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Sequential Pulsed Electric Field and Ultrasound Assisted Extraction of Bioactive Enriched Fractions from Button Mushroom Stalks

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Abstract : Edible mushrooms possess numerous functional components like homo- and hetero- β-glucans [β(1→3), β(1→4) and β(1→6) glucosidic linkages], chitins, ergosterols, bioactive polysaccharides and peptides imparting health beneficial properties to mushrooms. Some of the proven biological activities of mushroom extracts are antioxidant, antimicrobial, immunomodulatory, cholesterol lowering activity by inhibiting a key cholesterol metabolism enzyme i.e. 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCR), angiotensin I-converting enzyme (ACE) inhibition. Application of novel extraction technologies like pulsed electric field (PEF) and high power ultrasound offers clean, green, faster and efficient extraction alternatives with enhanced and good quality extracts. Sequential PEF followed by ultrasound assisted extraction (UAE) were applied to recover bioactive enriched fractions from industrial white button mushroom (Agaricus bisporus) stalk waste using environmentally friendly and GRAS solvents i.e. water and water/ethanol combinations. The PEF treatment was carried out at 60% output voltage, 2 Hz frequency for 500 pulses of 20 microseconds pulse width, using KCl salt solution of 0.6 mS/cm conductivity by the placing 35g of chopped fresh mushroom stalks and 25g of salt solution in the 4x4x4cm3 treatment chamber. Sequential UAE was carried out on the PEF pre-treated samples using ultrasonic-water-bath (USB) of three frequencies (25 KHz, 35 KHz and 45 KHz) for various treatment times (15-120 min) at 80°C. Individual treatment using either PEF or UAE were also investigation to compare the effect of each treatment along with the combined effect on the recovery and bioactivity of the crude extracts. The freeze dried mushroom stalk powder was characterised for proximate compositional parameters (dry weight basis) showing 64.11% total carbohydrate, 19.12% total protein, 7.21% total fat, 31.2% total dietary fiber, 7.9% chitin (as glucosamine equivalent) and 1.02% β-glucan content. The total phenolic contents (TPC) were determined by the Folin-Ciocalteu procedure and expressed as gallic-acid-equivalents (GAE). The antioxidant properties were ascertained using DPPH and FRAP assays and expressed as trolox-equivalents (TE). HMGCR activity and molecular mass of β-glucans will be measured using the commercial HMG-CoA Reductase Assay kit (Sigma-Aldrich) and size exclusion chromatography (HPLC-SEC), respectively. Effects of PEF, UAE and their combination on the antioxidant capacity, HMGCR inhibition and β-glucans content will be presented.

Keywords : β-glucan, mushroom stalks, pulsed electric field (PEF), ultrasound assisted extraction (UAE)
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