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A normalised real-time PCR method to quantify soybean protein material in meat products at trace amounts and as affected by thermal processing

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Over the last years, food allergies have been faced with growing interest since they are increasingly recognized as an important problem of public health. In order to protect consumers, proper legislation has been issued regarding foods known to cause allergic reactions in sensitized individuals. Accordingly, in EU, Directive 2007/68/EC requires the mandatory discrimination of all allergenic foods from the rest of the ingredients present in labels, regardless of their quantity. Soybean is among the fourteen groups of potentially allergenic foods whose presence in processed foods must always be declared [1]. Nowadays, soybean is frequently used by the food industry in several different processed foodstuffs, including meat products such as sausages, hamburgers or hams. Due to its wide use and considering that nowadays several food products can be processed in the same facility, even with such regulations in practice, unintentional cross-contamination with soybean and or/mislabelling can occur. Thus, for labelling compliance monitoring and to assure consumer's protection, the development of adequate methodology for soybean detection is of utmost importance.

This work aimed at developing a methodology based on real-time polymerase chain reaction (PCR) system with adequate sensitivity for the quantitative analysis of soybean as a potential hidden allergen in meat products. For this purpose, different binary model mixtures of pork meat spiked with known amounts of soybean protein isolate (SPI) or soybean protein concentrate (SPC) ranging from 10% to 0.001%, with and without thermal 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 10 mg/kg of SPI or SPC in pork meat. All real-time PCR assays presented excellent performance parameters, which were similar for both types of protein material in binary mixtures. The method appropriateness for quantification was demonstrated by the adequacy of linearity (R^2>0.98) and PCR efficiency (ranging from 92.2%-102.7%). Heat processing did not significantly affect the performance of the method since in both cases 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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