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Title: Technology for the Manufacture of Micronutrients (Fe, Se, Ca, and Vitamin D_2) Enriched Low Moisture Meat Analogue

Abstract

Given population growth and resource constraints, providing a healthy, protein-rich diet is a major challenge, especially for vegetarians. Meat, while a protein preference, is negatively associated with health and the environment. Meat analogues are the preferred alternative but may contain allergenic soy and wheat and cannot provide taste, texture, flavour and nutrition similar to meat due to their limiting amino acids. Thus, there is a need for a better and more sustainable protein source that provides nutritional value, texture, and taste, just as meat does. Mycelium is one such novel source of protein that has not been explored much. It has shown enormous potential in mimicking meat due to its texture similar to meat and can be a good choice in the vegetable meat industry. The experimental plan included optimization of submerged fermentation conditions (25-30 °C temperature; 5-7 pH; 100 rpm agitation speed) for growing micronutrient (Fe, Se, Ca, and vitamin D_2) enriched mycelium (*Pleurotus eryngii*) and its characterization. Pressing as a novel pre-treatment was optimized (force 3-5 kN; pressing time 20-50) and evaluated its effect on drying time and microstructure followed by optimization of vacuum (VD; temperature 40-60 °C; pressure 260-60 mmHg) and microwave (MWD; power 200-500 W) drying and its comparison for the selection of best extrusion feed. In extrusion, mycelium incorporation was tested from 0 to 40% w/w and pea protein isolate (100-60% w/w) for better texturization and optimization of extrusion process conditions using die head temperature, (T_{DHT} 130-150 °C), screw speed (SS 30-50 rpm), feeder screw speed (FS 6-10 rpm) and feed moisture content (FM 25-35% wb) to find the impact on system parameters; volumetric expansion ratio (VER), and water/oil absorption capacity (WAC/OAC). Further, characterization, sensory analysis, storage study at accelerated conditions and shelf life were evaluated. The results showed superior biomass yield (12.32 g dw/l) with 28 °C temperature and 6 pH with Fe (41.93 mg/100 g), Se (331.32 µg/100 g), Ca (255.14 mg/100 g), and vitamin D_2 (314.59 µg/g dw) in 5.73 % moisture. The optimum pressing condition was 5 kN force for 30 s, reducing the moisture from 88.45 to 52% wb and drying time by 50 % in both VD and MWD. The best VD condition was 60 °C and 60 mmHg, while the best MWD condition was 400 W. In comparison study, MWD showed better results with higher WAI (6.74 g/g), flowability (carr's index 19.64% and hausner ratio 1.244), and bioactives, thus being selected for extrusion feed. A new mathematical model for MWD was developed. The formulation, viz., 30% w/w mycelium and 70% w/w provided better texturization with better physicochemical, textural and rehydration properties with the good nutritional profiling. Based on numerical optimization for meat analogue, the optimum extrusion condition for final formulation (30% w/w mycelium, 60% w/w pea protein isolate, 10% w/w corn starch) was 30-32 % wb FM, 140-141 °C T_{DHT}, 39-40 rpm SS, and 10 rpm FS. The validation trial showed less than 5% variation between the predicted and experimental values of responses. The developed low-moisture meat analogue (LMMA) contain 54.01 g protein, 8.45 g fibre, 20.89 mg Fe, 70.4 mg Ca, 66.15 µg Se in 100 g dw, and 59.46 µg/g dw vitamin D_2. The shelf life of the LMMA in metalized polyester pouches was 20 weeks. The economic feasilbity of process was ascertained by sensitivity analysis.

Keywords: Mycelium; Low-moisture meat analogue; Extrusion; Drying; Process optimization; Model development; Shelf life prediction