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THE DEVELOPMENT OF NEW METHOD OF THE DETERMINATION OF BACTERIAL TRANSGLUTAMINASE ACTIVITY USING FIBRINOGEN AS A SUBSTRATE

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Introduction. Bacterial transglutaminase (TG) is widely used in food industry, because it is a calcium-independent enzyme. The determination of TG activity is an important issue that is necessary for adequate use. However the control of TG activity on site is complicated. Most of manufactures do not control TG activity during the use that can much affect the efficiency of its application in food industry. That is why the aim of our work was to develop a simple and accurate method of TG activity determination using human fibrinogen as a substrate

Methods. Fibrinogen was purified from human blood plasma. Thrombin (50 NIH/mL was purchased from Sigma (USA). For preparation of polymeric fibrin 0.3 mg/ml of fibrinogen was mixed with 0.5 NIH/ml of thrombin. Samples were incubated at 37 °C during 30 minutes in the presence of TG or equivalent volume of buffer. Cross-linking was detected using SDS-PAGE in the presence of 0.2% mercaptoethanol. The intensity of non cross-linked protein bands was estimated using densitometric software TotalLab TL100. Alternatively polymeric fibrin clot was removed from incubation media and dissolved in 0.125% acetic acid. Optical density of dissolved fibrin was monitored by spectrophotometer POP (Optizen, Korea).

Results and Discussion. SDS-PAGE demonstrated that 0,5 IU/ml of TG can cross link Aα-chain of fibrin. It's fibrin-specific activity was estimated as 5.0 ± 0.6 ug/min at the initial stages. Longer incubation or using of samples of TG with higher activity leaded to cross-linking of all three chains of fibrin. Polymeric fibrin is being cross-linked by TG effectively; however the sites of cross-linking are differed from those known for factor XIIIa. The cross-linking of fibrin by TG is time- and concentration-dependant.

Modified spectrophotometric method allowed us to obtain the calibration curve for the estimation of TG activity in International Unit based on the fibrin-specific activity. Estimation of activity was accurate in the range of concentrations from 0.17 to 0.8 IU/ml. This calibration curve allows estimating the enzymatic activity of commercially available TG.

Conclusions. Polymeric fibrin is useful substrate for estimation of TG activity. The simple and effective method for TG activity was developed and approved.

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