster mushrooms must be determined [85].

The UV–Vis light absorption spectra of the three pigments revealed high absorbance in the UV region. As confirmation that the mushroom pigments are acceptable as active photo-energy conversion molecules in DSSC, the optical density dropped with increasing wavelength, which is typical of the melanin's absorption profile [52]. However, the maximum absorbance of the pigments recovered from three oyster mushrooms was 235 nm, which differed slightly from the melanin measured from other species, such as *Osmanthus fragrans* seeds, which had a maximum absorbance of 195 nm [124], 215 nm for *Auricula* [125], and 280 nm for *P. cysteus* [21,124]. The changes in maximum absorbance could be attributed to a difference in melanin supply, which slightly changes the natural structure of melanin.

7. Safety, Production, Limitations, and Future Strategy of Medicinal Mushroom Dyes in DSSC

In first- and second-generation solar cells, crystalline and poly-crystalline silicon solar cells are utilised. For more than a half-century, silicon solar cells have been the dominant technology, accounting for nearly 90% of photovoltaic energy [126]. To solve the shortcomings of first- and second-generation solar cells, researchers began constructing a new generation of solar cells known as third-generation or emerging photovoltaics. Emerging photovoltaics are divided into the following categories:

- Dye-sensitised solar cells (DSSC);
- Solar cells with bulk heterojunction (BHJ);
- Solar cells with quantum dots (QDSSC);
- Solar cells made of perovskite.

DSSC have emerged as one of the most efficient third-generation photovoltaic cells due to many advantages over silicon-based solar cells in terms of manufacturing simplicity, cost-effectiveness, adaptability, and transparency [127,128]. Khaled Hamdani and colleagues reached a 14% efficiency record using nanoparticle 90 cells [128]. Various metal-free organic dyes have been reported with 6–13% efficiency [129]. Due to their push-pull nature, higher molar extinction coefficient, red-shifted absorption spectrum, intramolecular charge transfer (ICT), ease of electron transfer from excited state to TiO₂ conduction band, and feasible charge transfer from donor (D) to acceptor (A), metal-free organic dyes have shifted the focus away from metal-based organic dyes. Organic sensitizers conjugated with diverse donors, such as coumarin, triphenylamine, indoles, and carbazoles via stilbenes, azobenzenes, biphenyls, and imines, displayed significant solar efficiency.

The sensory quality of a mushroom is mainly determined by its colour. In DSSCs, colourants can help the active photo-energy conversion molecules. Synthetic dyes were once preferred over natural dyes because of their superior chemical and physical stability, colour intensity, and, most importantly, reduced cost [130]. Due to safety concerns and the health benefits of natural nutrients, non-synthetic additives are the current industry

trend [131,132]. Natural colourants are also useful in DSSC, as evidenced by the UV–Vis light absorption spectra of the three pigments [52].

The most widely studied and used colourants are carotenoids [133]. When compared to other natural colours, they are relatively thermostable compounds. The main instability constraints are oxygen and acidic degradation [134]. Encapsulation techniques, on the other hand, can allow them to be used in hydrophilic foods while limiting losses due to oxygen and acidic media exposure [135]. The pigments that give yellow, orange, and red their colours are called carotenoids, obtained from corn, carrot, papaya, tomato, and watermelon as well as algae and insects [134,136]. Other natural colourants are also used, but because of their low chemical and physical stability their application is limited. The most fundamental hurdle to employing natural colourants is a lack of process homogeneity. Because they are natural additives, the raw materials may have a lot of variation, making colour standardisation problematic [82].

Furthermore, depending on the product, colour loss during processing or storage can vary. As a result, natural colourant-containing products have a shorter shelf life to avoid standardisation concerns during storage. To maintain the physical and chemical durability of natural colourants and the ultimate appeal of coloured meals, more effective approaches are necessary. Despite challenges with physical and chemical stability, these colourants are employed in a wide range of goods because more solutions are being developed to improve their stability, particularly in the widely available yellow, red, and green naturally coloured dyes.

8. Conclusions

It is possible to employ medicinal mushrooms as a dye sensitizer in solar cell layers since they are safer, more cost-effective, bulkier, and of higher consistency in quality than conventional natural dyes. Meanwhile, these dyes provide health-promoting factors when introduced as food colourants, which also boost physicochemical and sensorial characteristics of food products when used in low quantities. When a dye is used as a single source, it helps to combine the efforts of achieving zero hunger and affordable energy in the pursuit of sustainable development. Because they are completely biodegradable in nature, the dyes identified provide a more environmentally friendly approach to waste disposal.

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