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Food allergens: screening analysis of soybean and peanut by ELISA, real-time PCR and digital PCR

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Introduction

In Europe, soybean and peanut are two of the fourteen substances that must be labeled according to Regulation (EU) No 1169/2011 and checked using analytical methods that ensure adequate sensitivity and specificity.

The available methods for food allergens detection and quantification are proteomic, immunological and DNA-based tests such as mass spectrometry (MS), enzyme-linked immunosorbent assay (ELISA) and lateral flow devices, polymerase chain reaction (PCR), respectively, and related drawbacks (1-3).

Many studies about the detection of food allergens were focused on qPCR results, and there are no available data on ddPCR.

The aim of the study was to evaluate the performances of different screening methods for various food matrices.

Methods

Three different screening methods were used for various food matrices: ELISA, real-time PCR (qPCR), and droplet digital PCR (ddPCR),

The development of DNA-based tests consisted in several steps:

- ✓ evaluation of optimal test portion
- ✓ assessment and validation of DNA extraction method
- ✓ selection of target DNA sequences (four for both peanut and soybean)
- ✓ optimization and verification of qPCR methods
- ✓ application of selected protocols in ddPCR (one for peanut and two for soybean)
- ✓ analysis of market samples.

Results

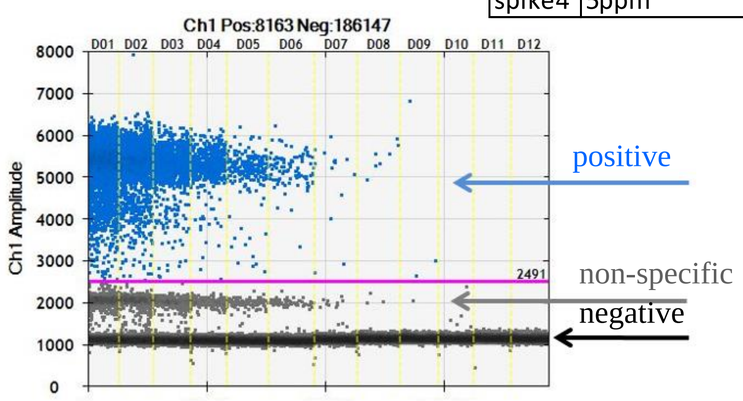
PCR results for soybean allergen show better sensitivity compared with those obtained with commercial ELISA kit for processed and unprocessed products.

	qPCR	ddPCR
+++	11-25 Cq	> 700 copy number
++	26-30 Cq	31-300 copy number
+	>31 Cq	< 30 copy number

* doubt

** diluted sample

		peanut				soybean					
		ddPCR	qPCR			ddPCR	qPCR				ELISA
market samples		**Arah2-PG**	**Arah2-PG**	**Biotecon**	**R-Biopharm**	**LE-PG**	**Glym5-PG**	**LE-PG**	**Biotecon**	**R-Biopharm**	**Euroclone**
ID	type	copy n.	mean Cq	mean Cq	mean Cq	copy n.	mean Cq	mean Cq	mean Cq	mean Cq	ppm
D1	peanut butter	+++**	+++	+++	+++						
D2	snack choco	++	++	+++	+++						
D3	roasted peanut	+++	+++	+++	+++						
C3	waffles					+++	+++	+++	+++	+++	+
A1	polenta (corn fluor)					+	+	+	++	+++	-
B1	baking powder					+	+	+	++	++	-
A2	breadcrumbs					+	+	+	++	+++	-
S1	polenta (corn fluor)					+	+	+	++	+++	+
S2	polenta (corn fluor)					+	-	-	++	++	-
S3	crackers					+*	+	-	++	++	-
S4	biscuits	-	-	-	-	-	-	-	-	+	-
spike1	10000ppm	++	++	+++	+++	+++	++	++	+++	+++	+
spike2	100ppm	+	+	++	++	++	++	+	+++	+++	+
spike3	10ppm	+	+	++	++	+	+	+	++	+++	-
spike4	5ppm	-	-	+	++	+	+	+	++	+++	-



The tested qPCR and ddPCR methods exhibit same sensitivity, furthermore the droplet digital PCR was useful to identify a non-specific soybean target, which was not detectable using real-time PCR with hydrolysis probes.

Discussion

The applied technologies can also provide quantitative results for peanut and soybean allergens, even though not required according to the current European legislation. Anyhow, a quantitative evaluation of the protein fractions eliciting allergic reactions could be useful and appropriate.

The results of this study show the potentiality of digital PCR as allergen analysis and provide a future chance to screen simultaneously several targets maintaining a high specificity, without losing sensitivity such as in multiplex real-time PCR.

Acknowledgments

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