The present study embarked on the objective of optimizing protein extraction from freeze dried fish waste (FD-FW), sardine (Sardina pilchardus). Introduction of freeze drying prior to extraction was aimed at reducing the risk of protein loses in fish waste (FW) during frozen storage before the extraction process. Response surface methodology (RSM) was used to study the effect of independent variables, namely time (30-60 minutes), pH (7-11), rotation speed (100-300 rpm), and NaOH: substrate ratio (1-3) on protein extraction from FD-FW. From RSM-generated model, the optimum conditions for extraction of protein from FD-FW were identified to be at pH 10.56 in 48.61 minutes reaction time, with rotation speed of 104.77 rpm and NaOH: substrate ratio of 1.54. Predicted protein yield was 85.02 mg/ml while an experimental protein yield was 83.51 mg/ml as revealed by confirmatory studies.

**Keyword:** Sardine pilchardus; Freeze drying; Freeze dried fish waste; Response surface methodology