

The two pilot tests carried out demonstrated the application of mushroom cultivation on a lavender farm following the circular economy principle. These molecules of steroid origin are similar to those found in human bile salts and mammals in general. This variable production probably depends on the fact that the domesticated strain, which has been cultivated for generations in a protected and controlled environment through cloning of the mycelium—a type of asexual reproduction that does not allow genetic mixing—depresses or silences in successive generations various secondary metabolic pathways that are no longer useful for maintaining physiological activity. The substrate enriched with lavender, a plant particularly rich in terpenes and other molecules it produces as antibacterial and antifungal agents, stimulates the fungus to activate its secondary metabolism, as evidenced by the presence of compounds that are not part of the fungus's usual primary metabolomic pattern. It should be noted that epoxides of fatty acids were only present in fungi cultivated on enriched substrates, and were absent or present in trace amounts in fungi grown on only straw, confirming that lavender acts as a stressor, and suggesting the role of fatty acids epoxides as biomarkers of stress in Basidiomycota. The evolution of these peptides can be seen in the large cyclized peptide molecules produced by some fungi, such as amatoxins of the *Amanita* genus, a clear evolutionary trend that leads from simple molecules to complex cyclic peptides with defensive functions. In particular for linoleic and linolenic acid, in the samples cultivated on substrates enriched with lavender, we noticed a partial decrease in the content of polyunsaturated fatty acids in favor of the presence of fatty acid epoxides. Its action is highly relevant regarding free radicals and peroxide ions, and largely justifies the powerful antioxidant action of *P. ostreatus* extracts analyzed on cell cultures treated with oxidizing agents (personal data). These molecules are produced by the fungus in response to environmental stresses through the activation of the monooxygenase domain of cytochrome P450, which is common to all fungi and has been highly preserved during evolution [32]. The vegetal waste partially inhibited the mycelial development, increasing the incubation time when grown on the substrate with lavender, and, later, the development of basidiomata (lowering the production yield compared with the control). Although weight data from the different growth phases of the fungus showed that the fungus grown on straw generally produced greater biomass, HPLC–MS and tandem mass analysis showed that the levels of *n*-acetylglucosamine and its precursors remained constant in each sample, independently of the type of substrate used for growth. Of note is the presence of fatty acid epoxides, particularly those of myristoleic, linoleic, and linolenic acids produced by the fungus grown on lavender-enriched substrates. Based on the average yields reported in Table 1, there were significant differences in the weight of basidiomata produced on the substrates tested ( $p < 0.001$ ), but there were no differences in the biomass of the produced sporomata between the two strains tested. These differences in biomass production suggest the use of lavender residues to grow mushrooms may be unsuitable because, as shown in Figure 2, the *Y* value is inversely proportional to the lavender waste content in the substrate. Because the substrate containing 30% lavender residue had slightly lower yields (ranging from 1 to –5.4%) than those observed in samples without lavender residue, this substrate may provide an appropriate balance between biomass production and improved metabolomic profile. Of the many molecules extracted and characterized, significant interest exists in the amino acids, glucosides, and small dipeptides, that are particularly known in the fungi kingdom. The

samples of *P. ostreatus* grown on substrate enriched with lavender also showed the presence of numerous molecules derived from fatty acids, in particular a series of fatty acid epoxides. In contrast to plants, in which the role of peroxygenases in the production of fatty acid epoxides is known, in fungi it appears that only the monooxygenase domain of cytochrome P450 is involved in their synthesis [34]. Sevtoxic and tumor growth-promoting activities are known [36], so further investigation to verify the activity of fatty acid epoxides produced by *P. ostreatus* would be useful. These calculations revealed that the yields on a substrate composed of 100% of lavender residue were lower than those on the control substrate composed exclusively of straw. There are myriad roles for these peptides and their derivatives; it has been shown that these peptides have antifungal, antimicrobial, immunostimulant, and growth-promoting properties [30]. This process, as already proposed by other authors, enables farmers to reuse the agricultural waste produced during the main production cycle to obtain a new product [11,20,26]. The particular substrate leads to distinct metabolomic profiles, a phenomenon that improves the commercial value compared with mushrooms grown on traditional substrates. However, as discussed below, the metabolomic characteristics and added nutraceutical value provided by the lavender to the cultivated basidiomata could compensate for a small drop in production. Specifically, we found that the wild strain is a slightly better producer of metabolites in response to the presence of an environmental substrate than the domesticated strain, if dried extracts and just secondary metabolites are considered. These metabolic pathways are not silenced in the wild strain, because in nature it is in direct contact with environmental stressors, and direct competitors and pathogens. Moreover, it is evident that a balanced intake of polyunsaturated fatty acids is fundamental for human health. Fungi are an excellent source of polyunsaturated fatty acids, whose role has long been recognized in the prevention of inflammatory heart and other diseases [31]. This trend was also confirmed by the presence of cholic acid in the samples of fungi cultivated on substrates enriched with lavender. Nevertheless, the role of fatty acid epoxides in the regulation and suppression of inflammatory processes has been recognized [35]. These data are consistent with the feasibility study carried out as part of the FINNOVER project ([www.interreg-finnover.com](http://www.interreg-finnover.com) accessed on 3 May 2021). Therefore, the condition by which peptides and dipeptides are produced in *P. ostreatus* appears to be archaic or, at least, less evolved [30]. These molecules are probably involved in the fatty acid mobilization inside fungal cells, consistent with the presence of lipid drops in the fungal cells. This difference compensates for the lower yield of mushrooms.