Abstract

Milk is a food produced and consumed in large quantities around the world and therefore its safe consumption requires an acceptably low level of microbial contamination. Milk should be also sufficiently stable in storage. Nowadays there are known more than 250 foodborne infections in the world. The bacterial safety limits in milk vary by country, but they are generally not higher than 10^3 cells per ml of sample depending on the type of bacteria. Consequently, sensors based on piezoelectric transduction were developed to quickly determine if milk samples have undergone contamination with an unsafe level of bacteria. We have been designed sensors based on aptamers as receptors immobilized on the sensory surface of a high frequency acoustic wave device (around 1 GHz) of electromagnetic piezoelectric acoustic sensor (EMPAS), capable of detecting *Escherichia coli*. The surface was functionalized by molecules with antifouling properties onto which the DNA aptamers were immobilized. Such sensor was able to selectively detect *E. coli* directly in raw cow's milk with a detection limit of 8 CFU/mL, far below the safe limits for dairy products. A food as rich in nutrients as milk can also deteriorate due to the presence of enzymes. Proteases can carry out proteolysis of the proteins present in milk, altering its nutritional properties, making it inedible or unsuitable for technological processing. Consequently, it is essential to monitor this enzymatic activity designing a sensor capable of detecting the proteolysis by the enzyme carried out on the β-casein previously adsorbed on the surface of a quartz crystal multi-harmonic microbalance (QCM). The sensor was tested for trypsin and plasmin. We have shown that addition of increasing concentrations of protease caused the progressive removal of the β-casein layer which in turn increases the resonant frequency of the QCM. The method allowed the detection of enzymes with high sensitivity in the sub-nanomolar range between 0.1 nM and 20 nM, and the study of the kinetics of β-casein proteolysis by means of an inverse Michaelis-Menten model. The basic constants of the enzymatic reaction were also determined. Furthermore, a model was used to study the viscoelastic properties of the protein adlayer after its formation and after proteolytic cleavage, in which it was found that β-casein is preferably adsorbed on hydrophobic surfaces as an asymmetrical bilayer, with a denser, thinner innermost layer and a less tightly bound, thicker outermost layer. It has been shown that applied detection methods can represent reliable, fast, very sensitive and low cost systems applicable for the rapid analysis of food safety.

Keywords: biosensor, trypsin, plasmin, aptamer, β-casein, acoustics methods, antifouling, viscoelastic properties