

**Profiling, identification and biotransformation of bioactive isoflavone isomers in
fermented soymilk using endogenous and exogenous β -glucosidases**

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Dietary supplements based on soy based foods and beverages are increasingly gaining prominence all over the world. In this study, liquid chromatography coupled with positive electrospray ionisation tandem mass spectrometry (MS/MS) and a Varian model HPLC attached to an Amperometric electrochemical detector were used for the quantitation and characterisation of isoflavones in fermented soymilk made from soy protein isolate SUPRO 590. Microorganisms possess endogenous β -glucosidases which can be utilised to hydrolyse predominant isoflavone β -glucosides in soymilk to improve biological activity. In our earlier study, probiotic organisms including *Bifidobacterium*, *Lactobacillus acidophilus* and *Lactobacillus casei* increased the concentration of bioactive isoflavone aglycones in soymilk during fermentation. β -glucosidase activity of enzyme was determined using *p*-nitrophenyl- β -D-glucopyranoside as a substrate. Aglycone equivalent ratio, a key factor in the delivery of health benefits of isoflavones, was also monitored during fermentation. Otieno-Shah (O-S) index was used as a measure to determine hydrolytic effectiveness of the enzyme during 4 h incubation of soymilk with the β -glucosidases. The isoflavones were found to produce characteristic radical ions as well as molecules of H₂O, CO₂, a sugar unit, and an alcohol through collision-induced fragmentation. Product ion fragments revealed unique fragmentation pathways for each isoflavone isomer. The occurrence of aldehydes such as pentanal, ethanal and methanal was shown to be specifically linked with the presence of aglycones, daidzein, genistein and glycitein, respectively. Exogenous enzyme had faster rate of isoflavone glucoside hydrolysis as compared to the endogenous enzyme. Highest O-S indices were obtained after 4, 3 and 2 h of incubation of 50 mL of soymilk with 0.2% v/v of with 40, 50 and 80 μ g/mL of enzyme solution, resulting into aglycone percent concentrations of 83.0, 85.6 and 83.7%, respectively. Conversely, highest O-S indices and aglycone percent concentration of soymilk fermented with endogenous enzyme were too low compared to those of exogenous enzyme over the same 4 h incubation period. Optimum aglycone equivalent

ratios coincided with highest O-S indices and highest aglycone concentrations in soymilk hydrolysed with exogenous enzyme. This was the same for endogenous enzyme, but only after an extended period of fermentation of 24 h. Thus positive ion fragmentation is important in qualitative mapping of isoflavone isomers and revealing the occurrence of other related compounds such as aldehydes in soymilk. Obtaining highest aglycone concentration as well as optimum aglycone equivalent ratio could be two crucial factors important for improving the biological activity of fermented soymilk.

Keywords: LC MS/MS, product ions, Otieno Shah (O-S) index, Aglycone equivalent ratio, β -glucosidase activity