Polymerase chain reaction for the detection of allergens: the case of soybean in processed meat products

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Introduction

Since vegetable proteins are considerably cheaper than muscle proteins, they are frequently used as meat extenders to reduce the cost of the final product. Due to several interesting characteristics, soybean is reported to be the most widely used vegetable protein in the meat industry. Nevertheless, soybean is included in the group of 12 ingredients potentially allergenic, which should therefore be labelled according to the Codex Alimentarius FAO/WHO and the European Commission (Directive 2003/89/EC). It has been described that amounts of soy bellow 0.1% and 1% (w/w) can lead to allergic reactions in sensitive consumers [1]. The analytical methods used for soybean detection in foods rely mainly on protein and DNA analysis. However, it has been referred that protein-based methods can be less sensitive in the evaluation of thermally processed foods because of protein denaturation. Recently, the analysis of DNA coupled with polymerase chain reaction (PCR) presents a fast, sensitive and highly specific alternative to protein-based methods.

The aim of the present work was to develop PCR techniques able to detect the addition of soybean in highly processed meat products, such as Frankfurt sausages.

Material and methods

Sampling

Reference binary mixtures containing of soybean (w/w) in pork meat were prepared to a final weight of 100 g (Table 1). To evaluate the effect of thermal treatment, identical binary mixtures were prepared and submitted to heat treatment in an autoclave at 121ºC for 5 min. The samples included Frankfurt sausages (18 samples) purchased in local supermarkets. The samples comprised glass bottles and canned sausages, containing pork or poultry as the main meat ingredient.

DNA extraction

The DNA was extracted and purified using the Wizard® DNA Clean-up system (Promega) with minor modifications as described by Mafra et al. (2008) [2]. Concentration and purity of extracts were determined by UV spectrophotometry.

PCR amplification

DNA amplification was performed by PCR targeting the lectin gene as a marker for soybean, with the primers GM03 (GCC CTC TAC TCC ACC CCC ATC C) and GM04 (GCC CAT CTG CAA GCC TTT TGG TG) producing fragments of 118 bp. Samples whose bands were very light with GM03/GM04 primers were also amplified using LE1 (CAA AGC AAT GGC TAC TTC AAA G) and LE2 (TGA GTT TGC CTT GCT GGT CAG T) primers, producing shorter fragments (103 bp). The PCR components and amplification conditions are presented on tables 2 and 3, respectively

Table 1 – Percentages of pork meat and soybean protein used in reference binary samples analysed

Binary mixtures	Soybean (g)	Pork (g)	Total (g)	% Soybean
ps0	100	0	100	100
ps1	50	50	100	50
ps2	25	75	100	25
ps3	10	90	100	10
ps4	5	95	100	5
ps5	2.5	97.5	100	2.5
ps6	1	99	100	1
ps7	0.5	99.5	100	0.5
ps8	0.1	99.9	100	0.1
ps9	0	100	100	0

Table 2 – PCR Components

Components	Volume (µL)	
	LE1/LE2	GM03/GM04
Water	13.8	14.8
Buffer (10x)	2.5	2.5
$MgCl_2$ ()	2.0	1.5
dNTP (each)	2.0	2.0
Primers (each)	1.25	1.0
Taq Polimerase (5U/µL)	0.2	0.2
DNA extract	2.0	2.0
Total reaction volume	25.0	25.0

Table 3 – PCR Amplification conditions

Target	*Soybean*			
Primers	*LE1/LE2*		*GMO3/GMO4*	
Steps	**Temp.**	**Time**	**Temp.**	**Time**
Denaturation	94ºC	4 min	95ºC	5 min
Amplification	94ºC	30 seg	95ºC	30 seg
	60ºC	30 seg	65ºC	30 seg
	72ºC	30 seg	72ºC	1 min
Nº of cycles	35		37	
Final extension	72ºC	4 min	72ºC	5 min

Results

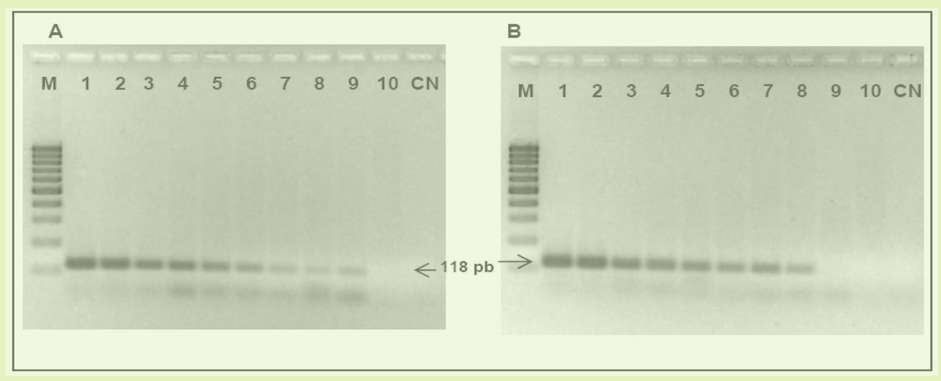

Figure 1 – Agarose gel electrophoresis of PCR products of soybean lectin gene of binary mixtures of pork meat and soybean textured protein in raw **(A)** and autoclaved during 5 min at 121ºC **(B)**. M: 100 bp ladder; lane 1: 100% soybean; lanes 2–9: 0.1%, 0.5%, 1%, 5%, 10%, 25%, 50%, 75% (w/w) of soybean in pork meat, respectively; lane 10: 100% pork meat; CN: negative control.

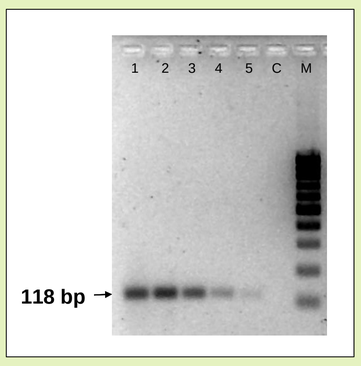

Figure 3 - Agarose gel electrophoresis of PCR products of soybean lectin gene of a 100% soybean extract serially diluted. Lane 1, 100 ng; lane 2, 10 ng; lane 3, 1 ng; lane 4, 0.1 ng; lane 5, 0.01 ng; C, negative control; M, 100 bp ladder.

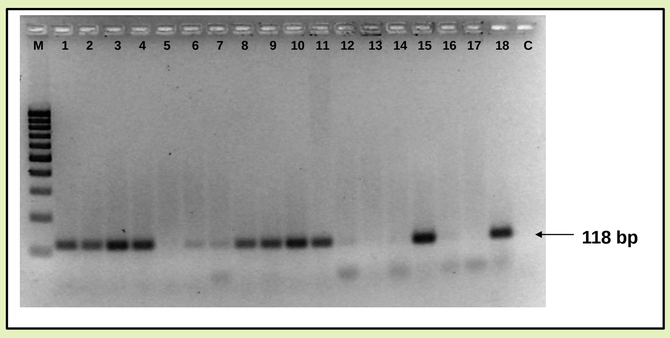

Figure 2 - Agarose gel electrophoresis of PCR products of soybean lectin gene of commercial Frankfurt type sausages (Lanes 1-18). M, 100 bp ladder; C, negative control.

Table 4 - Main and/or relevant ingredients of Frankfurt type sausages analyzed and PCR results for soybean lectin gene detection (GM03/GM04)

Sample	Protein declared		PCR
	vegetable	soybean	
1	X		++
2		X	++
3		X	+++
4		X	+++
5	X*		-
6	X*		+
7		-	+
8		X	++
9		X	++
10		X	+++
11	X		++
12	-		+
13	-		-
14	-		-
15	X		+++
16	-		+
17	-		-
18	X		+++

+++ strong positive; ++ medium positive; + weak positive; - negative; *hydrolyzed vegetable protein.

Conclusions

- ✓ The results showed that soybean detection was successfully achieved in all binary raw mixtures until the level of 0.1% (Fig. 1A) and 0.01 ng of DNA (Fig. 3).
- ✓ In autoclaved samples, the levels of amplification were observed for up 0.5% of soybean, probably due to DNA degradation induced by thermal treatment (Fig. 1B).
- ✓ The application of PCR technique to commercial Frankfurt type sausages evidences the presence of soybean in several samples (Table 4). Soybean was detected in all the 9 samples labelled as containing soybean protein. Sample 1, having vegetable protein as a declared ingredient, was also positive to soybean.

- ✓ Positive detection for soybean was also obtained for samples 7, 12 and 16, although soybean ingredients were not referred in those labels (Table 4). **This could represent a health concern for sensitive consumers.**
- ✓ It could be concluded that the developed PCR technique was successfully applied to 18 processed samples and that it could be used in routine analysis to detect the presence of soybean in compliance with the label statements.

References:

[1] Koppelman, S.J.; Lakemond, C.M.M.; Vlooswijk, R.; Hefle, S.L. (2004). Detection of soy proteins in processed foods: literature overview and new experimental work. J. AOAC Int. 87:1398-1407.
[2] Mafra, I.; Silva, S.A.; Moreira, E.J.M.O.; Silva, C.S.F.; Oliveira, M.B.P.P. (2008). Comparative study of DNA extraction methods for soybean derived food products, Food Control, 19:1183-1190.