This work aimed to compare the electrophoretic profile of myofibrillar and sarcoplasmic proteins of fillet and mechanically separated meat (MSM) of fresh Nile tilapia (Oreochromis niloticus). This byproduct of the tilapia fillet (MSM) has been used as an alternative substrate in the development of thermally processed products. A fingerprint of the fillet and the byproduct’s polypeptide chains was obtained using the technique of electrophoresis on polyacrylamide gel containing sodium dodecyl sulfate (SDS-PAGE). The comparative analysis of electrophoretic profile showed a lower intensity of staining for all the polypeptide chains of the MSM compared to those observed in the fillet. Of the three polypeptide chains that make up the actomiosin tissue (myofibrillar proteins), the presence of myosin light chain (17.64 kDa), myosin heavy chain (224.56 kDa) and actin (39.18 kDa) could be highlighted. The sarcoplasmic protein fraction was characterized by the presence of 16 polypeptide chains. These results show that the use of MSM as a raw material can generate a product qualitatively similar to the fillet, but with lower amount of protein in its formation. In order to evaluate the protein integrity, MSM pâté was used as a food model. The evaluation of protein hydrolysis during storage was performed by spectrophotometric and electrophoretic analysis. No protein hydrolysis during 180 days of storage of MSM canned pâté was observed. So, it could be concluded that MSM has potential in agribusiness application by presenting nutritional similarity to the tilapia fillets and protein stability during storage.