Soybean has high isoflavone content with recognized for health benefits. Aglycone isoflavones have highest antioxidant activity and are more bioavailable than their glycoside forms. Fermentation process can be an alternative for glycoside isoflavone conversion into aglycones achieved by the catalytic action of β-glucosidase. The aim of this work was to investigate the effect of fermentation process with *Monascus purpureus* on defatted soybean flour (DSF) for the conversion of isoflavones into aglycones. For the semi-solid fermentation, the DSF was dispersed with distilled water (1:1; w:v), initial pH 6.0, and autoclaved at 121 °C for 15 min. The substrate was inoculated with suspension of $10^7$ spores, and incubated at 30 °C for 48h. A control was prepared without inoculum. The bioprocess was monitored by the β-glucosidase activity using p-NPG as substrate. Isoflavones were determined using Waters Acquity UPLC® system (reverse phase column Acquity UPLC® BEH C18, 2.1mm x 50.0mm x 1.7µm particles) with a non-linear gradient with an initial phase of 90%/10% acidified water/acetonitrile. Total run time was 12min, the flow rate, 0.7mL min$^{-1}$ and 35°C. The results indicated that the DSF induced β-glucosidase production by fungus and showed statistically significant difference (p<0.05): fermented DSF (150.62±0.38UA g$^{-1}$); DSF (39.80±1.06UA g$^{-1}$) and control (24.22±0.04UA g$^{-1}$). The concentration of aglycone isoflavones in relation to total isoflavones was 17.24% in the control, 14% in the DSF and 82.08% in the fermented DSF. The fermentation process of DSF with the fungus *M. purpureus* promoted the transformation of isoflavone glycoside into aglycones forms in 5.86 times.