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Development of biosensor for detection proteases activity by means of thickness shear mode transducer with immobilized casein

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Degradation of milk proteins plays a key role in flavour and consistency changes of milk and cheese. Control above desirable cheese ripening, undesirable gelation or bitterness after ultra high temperature (UHT) casein breakdown is important goal for food industry. Serine protease plasmin has been always connected with milk proteolysis thanks to its abundant transfer from blood circulation into bovine milk. Main goal of our work was to study mechanism of a degradation of β -casein by plasmin and trypsin proteases. Thickness shear mode method (TSM) allowed us analyzing changes in frequency and in motional resistance of casein layer caused by proteases cleavage. After enzymatic reaction with plasmin, short fragments of casein were cleaved by protease causing increase in resonance frequency of TSM transducer. Plasmin detection was performed in the range of concentration 1-20 nM, which corresponding to protease concentration causing changes of milk quality. The casein layer has been substantially affected also by trypsin. Detection limit of TSM transducer for plasmin was found to be 0,65 nM. As opposed to plasmin and trypsin, thrombin, which doesn't cleave casein, caused only small decrease in frequency and increase in motional resistance. This is an evidence of weak nonspecific adsorption of thrombin on casein layer. Topography of casein layers and its changes following plasmin cleavage was also studied by atomic force microscopy.

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