BOOK OF ABSTRACTS

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Development of a highly sensitive real-time PCR system for the quantification of soybean as a potential allergenic ingredient

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Soybean is a food ingredient with both techno- and biofunctionality properties, whose use has been increasing considerably in the past decades [1]. Besides its numerous applications, soybean is widely used by the food industry in processed foodstuffs such as sausages, hamburgers or hams. However, since soybean is considered one of the most common foods known to cause allergic reactions in sensitized individuals, the European Union established legislation aiming to protect these patients. According to the Directive 2007/68/EC, soybean plus 13 other groups of foods must always be labeled independently of its amount. For labeling compliance monitoring, the development of adequate methodology for soybean detection is of utmost importance.

In this work, we propose developing a molecular approach based on real-time polymerase chain reaction (PCR) system with adequate sensitivity for the quantitative analysis of soy as a potential allergen in meat products. For this purpose, different model samples of pork meat spiked with known amounts of isolated or concentrated soy protein, ranging from 10% to 0.001%, with and without heat treatment were prepared. The reference mixtures were used to develop a calibration model based on real-time PCR using primers and hydrolysis probes specifically designed to target eukaryotic reference (universal) and lectin (specific for soybean) genes.

The proposed system presented high specificity and sensitivity allowing a relative quantification of 50 mg/kg of isolated or concentrated soybean in pork meat. The performance of the technique demonstrated its appropriateness for quantification by the adequacy of linearity and (R^2>0.98) and PCR efficiency (~100%) parameters for real-time PCR, similar for both types of protein material in binary mixtures. Heat processing did not affect the performance of the method that allowed reaching the same relative sensitivity. It also enabled amplifying soybean until 2.44 pg (2.2 DNA copies). The normalized technique for the quantification of soybean was successfully validated by its application to blind reference mixtures, indicating a high proximity between the actual and the estimated values. In summary, the proposed normalized system presented adequate sensitivity for the quantification of soybean as a potential hidden allergen in foods.

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