

Development of functional food product using mushroom (*Calocybe indica*) mycelia fermented agro-waste

Abstract Mushrooms are edible fungi rich in nutraceuticals possessing various therapeutic benefits like anti-diabetic, anti-proliferative, hepato-protective, anti-hypertensive, anti-angiogenic, antidepressive, etc. However, cultivation of mushrooms requires a specific substrate, extensive time and labour cost, and hence makes it unsuitable for the commercialization of mushroom fortified products. In this context, the cultivation of mushroom mycelia in agro-industrial wastes under a submerged fermentation process can be a promising alternative. Various agro-industrial wastes (ripe banana peel, green banana peel, orange peel, de-oiled groundnut cake, and de-oiled mustard cake) were screened as potential sources for the development of cost-effective functional food formulations. A novel submerged fermentation technique using *Calocybe indica* for enhancing the nutraceutical attributes of ripe banana peel powder (BPP) and de-oiled groundnut cake powder (DGCP) in combination was tested. An optimized aqueous media (through RSM) containing 6.6 g/l BPP and 7.5 g/l DGCP produced 5.3 g/l dry mycelial biomass of *C. indica* in 10 days. The fermented agro-waste (13.73 g/l, dry wt.) was found to improve its protein (9.37 to 17.06%), fibre (7.47 to 22.12%), total glucan content (11.16 to 27.62%, of which 24.67% were β -glucan content), minerals, total phenols, flavonoids (40.64 to 98.37 mg QE/g with its radical scavenging activity (IC₅₀, 94.86 to 28.43 mg/ml). A reduction in tannin, phytic acid (26.25 to 6.84 mg/100g) and oxalate was also observed after the fermentation process. Nonetheless, the fermented agro-waste also revealed an increase in Ergosta-compounds, (3.57%) which include Ergosta-5,7,9(11),22-tetraen-3-ol,(3.beta.,22E)- (1.87%), Ergosta-5,7,22-trien-3-ol,(3.beta.,22e)- (1.24%) and Ergosta-7,22-dien-3-ol,(3.beta.,5.alpha.,22E)- (0.46%). Functional attributes were found to get significantly affected by a reduction in water holding capacity (34.29 xiv to 30.97 ml/g), least gelation concentration (18 to 12.5%), bulk density (6.82 to 2.28 g/ml) and swelling volume (9.5 to 6.1 ml/g) with an increase in β -glucan (8 to 24.57%), dispersibility (78.27 to 92.92%), oil holding capacity (0.59 to 1.73 ml/g). GC-MS analysis confirmed the enriched levels of bioactive compounds namely, (S)-(-)-Perillyl alcohol, α -Longipinene, isozonarol, widdrol, hydroxy valerenic acid, reynosin, β -Eudesmol, lupenone, etc. in fermented agro-waste mixture. The mushroom mycelia grown in optimized agro-waste induced media was found to get enriched with protein (24.22 – 31.81%), fat (3.48 – 5.91%), fibre (5.46 – 11.35%), ash (4.80 – 6.02%) and β -glucan (22.05 – 34.62), various minerals, amino acids and vitamin D (194.81 μ g/g DW at 25 min to 264.17 at 20 min). The MTT assay of the mushroom revealed an enhanced anti-proliferative activity from 14.51 – 78% of HeLa cell lines when mycelium was cultivated in agro-waste. Nutraceutically enriched PoF-mixed-Ag was utilized for the preparation of two different types of halwa mixes namely suji substituted halwa mix and composite halwa mix. The composition of both the halwa mixes was optimized using hybrid PCA-RSM. The composite halwa was well accepted by the consumers, enriched with protein (22.80% wb), fat (5.75% wb) fibre (5.01% wb), ash (2.11% wb) and β -glucan (12.52 % W/W). The composite halwa was found to be a good source of TPC (476.33 mg GAE/100g), and TFC (521.45 mg QE/100g) and showed the lowest IC₅₀ value of 24.52 mg/ml For DPPH scavenging activity. The value-added composite halwa mix was well appreciated by rural consumers (both adults and children). It

was observed that mushroom mycelia fortified food products are well accepted by the consumers; however, the umami flavour of the mushroom is not well appreciated. An integrated strategy for the development of *C. indica* mycelia-fortified functional food products has been presented here xv for promoting human health, protecting environment utilizing agro industrial waste materials and overall economic development.