

Original article

A PCR assay to detect trace amounts of soybean in meat sausages

Sónia Soares,^{1,2} Isabel Mafra,^{1*} Joana S. Amaral^{1,2} & Maria Beatriz P.P. Oliveira¹

¹ REQUIMTE, Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, Porto 4099-030, Portugal

² ESTiG, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, 5301-857 Bragança, Portugal

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Summary Soybean proteins are the most widely used source vegetable proteins in the meat industry because of several interesting characteristics. As soybean is included in the group of ingredients potentially allergenic, if not declared, it can be considered a hidden allergen, representing a potential risk to sensitised individuals. The aim of this work was to optimise and apply DNA-based techniques for soybean detection in meat products, as alternative to the currently used protein-based methods. The optimised polymerase chain reaction (PCR) protocol targeting the soybean lectin gene enabled the detection of the addition of 0.1% and 0.5% of hydrated textured protein, which corresponded to 0.01% and 0.06% (w/w) of soybean protein in unprocessed and heat-processed pork meats, respectively. The established PCR technique, when applied to commercial meat sausages (eighteen samples), confirmed the presence of soybean declared in nine samples and indicated the presence of soybean in four samples with no labelled information about soybean. Additionally, the event-specific PCR detection of Roundup Ready[®] soybean was also performed, enabling the detection of transgenic DNA in three samples.

Keywords GMO, meat sausages, PCR, processed meat, soybean detection.

Introduction

In many developed countries, meat is still considered as a premium quality protein source, not only for its nutritional composition but also for its organoleptic characteristics. Nevertheless, because of the high prices of animal proteins, especially those from high quality meat sources, the industry frequently adds vegetable proteins to meat products. Because vegetable proteins have a considerably lower price than muscle proteins, their use as meat extenders reduces the cost of the final product (Belloque *et al.*, 2002).

Soybean protein is reported to be the most widely used vegetable protein in the meat industry owing to several interesting characteristics (Macedo-Silva *et al.*, 2001; Belloque *et al.*, 2002). Soybean protein is added for its emulsifier properties, preventing the coalescence of fat during heating, and also because it increases the capacity of water-holding and improves the texture of the final product (Macedo-Silva *et al.*, 2001; Castro *et al.*, 2007). Additionally, some health benefits, such as cholesterol levels reduction and decreasing in menopause symptoms, have been associated to soybean consumption (Riaz, 1999).

Because soybean is widely used in foodstuffs, it is considered as a particularly insidious hidden allergen in several products, such as pastries, bakery products, infant food, sausages, processed meats and hamburgers. Soybean is included in the group of twelve ingredients potentially allergenic, which should therefore be labelled according to the *Codex Alimentarius* FAO/World Health Organization (WHO) and the European Commission (Directive 2003/89/EC). Koppelman *et al.* (2004) referred that amounts of soy below 0.1% and 1% (w/w) can lead to allergic reactions. Such low quantities demand highly sensitive and reliable techniques able to detect and quantify minute amounts of soybean in processed foods. Moreover, in some countries, the addition of soybean proteins is restricted to a certain extent (Belloque *et al.*, 2002). For example, the US regulation for meat products containing soybean proteins allows a level of 3.5% of soybean flour and 2% of soybean protein isolate for sausages (Belloque *et al.*, 2002); in Spain, the limit of soybean protein authorised corresponds to 3% (referred to the product as dry basis) (Castro *et al.*, 2007); and in Brazil, a maximum of 0.04% of soybean protein is authorised for mortadella, hot-dog sausage, hamburger and fried chicken steaks (Brod & Arisi, 2007).

Another important issue to consider is the use of genetically modified (GM) soybean in foodstuffs.

*Correspondent: Fax: +351 222003977;
e-mail: isabel.mafra@ff.up.pt

Because GM crops entered the food chain, a scientific and public debate concerning their safety and the need for labelling information arise, especially in Europe. The latest EU regulations (Commission Regulation (EC) (2003a,b) impose the compulsory labelling for food products containing more than 0.9% of authorised GM material. Although the issue of GMO is of unquestionable importance, considering the potential threatening to soy-allergic consumers, there is a clear need for sensitive methodologies that can detect even traces of soybean, either GM or not, in meat products.

Various methods have been applied in soybean detection in food products. Several immunological assays have been developed to detect soy allergens in foods, being mostly designed to determine adulteration of meat products with soy proteins (Macedo-Silva *et al.*, 2001; Poms *et al.*, 2004). The currently available AOAC official method for measuring soybean proteins in raw and heat-processed meat products consists of using an enzyme-linked immunosorbent assay (ELISA) method (AOAC Official Method). Like other immunological methods, ELISA is based on the reactivity between an antigen (soybean proteins, generally glycinin and β -conglycinin) and an anti-serum that recognises the specific proteins. Belloque *et al.* (2002) referred that one major limiting step of this technique applied to soybean detection was the need of developing an appropriate antiserum, especially when heat-processed foods are considered. Other disadvantages pointed to the AOAC official method were its complexity, delaying and cost, besides the fact of being considered only a semi-quantitative method (Belloque *et al.*, 2002; Castro-Rubio *et al.*, 2005). Other protein-based methods have been reported for soybean detection in meat products, namely electrophoretic and chromatographic techniques (Belloque *et al.*, 2002). However, some authors referred that protein-based methods can be significantly less sensitive in the evaluation of thermally processed foods because of protein denaturation and specific epitope alterations (Saez *et al.*, 2004; Rodríguez *et al.*, 2005).

The higher stability of nucleic acids compared to proteins and their ubiquity in every type of cells makes DNA molecules the target compounds for species identification in several recent works (Mafra *et al.*, 2008a). Moreover, sensitivities of DNA-based methods, namely polymerase chain reaction (PCR), generally show lower limits of detection (Poms *et al.*, 2004). However, only few reports describe the PCR detection of soybean in meat products (Meyer *et al.*, 1996; Brod & Arisi, 2007), without reporting sensitive assays, as they still rely basically on protein-based methods (Belloque *et al.*, 2002; Koppelman *et al.*, 2004).

The aim of the present work was to apply the PCR technique for the specific detection of soybean in meat products. Because pork meat is a common and major ingredient present in meat sausages such as frankfurters,

which are generally heat treated, the method was optimised using binary mixtures of pork meat and soybean, either as raw or autoclaved, to establish sensitive assays. Several commercial samples of frankfurters/hot-dog sausages and other meat sausages were tested and the compliance of the labels checked. The samples positive to soybean were also tested for the presence of GM soybean.

Materials and methods

Sample preparation

A textured soybean (46% protein, determined by Kjeldahl method) sample was purchased in a local supermarket, processed following the commercial instructions (hydrated and microwave cooked, total moisture 78%) and squeezed to eliminate as much water as possible. The final protein content of the hydrated textured soybean determined by Kjeldahl was 12.5% (w/w). Pork muscle samples were acquired in a local retail market. Reference binary mixtures containing 0.1%, 0.5%, 1%, 5%, 10%, 25%, 50% and 75% (w/w) of hydrated textured soybean in pork meat were prepared to a final weight of 100 g. The binary mixtures were triturated and homogenised using a blender after the addition of 15 mL of sterile phosphate-buffered saline solution (136 mM NaCl, 1.4 mM KH_2PO_4 , 8.09 mM $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 2.6 mM KCl, pH 7.2). To avoid contaminations, each mixture was processed separately using different material and different blender containers previously treated with DNA decontaminator solution. To evaluate the effect of thermal treatment, identical binary mixtures were prepared and submitted to heat treatment in an autoclave at 121 °C for 5 min.

Certified reference materials (CRM) from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) were used as standards for testing the presence of GMO soybean and consisted of dried soybean powder with 0%, 0.1%, 0.5%, 1%, 2% and 5% (w/w) of Roundup Ready[®] soybean (Fluka, Buchs, Switzerland).

The analysed samples included meat sausages, from which some were frankfurters (samples 1–4 and 10), hot-dog sausages (samples 5–9 and 12–18) and one barbeque sausage sample (sample 11) purchased in local supermarkets. The samples comprised glass bottles and canned sausages, containing pork, chicken or turkey as the main meat ingredient.

DNA extraction

DNA was extracted using the Wizard method with minor modifications as described by Mafra *et al.* (2008b). Briefly, the grounded and homogenised samples (200 mg for binary mixtures and commercial samples

and 275 mg for cooked binary mixtures) were transferred to a 2-mL sterile reaction tube followed by the addition of 860 μL of TNE extraction buffer (10 mM Tris, 150 mM NaCl, 2 mM EDTA, 1% SDS), 100 μL of 5 M guanidine hydrochloride solution and 40 μL proteinase K solution (20 mg mL^{-1}). After the incubation at 60 °C for 3 h, with occasional stirring, the suspension was centrifuged (15 min, 18 514 g) and 500 μL of the supernatant were mixed with 1 mL of Wizard® DNA purification resin (Promega, Madison, WI, USA). The mixture was eluted through a column and the resin was washed with 2 mL isopropanol solution (80% v/v). After drying the column, the DNA was eluted by centrifugation (1 min 10 000 g) with 100 μL of Tris-EDTA buffer (10 mM Tris, 1 mM EDTA) at 70 °C to a new reaction tube. The extractions were performed in duplicate for each sample and binary reference mixture. All extractions included a blank extraction for the control of reagents and contaminations during extraction procedure.

DNA quantification and purity

The quality of extracted DNA was analysed by electrophoresis in a 1.0% agarose gel carried out in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) for 40 min at 120 V, stained with ethidium bromide (0.4 $\mu\text{g mL}^{-1}$ for 5 min) and destained in distilled water for 20 min. The agarose gel was visualised under UV light and a digital image was obtained using a Kodak Digital Science™ DC120 (Rochester, NY, USA).

The DNA was quantified by spectrophotometry using a Shimadzu UV-160A spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The DNA concentration was determined by UV absorbance at 260 nm (1 absorbance unit corresponds to 50 $\mu\text{g mL}^{-1}$ of dsDNA). The purity of the extracted DNA was determined by the ratio of the absorbance at 260 and 280 nm (ISO 21571, 2005; Somma, 2006).

PCR conditions

The amplifications by polymerase chain reaction (PCR) were carried out in 25 μL total reaction volume containing 2 μL of DNA (50–100 ng) extract, 15 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.5 μM of each primer, 0.2 mM of each dNTP (Invitrogen, Carlsbad, CA, USA), 1.5 mM of MgCl_2 when using primers GM03/GM04 and 2 mM of MgCl_2 when using primers RRS-3J1/RRS-3J3 (Table 1) and 1 U of DNA polymerase AmpliTaq Gold® (Applied Biosystems, Branchburg, NJ, USA). The primers were synthesised by Eurofins MWG Operon (Ebersberg, Germany).

The PCR amplifications were performed in a thermal cycler PTC-100 (MJ Research, Inc., Watertown, MA, USA). The following programmes were used 5 min at

95 °C, 37 cycles of 30 s at 95 °C, 30 s at 65 °C and 60 s at 72 °C, and 5 min at 72 °C for primers GM03/GM04; 4 min at 94 °C, 37 cycles of 30 s at 94 °C, 30 s at 60 °C and 60 s at 72 °C, and 4 min at 72 °C for primers RRS3J1/RRS3J3. The amplified fragments were analysed by electrophoresis in a 2.0% agarose gel carried out in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) for 60 min at 120 V, stained with ethidium bromide (0.4 $\mu\text{g mL}^{-1}$ for 5 min) and destained in distilled water for 30 min. The agarose gel was visualised under UV light and a digital image was obtained using a Kodak Digital Science™ DC120. Each extract was amplified at least in duplicate assays.

Real-time PCR amplification

The amplifications by real-time PCR were carried out in 20 μL mixture containing 2 μL (50 ng) of DNA extract, 1 $\times$ of iQ™ Supermix (Bio-Rad Laboratories, Hercules, CA, USA), 900 nM of each primer (Lectin-F/Lectin-R) and 100 nM of probe (Lectin-TMP) (Table 1). The real-time PCR assays were performed on a fluorometric thermal cycler iCycler iQ™ Real-time Detection System (Bio-Rad Laboratories) using the following conditions: 95 °C for 1 min, 50 cycles at 95 °C for 30 s and 60 °C for 1 min, with collection of fluorescence signal at the end of each cycle. Data were collected and processed using an iCycler iQ™ Real-Time Detection System Software version 3.1 (Bio-Rad Laboratories). Each sample was amplified in triplicate assays.

Results and discussion

Qualitative and quantitative analysis of extracted DNA

The quality of DNA extracts from binary pork meat/soybean reference samples was examined by agarose gel electrophoresis (Fig. 1). This enabled the comparison of DNA integrity before and after heat treatment by autoclaving. Variations of DNA fragment length, a parameter for DNA integrity, are dependent on the material under examination, the degree of processing and the DNA extraction method. In general, the raw mixtures showed an intense smear of short and long DNA (> 10 000 bp) fragments with a high DNA amount ranging between 2000 and 10 000 bp (Fig. 1a). This suggests that DNA was not excessively degraded during the Wizard extraction protocol, as previously reported for soybean products (Mafrá *et al.*, 2008b). The extract derived from 100% soybean material (lane 1) shows a different pattern with a high amount of degraded DNA, which can be justified by the processing necessary to prepare textured soybean. The intensity of DNA smears was significantly decreased in all the autoclaved samples containing pork meat (lanes 2–10) (Fig. 1b) because of the heat treatment. The samples

Table 1 Oligonucleotide primers used in the present work

Primer	Sequence (5'-3')	Target	Amplicon (bp)	Reference
GM03	GCC CTC TAC TCC ACC CCC ATC C	<i>Lec1</i>	118	ISO 21569 (2005), Meyer <i>et al.</i> (1996)
GM04	GCC CAT CTG CAA GCC TTT TGG TG			
RRS-3J1	GCA TCT ACA TAT AGC TTC TCG TTG	NOS 3'UTR/plant junction	106	Costa <i>et al.</i> (2010)
RRS-3J3	AAC TTC TCG ACG ATG GCC G			
Lectin-F	TCC ACC CCC ATC CAC ATT T	<i>Lec1</i>	81	ISO 21570 (2005)
Lectin-R	GGC ATA GAA GGT GAA GTT GAA GGA			
Lectin-TMP	FAM-AAC CGG TAG CGT TGC CAG CTT CG-BHQ2			

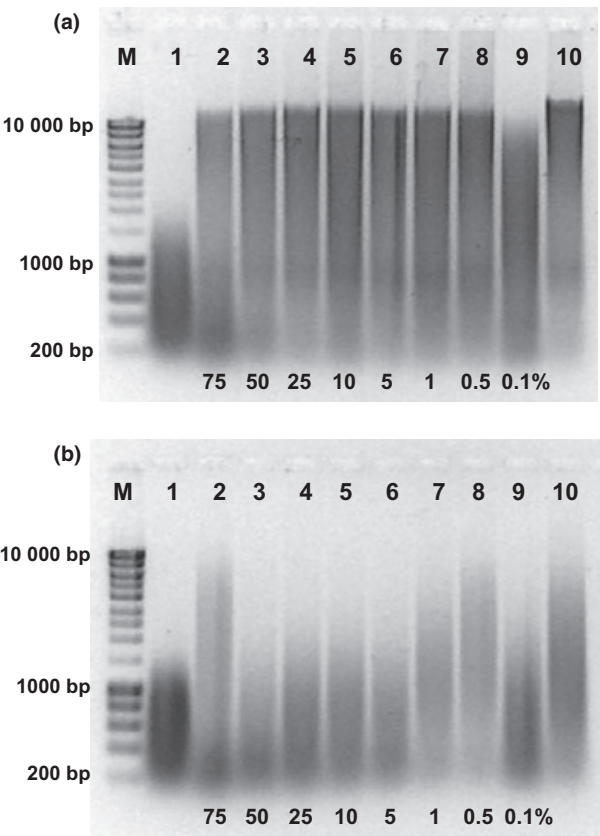


Figure 1 Agarose gel electrophoresis of DNA extracts from binary mixtures of pork meat and hydrated textured soybean. (a) Unprocessed and (b) autoclaved. M, Hyperladder I (Bioline, London, UK); lane 1, textured soybean; lane 2–9, different proportions of hydrated textured soybean in pork meat (w/w); lane 10, pork meat.

depicted in Fig. 1b show a higher shearing degree and, consequently, smaller length of DNA fragments than in raw samples, indicating DNA degradation. The high amount of DNA between 2000 and 10 000 bp found in raw mixtures was mostly lost in the majority of samples after heat treatment.

The extracts of commercial sausages showed an excessively sheared DNA for most samples exhibiting

patterns similar to those of autoclaved binary samples (Fig. 1b). An exception can be observed for sample 11, corresponding to a barbecue sausage sample, raw and vacuum packed, which showed prominent fragments above 10 kb, consistent with the lower degree of heat processing (data not shown).

The purity of the extracts obtained for all the reference binary mixtures was high because the ratio A_{260}/A_{280} ranged between 1.7 and 1.8. A higher variability of the same ratio was found for the extracts of commercial samples (1.4–2.0). The DNA yields obtained ranged between $473\text{--}1155\text{ ng }\mu\text{L}^{-1}$ and between $334\text{--}1308\text{ ng }\mu\text{L}^{-1}$ for the raw and autoclaved standard binary mixtures, respectively, and between $243\text{--}867\text{ ng }\mu\text{L}^{-1}$ for the sausage samples. The high levels of purity together with the high DNA yields obtained with the Wizard extraction method described in this work, suggest the adequacy of this protocol for meat-based products, as previously demonstrated for other vegetable food matrices (Mafra *et al.*, 2008b).

PCR detection of soybean lectin gene

In this study, the primers GM03/GM04 were used to amplify an internal fragment (118 bp) of the soybean lectin gene (*Lec1*) because of their high specificity, previously demonstrated by sequence similarity search in NCBI databases and to the absence of PCR amplification using DNA from other crop plants (legumes, cereals, vegetables) or from beef and pork (ISO 21569, 2005) (Fig. 2). The PCR conditions used in this work were optimised by the amplification of DNA extracts from raw binary standard mixtures, enabling the detection of 0.1% hydrated textured soybean protein in pork meat (Fig. 2a). For autoclaved binary samples (Fig. 2b), the level of PCR amplification was similar for all standard mixtures until the proportion of 0.5% of soybean, with no visible fragment for 0.1% of added hydrated textured soybean. This suggested that heat treatment affected the DNA integrity, as noted previously in the evaluation of genomic DNA. Considering that the average protein content of the hydrated textured soybean added to meat was 12.5% (w/w), this result corresponds to a level of soybean protein

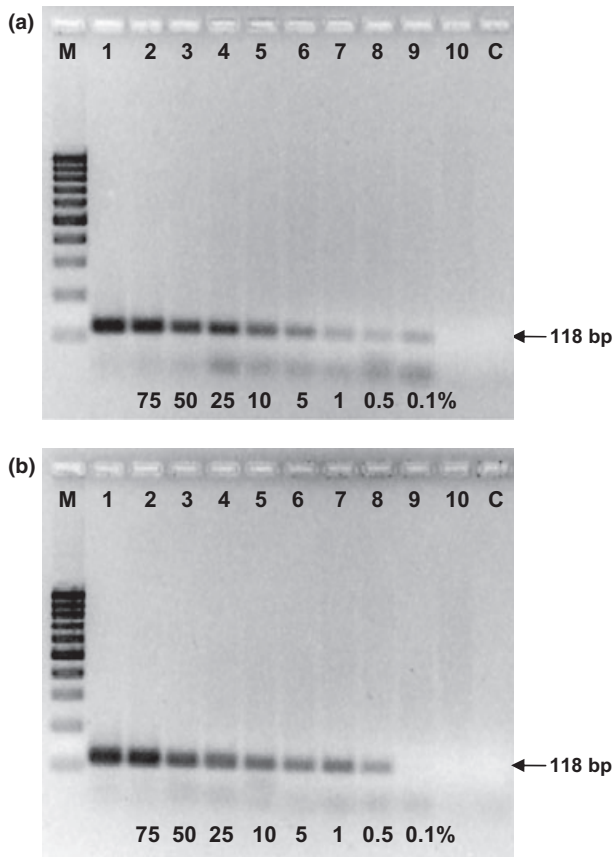


Figure 2 Agarose gel electrophoresis of PCR products targeting soybean lectin gene amplification of binary mixtures of pork meat and hydrated textured soybean. (a) Unprocessed and (b) autoclaved. M, 100-bp ladder (Bioron, Ludwigshafen, Germany); lane 1, textured soybean; lane 2–9, different proportions of hydrated textured soybean in pork meat (w/w); lane 10, pork meat; C, negative control.

detection of 0.01% and 0.06% in unprocessed and heat-processed binary mixtures, respectively. Higher detection levels of soybean proteins in heat-processed meat products were obtained by using chromatographic techniques (0.07–0.9%) (Castro *et al.*, 2005, 2007; Castro-Rubio *et al.*, 2005; García *et al.*, 2006). This result indicates that DNA-based methods can be used as highly sensitive alternative techniques to the protein-based methods, generally used for soybean detection in meat products (Belloque *et al.*, 2002; Koppelman *et al.*, 2004).

A great number of reports can be found in the literature regarding the detection of soybean in foods by PCR, specifically for the detection of GM soybean. In spite of that, they do not refer to the level of species-specific soybean detection and rather to the level of GMO detection. Examples of several DNA-based methods proposed for the detection of RR soybean

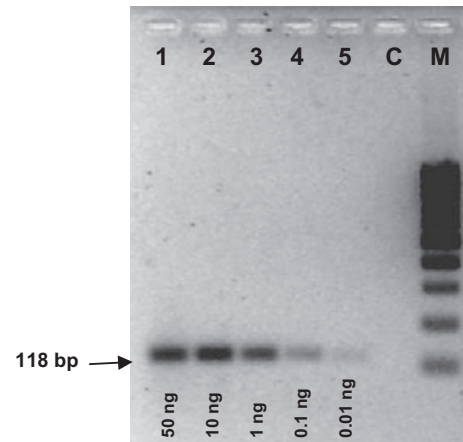


Figure 3 Agarose gel electrophoresis of PCR products targeting soybean lectin gene of different amounts of soybean DNA (lanes 1–5). M, 100-bp ladder (Bioron, Ludwigshafen, Germany); C, negative control.

include end-point PCR detection (Forte *et al.*, 2005; Liu *et al.*, 2005; Abdullah *et al.*, 2006; Kakihara *et al.*, 2006; Ferrari *et al.*, 2007), nested PCR (Brod & Arisi, 2007; Brod *et al.*, 2007), quantitative real-time PCR (Andersen *et al.*, 2006; Brod & Arisi, 2008; Harikai *et al.*, 2009; Moriuchi *et al.*, 2007; Murray *et al.*, 2009; Terry *et al.*, 2002) and microarray PCR (Leimanis *et al.*, 2006; Hamels *et al.*, 2009). Because the reported studies focus mainly the detection of GMO in soybean-derived food products, there is still scarce literature concerning the specific PCR detection of trace amounts of soybean in meat sausages. As several products based on soybean protein are added to a wide range of processed foods such as meat products, it is very important to determine the level of PCR detection, especially because it is often a minor ingredient. Meyer *et al.* (1996) proposed one of the first PCR techniques for the specific detection of soybean in a wide range of soybean products, reaching a detection level of soybean in meat products of 1%. Comparing with the present results, the soybean detection was much improved, which is of utmost importance considering the need of detecting trace amounts of soybean because of its potential allergenicity. The sensitivity of soybean-specific PCR assay was determined by amplification of a DNA extract of soybean textured protein 10-fold serially diluted. The obtained results can be observed in Fig. 3, where it can be noted the amplification of the lowest level of 10 pg of soybean DNA. Further dilutions were also tested, but with no detectable amplification (data not shown).

The optimised PCR conditions were then applied to eighteen commercial samples of meat sausages to verify the presence of soybean in compliance with the label statements (Table 2 and Fig. 4). As expected, soybean

Table 2 Main and/or relevant ingredients of commercial meat sausages analysed and PCR results for soybean lectin gene and RR soybean detection

Sample	Species declared		Protein declared				PCR results		
	Turkey	Chicken	Pork	Milk	Vegetable	Soybean	Soybean <i>Lec1</i> gene		
							GM03/GM04	Ct ^b	RR soybean ^a
1			X		X		++	27.7 ± 0.07	–
2			X	X		X	++	28.2 ± 0.05	–
3			X			X	+++	27.6 ± 0.06	+
4			X	X		X	+++	28.4 ± 0.29	+
5	X	X	X	X	X ^c		–	NA	
6			X	X	X ^c		+	NA	–
7	X	X	X	X			+	NA	–
8			X	X		X	++	30.8 ± 0.13	–
9	X			X		X	++	30.9 ± 0.42	–
10	X	X		X		X	+++	28.6 ± 0.21	+
11			X			X	++	29.4 ± 0.18	–
12			X				+	NA	–
13			X	X			–	NA	
14			X	X			–	NA	
15	X	X				X	+++	27.8 ± 0.10	–
16	X	X	X	X			–	NA	
17	X						–	NA	
18		X		X		X	+++	30.3 ± 0.36	–

NA, no detectable amplification by real-time PCR; –, not detected; +, positive detection.
^aThe PCR assays for RR soybean were only performed for the samples positive to soybean lectin gene.
^bCt – cycle threshold of real-time PCR assays (mean ± standard deviation).
^cHydrolysed vegetable protein.

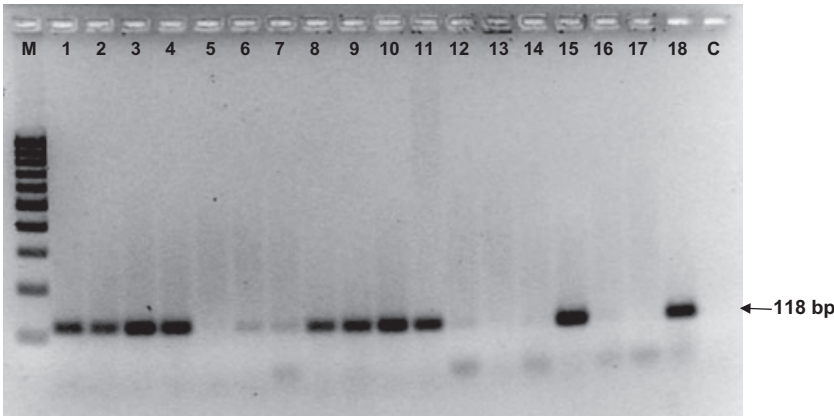


Figure 4 Agarose gel electrophoresis of PCR products targeting soybean lectin gene of commercial meat sausages (lanes 1–18 correspond to samples in Table 2). M, 100 bp ladder (Bioron, Ludwigshafen, Germany); C, negative control.

lectin gene was detected by PCR using the primers GM03/GM04 in all the nine samples labelled as containing soybean protein. These results were confirmed as positive by real-time PCR using a specific TaqMan probe. Sample 1, having vegetable protein as a declared ingredient, was also positive for soybean and

confirmed by real-time PCR. Sample 6, labelled as containing hydrolysed vegetable protein, tested also positive for soybean, although presenting a faint band that was not confirmed by real-time PCR. As referred, soybean protein is the most frequently used vegetable protein in the meat industry, though it is not expected

that consumers are aware of this information. Knowing that soybean is considered as a potential allergen, its presence should be clearly declared regardless of the amount according the EU legislation (Directive 2003/89/EC). Thus, the designation of 'vegetable proteins' can mislead the consumer, representing a potential risk for allergic individuals. Other weak PCR bands were obtained for samples 7 and 12 (Fig. 4), though not confirmed by real-time PCR (Table 2). The results for the amplification of the remaining samples (13, 14, 16 and 17) confirmed the absence of soybean.

PCR detection of RR soybean

In addition to the need of verifying the amount of soybean, it is also important to know whether the soybean is from GM crops, because the EU (Commission Regulation (EC), 2003a) impose the compulsory labelling for food products containing more than 0.9%. For the event-specific detection of Roundup Ready® (RR) soybean, the primers RRS-3J1/RRS-3J3 were specifically used to target the plant genome and NOS terminator junction zone, as alternative to others more frequently used such as GM07/GM08 (Querci *et al.*, 2006). This enabled to obtain a highly specific PCR product with an expected small DNA fragment (106 bp), adequate to amplify excessively degraded DNA from very processed foods (Costa *et al.*, 2010). The successful amplification of certified reference material extracts containing known amounts of RR soybean using primers RRS-3J1/RRS-3J3 is shown in Fig. 5. The DNA extracts from meat samples that tested positively for the lectin gene were further amplified for the presence of RR soybean. The event-specific PCR amplification showed faint bands in three samples (Fig. 5) and no detectable amplification in the other samples (Table 2). This suggests the presence of RR

soybean in samples 3 and 4 (Fig. 5, lanes 8 and 9) and traces in sample 10 (Fig. 5, lane 10).

Final remarks

The results indicate that the proposed PCR assay can be used as highly sensitive, fast and simple alternative to the protein-based methods generally used for soybean detection in raw and heat-processed meat products. The optimised species-specific PCR technique allowed the detection of 0.1% and 0.5% of soybean textured protein in raw and heat-processed meat, corresponding to levels of protein detection of 0.01% and 0.06%, respectively. The sensitivity of soybean-specific PCR assay reached a level of 10 pg of soybean DNA.

The application of the established PCR conditions to commercial meat sausages such as frankfurters allowed confirming the presence of soybean mentioned on the labels, but also verifying that one sample labelled as containing vegetable proteins can mislead the consumer as it included soybean. These findings suggest the usefulness of the proposed PCR technique in the detection of samples incorrectly labelled, which might be because of fraudulent practices or incorrect labelling during the industrial manufacture. The undeclared presence of soybean can mislead the consumers and has negative implications concerning health, in the case of sensitive consumers to potential allergens, such as soybean.

Another achievement of the present work was the event-specific PCR detection of RR soybean in three of the samples positive for soybean DNA.

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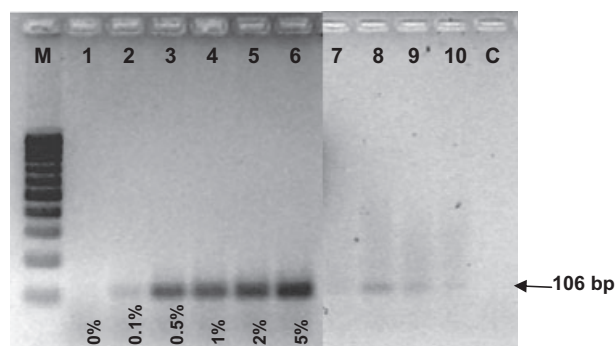


Figure 5 Agarose gel electrophoresis of PCR products targeting RR soybean (RRS-3J1/RRS-3J3) of certified reference materials (CRM, lanes 1–6) and meat sausages (lanes 7–10, samples 2, 3, 4 and 10, respectively). M, 100 bp ladder (Bioron, Ludwigshafen, Germany); C, negative control.

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