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Effect of Chitosan Oligosaccharide from Tenebrio Molitor on Prebiotics

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Abstract : Chitosan is used in various industries such as food and medical care because it is known to have various functions such as anti-obesity, anti-inflammatory and anti-cancer benefits. Most of the commercial chitosan is extracted from crustaceans. As the harvest rate of snow crabs and red snow crabs decreases and safety issues arise due to environmental pollution, research is underway to extract chitosan from insects. In this study, we used Response Surface Methodology (RSM) to predict the optimal conditions to produce chitosan oligosaccharides from mealworms (MCOS), which can be absorbed through the intestine as low-molecular-weight chitosan. The experimentally confirmed optimal conditions for MCOS production using chitosanase were found to be a substrate concentration of 2.5%, enzyme addition of 30 mg/g and a reaction time of 6 hours. The chemical structure and physicochemical properties of the produced MCOS were measured using MALDI-TOF mass spectra and FTIR spectra. The MALDI-TOF mass spectra revealed peaks corresponding to the dimer (375.045), trimer (525.214), tetramer (693.243), pentamer (826.296), and hexamer (987.360). In the FTIR spectra, commercial chitosan oligosaccharides exhibited a weak peak pattern at 3500-2500 cm-1, unlike chitosan or chitosan oligosaccharides. There was a difference in the peak at 3200~3500 cm-1, where different vibrations corresponding to OH and amine groups overlapped. Chitosan, chitosan oligosaccharide, and commercial chitosan oligosaccharide showed peaks at 2849, 2884, and 2885 cm-1, respectively, attributed to the absorption of the C-H stretching vibration of methyl or methine. The amide I, amide II, and amide III bands of chitosan, chitosan oligosaccharide, and commercial chitosan oligosaccharide exhibited peaks at 1620/1620/1602, 1553/1555/1505, and 1310/1309/1317 cm-1, respectively. Furthermore, the solubility of MCOS was 45.15±3.43, water binding capacity (WBC) was 299.25±4.57, and fat binding capacity (FBC) was 325.61±2.28 and the solubility of commercial chitosan oligosaccharides was 49.04±9.52, WBC was 280.55±0.50, and FBC was 157.22±18.15. Thus, the characteristics of MCOS and commercial chitosan oligosaccharides are similar. The results of investigating the impact of chitosan oligosaccharide on the proliferation of probiotics revealed increased growth in L. casei, L. acidophilus, and Bif. Bifidum. Therefore, the major short-chain fatty acids produced by gut microorganisms, such as acetic acid, propionic acid, and butyric acid, increased within 24 hours of adding 1% ($p<0.01$) and 2% ($p<0.001$) MCOS. The impact of MCOS on the overall gut microbiota was assessed, revealing that the Chao1 index did not show significant differences, but the Simpson index decreased in a concentration-dependent manner, indicating a higher species diversity. The addition of MCOS resulted in changes in the overall microbial composition, with an increase in Firmicutes and Verrucomicrobia ($p<0.05$) compared to the control group, while Proteobacteria and Actinobacteria ($p<0.05$) decreased. At the genus level, changes in microbiota due to MCOS supplementation showed an increase in beneficial bacteria like lactobacillus, Romboutsia, Turicibacter, and Akkermansia ($p<0.0001$) while harmful bacteria like Enterococcus, Morganella, Proterus, and Bacteroides ($p<0.0001$) decreased. In this study, chitosan oligosaccharides were successfully produced under established conditions from mealworms, and these chitosan oligosaccharides are expected to have prebiotic effects, similar to those obtained from crabs.

Keywords : mealworms, chitosan, chitosan oligosaccharide, prebiotics
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