Abstract

Mushrooms are rich in various nutritious and biologically active compounds such as polysaccharides including β-glucans, peptides, proteins, terpenoids, fatty acid esters, organic acids, sterols, alkaloids, phenolic compounds and are responsible for antioxidative, anticancer, antiviral, anti-inflammatory, antihyperlipidemic, immunomodulating and hypocholesterolemic properties. These properties can modify the physiological and metabolic functions of the human body and also have pro-health effects. However, the cultivation of mushrooms is tedious, requires a specific substrate approach, labour-intensive and takes 2 to 3 months. Moreover, its growth is season dependent and if the temperature is not controlled in the interior of the mushroom unit, it could only be grown during specific months of the year and hence makes it unsuitable for the commercialization of mushroom fortified products. In this context, the production of mushroom mycelia through submerged culture technique can be used as an alternative solution to produce higher mycelial biomass of mushrooms at industrial scale in a shorter period (8-10 days) in the fermenter enriched with nutraceutical properties. Also, growing mushroom mycelium under submerged culture conditions ensures its availability throughout the year, which requires limited space and fewer chances of contamination. At the same time, there exist certain limitations in this process e.g. the need for rather sophisticated equipment, expensive infrastructure and highly skilled personnel. In this study, mass production of fungal mycelial biomass of *Pleurotus eryngii* and *Calocybe indica* with enhanced nutritional values through submerged cultivation technique was achieved using one factor at a time (OFAT) approach. Both the physical (Temperature, pH, and RPM) and chemical (carbon sources, nitrogen sources, and C/N ratio) parameters were studied in shake flasks levels and further the optimized parameters were used for mass production of mushroom mycelia in fermenters (15 L, 100 L, 350 L). The harvested mushroom mycelia were then exposed to UV-

B irradiation for enhancement of vitamin D2. The highest biomass yield of *P. eryngii* in 15 L fermenter in batch mode under optimized conditions of agitation 200 rpm, aeration rate 0.1 vvm, temperature 28 °C, pH 6.5 and inoculum size 4% was 13.44±0.82 g/l. The batch phase was extended to fed-batch phase to increase biomass yield and the final biomass was increased up to 22.68 ± 0.8 g/l. *P. eryngii* mycelial biomass was further produced in 100 L fermenter in which biomass yield obtained was 12.54 ± 0.81 g/l. The highest biomass yield of *C. indica* in 15 L fermenter in batch mode under optimized condition of agitation 150 rpm, aeration rate 0.1 vvm, temperature 28 °C, pH 6.5 and inoculum size 4% was 9.54 ± 0.35g/l. Further, *C. indica* mycelia was also produced in large scale fermenter 350 L and the final biomass yield was 9.34 ± 0.81 g/l. The ideal UV-B irradiation conditions were determined for vitamin D2 synthesis in harvested mycelia using RSM. The effects of three independent variables namely, temperature (20-40 ^{o}C), ultraviolet-B intensity (0.6-1.2 W/m^2), and time of exposure (10-30 min) on vitamin D2 were investigated. Under optimal conditions of ambient temperature, 24.29 ^{o}C, UV-B intensity, 1.099 W/m^2, and exposure time, 23.88 min, the vitamin D2 got increased to 329.43 µg/g dry wt. Similarly, for *C.indica* the vitamin D2 was improved to 247±3.28 µg/g dry wt. The freeze-dried *P. eryngii* mycelia contained protein 24.52±0.53%, crude fat 6.78±0.81%, crude fiber 18.54±0.57%, total ash 7.82±0.61%, and carbohydrate 55.36±1.24% and rich in various amino acids like glutamic acid (3.05%), arginine (2.28%), threonine (2.03%), glycine (2.01%), and alanine (1.74%). While, freeze-dried *C.indica* mycelia was found to have protein 28.21±0.31%, crude fat 5.78±0.55%, crude fiber 14.24±0.37%, total ash 6.72±0.22%, and carbohydrate 57.06±1.13%. *C.indica* mycelia showed higher content of glutamic acid (4.01%), alanine (2.77%), leucine (1.89%), lysine (1.74%), isoleucine (1.68%), aspartic acid (1.68%), glycine (1.49%), Threonine (1.33%) and valine (1.33%). The effect UVB irradiation on nutritional quality of produced mycelia was also studied

and compared with their non UV-treated (control) mycelia. Ergosterol content in both the mycelia (*P. eryngii* & *C.indica*) got decreased significantly from 2.48 to 1.63 mg/g (*P. eryngii*) and from 2.11 to 1.46 mg/g (*C. indica*).

UV irradiation caused a significant ($p < 0.05$) improvement in mycelial beta glucans (%), i.e., from 26.18 to 32.21% (w/w) in *P. eryngii* and from 24.11 to 31.46 % (w/w) in *C. indica.* The antioxidant activity including FRAP, DPPH scavenging ability, TPC and TFC in UVB treated samples was also improved significantly ($p<0.05$) of both the mycelial extracts when compared with their control (non UVB treated) samples. Nutraceutically enriched mycelia were utilized for the preparation of two different types of food products namely instant noodles and meat analogues. The physico chemical properties, sensory evaluation and self-life studies were conducted for both the products. The noodle samples with 6% *P. eryngii* mycelia powder and with 15% *C. indica* mycelia powder were found acceptable. It was observed that mushroom mycelia fortified food products are well accepted by the rural consumers (both adults and children).