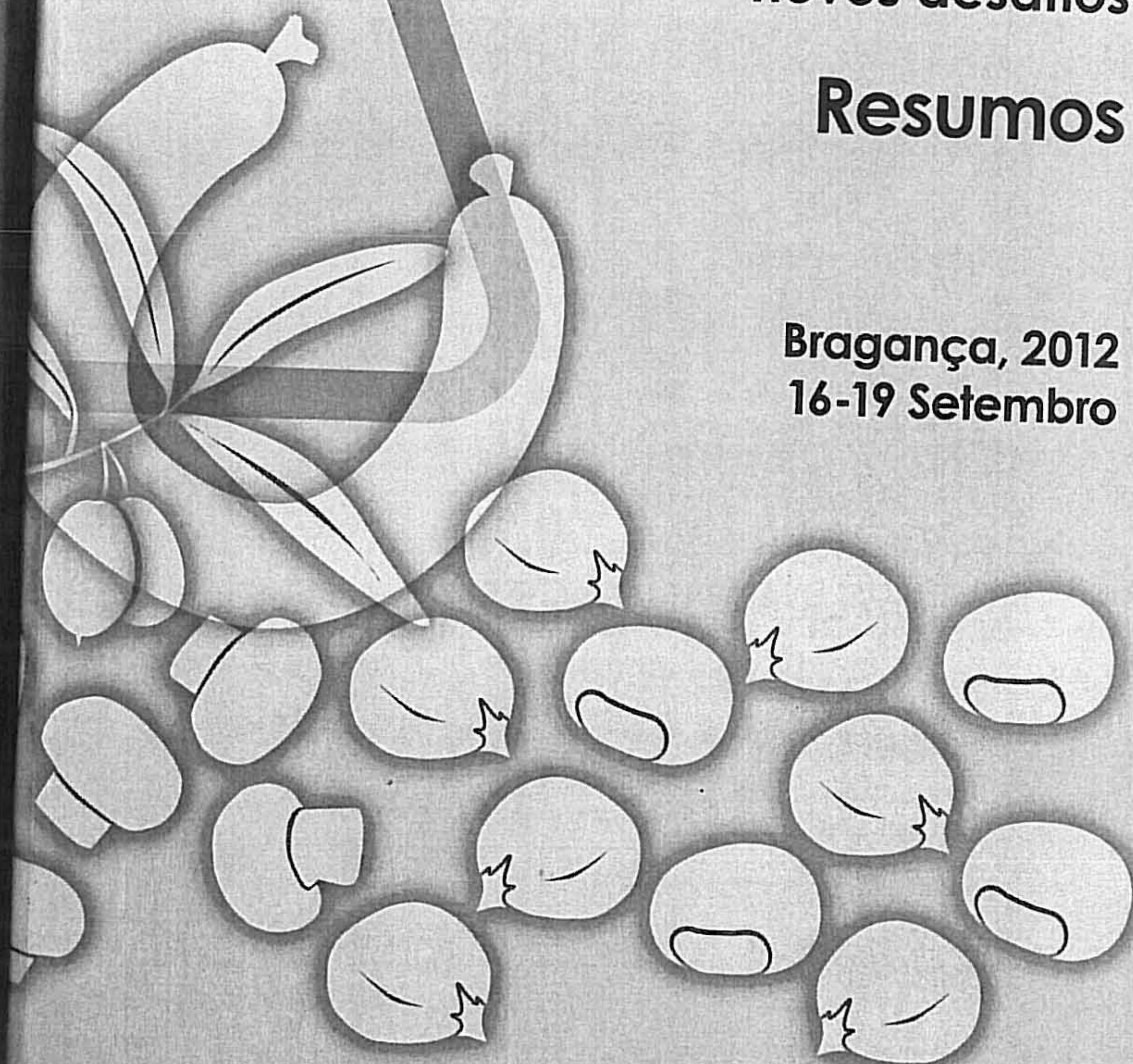
11º Encontro de Química dos Alimentos

Qualidade dos alimentos: novos desafios



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Livro de Resumos

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Detecting *Escherichia coli* O157:H7 by Quartz Crystal Microbalance with Dissipation (QCM-D)

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Escherichia coli O157:H7 is a foodborne pathogen classified as an Enterohemorrhagic *E. coli* being associated to foodborne outbreaks with high mortality. Since the traditional methods for its detection are often time-consuming, there is a need to develop new techniques that allow a rapid, simple, reliable, specific and sensitive detection for a quick and effective medical intervention, as well as to ensure food safety. In the last years different approaches have been suggested for this purpose including the use of biosensors based on different bioreceptors such as enzymes, antibodies and DNA. Recently, there has been a growing interest regarding DNA biosensors due to advantages such as DNA high specificity and stability and the possibility of developing devices that can be reusable after thermal melting of the DNA duplex [1].
Since these genosensors use immobilized DNA single strands to detect the complementary sequence by hybridization, it is very important to optimize the conditions used during probe immobilization and target hybridization. In this work, a DNA piezoelectric biosensing method for *E.coli* O157:H7 DNA detection was developed and optimized using a QCM-D to evaluate the immobilization/hybridization mass phenomena. A 21-mer oligonucleotide sequence (probe) and its complementary strain (DNA target) were selected from the *eae* gene of *E. coli* O157:H7. DNA probe was modified with a C6 alkanethiol group to improve immobilization by Self-Assembled Monolayer on the gold electrode surface. To prevent non-specific adsorption of DNA during the immobilization and hybridization steps, 6-mercapto-1hexanol (MCH) was used as a blocking agent. Different parameters such as probe concentration (0.25, 0.5, 1.0, 2.0μM) and incubation time (30 and 60min) on the immobilization step, MCH concentration (0.5 and 1.0μM) and incubation time (30 and 60min), target concentration (0.5 and 1.0mM) and hybridization temperature (22 and 30°C) were tested. Co-immobilization assays using a mixture of DNA thiol Probe and MCH, were also performed at 22 and 30°C. The best results regarding immobilization on the gold electrode at 22°C were obtained using 1μM of DNA probe and 30min of incubation. Co-immobilization of DNA thiol probe and MCH did not reveal a significant improvement. When using 1 μM target DNA, higher hybridization efficiency was observed at 30°C. Optical validation of the developed protocol was achieved by epifluorescence microscopy, used to identify different fluorescent labels on the DNA thiol probe and target. The proposed methodology can potentially be used for further applications, namely the development of an electrochemical DNA biosensor for rapid DNA analysis and pathogen detection.

References:

[1] FRR Teles, LP Fonseca, Talanta, 2008, 77, 606-623