ABSTRACT

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Title: Formation of carbonyl compounds by non-enzymatic protein glycation

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This diploma thesis was aimed at the optimization of carbonyl groups determination in biological material. Carbonyl groups are formed by effect of protein oxidation or glycation. As a model protein, I used bovine serum albumin for spectrophotometric and aspartate aminotransferase for electrophoretic method of assessment, which were glycated by several glycating agents (glucose; glucose + copper; fructose; fructose + copper; methylglyoxal). I optimized different parameters of spectrophotometric determination: solvent, concentration of reagent, amount of protein, and duration of incubation. I managed to find suitable conditions of this determination, which I have used in subsequent experiments with different glycating agents. At electrophoretic determination, I performed SDS-PAGE with following silver staining or immunoblotting with chemiluminescent detection. The results of spectrophotometric determination showed that methylglyoxal has the highest glycating ability, followed by fructose with addition of cupric ions, fructose itself, glucose with addition of cupric ions, and the slowest glycating agent is glucose. At electromigration methods, the mobility of glycated proteins decreased due to the increase in their molecular weight as a result crosslinks formation. Fructose with addition of cupric ions approved here to be the most effective glycating agent, where such a big increase in molecular weight of protein occurred after seven days incubation that they did not permeate to the gel at all.