Fasciola hepatica is a parasite of global socioeconomic importance, infecting both livestock and humans. Resistance to current treatments have prompted research into novel drug target discovery and vaccine development. Within their host, juvenile fluke excyst from infective metacercariae and migrate through host tissue. Newly excysted juveniles secrete three similar cathepsin B (FhCB) proteases known to be up-regulated during tissue migration and down-regulated thereafter, implicating infection-specific role(s). We have recombinantly expressed these three FhCBs in yeast (*Pichia pastoris)* and have activated these, both via auto- and trans-catalytic mechanisms. We have carried out a biochemical assessment of pH optima and substrate specificities, which showed differences between the three FhCBs. Inhibition profiles were determined using a library of cysteine protease inhibitors and inhibition constants have been determined for a number of inhibitors. We have also shown that FhCB1 and FhCB2 have the ability to digest *F. hepatica* Helminth Defence Molecules and host haemoglobin, similar to *Fasciola* cathepsin L. Enzyme kinetics also revealed fundamental differences in *Fasciola* cathepsin B biochemistry when compared to those described in mammalian species, suggesting fluke specific roles, potentially in parasite virulence.