Effect of thermal process on food allergen detection using ELISA Method

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Introduction

Food allergy is an increasing health problem of a potential high severity and of major concern for food manufacturers. European directive 2003/89/CE regulates food labelling and specifies that potentially allergenic food ingredients listed in its annex 3bis have to be labelled when present in food products.

The publication of this directive has lead to the development of allergen detection analysis mainly using immunological or molecular biology methods.

Among detection methods available for allergen testing in food products Enzyme-Linked ImmunoSorbent Assay (ELISA) is one of the most commonly used. This method is based on the recognition by antibodies, coupled with an enzyme, of proteins constitutive to allergenic ingredients. Many commercial ELISA kits reported to be usable on food matrices are available. The complexity of food products compositions and production processes may interfere with the performances of this method. This aim of this study is to evaluate the effect of thermal process on ELISA detection limit.

Methods

Three different "gluten-free" bakery products (brioche, cake, sandwich loaf) were prepared and contaminated in the dough before cooking with known levels with four different allergenic ingredients (wheat flour, peanut meal, milk powder and fresh egg). Allergen content in those ingredients was determined using the same ELISA kit than those used for samples analysis.

Brioche samples spiked with Peanut and Gluten were analysed before and after cooking using ELISA method. Sandwich loaf samples contaminated with egg and cake samples spiked with milk were analysed only after cooking.

All cooked samples were also analysed using in-house PCR methods.

Allergen	Peanut	Gluten	Egg white protein	Casein (cow's milk)
Kit name	Ridascreen Peanut	Transia Plate Gluten	Ridascreen Egg	Casein residue
Manufacturer	R-Biopharm	BioControl	R-Biopharm	Elisa Systems
Cat N°	R6201	GL0301	R6401	ESCASRD-48
Detection limit	2,5ppm peanut	10ppm gluten	2ppm egg white proteins	0,5ppm casein

Table N°1: Commercial ELISA Kits description. Detection limits are those reported by kits manufacturers. (ppm = mg/kg)

Allergen	Presentation	[analyte] determined using ELISA Kits
Peanut	Peanut meal	111% peanut
Gluten	Wheat flour	11% gluten
Egg	Fresh egg	9% Egg White proteins
Milk	Skim milk powder	38% Casein

Table N°2: Presentation of allergenic ingredients used for dough contamination. Allergen concentration determined using ELISA methods applied to food product analysis.

Results

A: Peanut

Method	[Peanut meal] ppm	10000	1000	500	100	50	25	12,5	5	1	0	LD reported by kit manufacturer
	[Peanut] ppm	11100	1110	555	111	55,5	27,8	13,9	5,6	1,1	0	
ELISA	Brioche dough	+	+	+	+	+	+	+	-	-	-	2,5ppm peanut
	Cooked brioche	+	+	+	+	+	-	-	-	-	-	
PCR	Cooked brioche	+	+	+	+	+	+	+	-	-	-	

B: Gluten

Method	[Wheat Flour] ppm	10000	1000	500	100	50	25	12,5	5	1	0	LD reported by kit manufacturer
	[Gluten] ppm	1100	110	55	11	5,5	2,8	1,4	0,6	0,1	0	
ELISA	Brioche dough	+	+	+	+	+	-	-	-	-	-	10ppm Gluten
	Cooked brioche	+	+	+	+	-	-	-	-	-	-	
PCR	Cooked brioche	+	+	+	+	+	+	+	+	-	-	

C: Egg

Method	[Fresh egg] ppm	10000	1000	500	100	50	25	12,5	5	1	0	LD reported by kit manufacturer
	[Egg white proteins] ppm	860	86	43	8,6	4,3	2,2	1,1	0,4	0,1	0	
ELISA	Cooked Sandwich Loaf	+	-	-	-	-	-	-	-	-	-	2ppm egg white proteins
PCR	Cooked Sandwich Loaf	+	+	+	+	+	+	-	-	-	-	

D: Milk

Method	[Skim milk powder] ppm	10000	1000	500	100	50	25	12,5	5	1	0	LD reported by kit manufacturer
	[Casein] ppm	3750	375	187,5	37,5	18,8	9,4	4,7	1,9	0,4	0	
ELISA	Cooked cake	+	+	+	+	-	-	-	-	-	-	0,5ppm casein
PCR	Cooked cake	+	+	+	+	+	+	+	-	-	-	

Table N°3: Thermal effect on ELISA detection of peanut (A) or gluten (B) in brioche samples and comparison of ELISA and PCR methods applied to peanut (A), gluten (B), egg (C) and milk (D) detection. Allergen amounts introduced in samples are expressed in terms of weight of allergen ingredient per food product total weight . Based on results of ELISA determination (Table N°2), these amounts have been converted into ELISA kit unit. (+): OD are significantly different than those observed for unspiked samples (0ppm) or DNA amplification. (-): OD are not significantly different than those observed for unspiked samples or no DNA amplification.

The results show that, except for gluten, none of the detection limits specified by kits manufacturers were achieved in cooked samples, detection limits in cooked products were 10 times (peanut) to 400 times (egg) higher. In case of peanut, it is also not achieved in crude dough. These discrepancies are probably essentially due to thermal process, as compared with PCR results, but may also be due to matrix effect: interferences between food products ingredients and either immunological or enzymatic reactions involved during ELISA testing.

Conclusion

The effect of thermal processes during food products manufacturing, may be damaging to allergen detection limits achieved by ELISA method. It has to be reminded that degradation affecting proteins targeted by antibodies in ELISA testing is not a guarantee that allergenic proteins are also degraded and would not give adverse reactions to allergic consumers through remaining epitopes.

Therefore it is crucial to evaluate ELISA performances, especially detection limit, using reference materials that include the process effect.

De facto, ELISA methods dedicated to cooked products analysis have to be validated using incurred samples.

Bibliography

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