The biochemical properties of hemoglobin (Hb) of Nile Tilapia (*Oreochromis niloticus*) were studied. Averages of hematocrits and Hb concentrations were about 25.80 ± 3.71% and 7.05 ± 1.08 g/dl, respectively. The Nile Tilapia Hb was determined by cellulose acetate electrophoresis. It was found that there were 9 – 11 isomers of Hb. Moreover, Hb can be classified into two groups; anodic, cathodic I and II, the proportion of these groups was approximate amount 45.72%, 47.62% and 6.66% of total Hb, respectively. By mass spectrometry, the molecular weight of native Hb was 62,954 daltons and the molecular weights of subunits were 15,340, 15,621 and 16,126 daltons. The anodic Hb was composed of 5 – 9 pIs (ranging from 5.28 – 6.80) and the cathodic Hb was composed of 8 – 10 pIs (ranging from 7.27 – 9.71).

The freshness and oxidation changes of Nile Tilapia kept in cold room at the averaged temperature of 2.97 ± 0.84 °C were studied. The chemical methods namely K-value, %methemoglobin, TBA number and hydroperoxide, the physical methods namely temperature, pH and color of meat and gill as well as the texture of meat and the sensory method were used. It was found that the freshness was continuously decreased and was rejected on the 9th day with a sensory evaluation score at 4.54 ± 0.25 (rejected point was < 5). While the K-value was continuously increased and was rejected on the 9th day at 77.64 ± 3.39% (rejected point was > 80%). The %methemoglobin and TBA number were continuously increased and were rejected on the 7th day at 74.40 ± 7.48% and 1.02 mg malonaldehyde/kg, respectively. The results indicated that blood was deteriorated at a faster rate than meat. It was also found that the changes of the K-value and the %methemoglobin had the same trend and highly related with an equation of \( Y = 0.7935X + 32.38 \) and \( R^2 = 0.9386 \). Therefore, the Hb oxidation of Nile Tilapia could be applied as an index for the freshness of Nile Tilapia.