PTs for gluten detection in artificially contaminated food: comparative analysis between spiking levels and assigned values

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INTRODUCTION

Food allergies are increasing worldwide and becoming a public health concern [1]. Due to the importance of the detection and/or determination of allergens in food, the number of laboratories interested in these analyses has gradually increased in recent years. The main purpose of these analyses is to ensure higher quality of allergen-free food, allowing the detection of allergens both during the different steps of production and in the final products. However, allergens determination is a challenge for laboratories, since food allergens are more or less denatured mixtures of non-defined proteins in complex matrices and their quantification is intrinsically related to the food preparation techniques: for processed food the extraction of denatured or altered proteins tends, in fact, to be difficult, due to their reduced solubility as compared to native proteins [2]. The objective of this work was to determine differences in the detection of gluten in artificially contaminated food, between baked matrices and non-baked ones. 13 proficiency testing schemes (PTs) has been organized using infant flour and cake samples spiked in gluten gathering an average of more than 20 laboratories. Infant flour samples were spiked with calibrated amount of wheat flour and sent to the laboratories without any heating process. Conversely, cake samples were prepared including a defined gluten content in the recipe before the cooking process and then baked. For each test, a statistical treatment of the data was performed according to ISO 13528 [3] and assigned (consensus) values were calculated from the participants' results obtained performing the enzyme linked immunosorbent assay (ELISA) technique. Assigned values of infant flour and cake samples were compared to the theoretical ones (spiking levels) to evaluated recovery rates.

MATERIALS and METHODS

Sample preparation:

- Infant flour samples were prepared spiking an allergen free flour (rice) with gluten in well controlled proportions. This flour was then homogenized and divided into series of samples using a carousel in association with a mixer.
- Cake samples were made using a mix of gluten free flours (rice flour, corn, potato and manioc starches, guar meal), sugar, egg, butter, milk, sodium bicarbonate and potassium tartrate. The targeted allergen was added during the paste preparation step, before baking. The prepared cakes were ground into fine powders and divided into series of samples using a carousel.

The theoretical gluten concentrations of all the manufactured batches (between 0 and 100 ppm) are indicated in Tables 1 and 2.

After the production and before the shipment to the laboratories, the homogeneity and stability of the samples for the duration of the test were checked, according to requirements of the ANNEX B of ISO 13528 standard [3].

Analyses:

The laboratories that participated in PTs were required to return their results, expressed as gluten content, in mg of gluten *per* kg of raw product, via a dedicated website after a period of 3 weeks, specifying the ELISA kit used for the gluten detection.

Statistical treatment:

The statistical treatments were conducted according to ISO 13528 standard [3]. The assigned values (x_{pt}) were estimated using the means of all results (except incoherent ones), obtained from the application of robust algorithm A.

RESULTS and DISCUSSION

Statistical data and gluten recovery rates of the tests on infant flour								
X mg/kg	x_{pt} mg/kg	$u(x_{pt})$ mg/kg	$s(x_{pt})$ mg/kg	$p(x_{pt})$	$CV(x_{pt})$ %	p_{CA}	p_{NQ} %	Recoveries %
-	-	-	-	-	-	13	85	-
15	22	1	4	22	18	22	0	147
41	36	4	11	14	31	15	7	88
47	50	5	14	11	28	14	7	106
97	98	14	35	10	36	13	23	101
102	87	6	18	14	21	20	30	85

Table 1. Summary of the statistical treatment of the data and recovery rates of gluten of the performed PT on infant flour (non-baked matrix)

Statistical data and gluten recovery rates of the tests on cake								
X mg/kg	x_{pt} mg/kg	$u(x_{pt})$ mg/kg	$s(x_{pt})$ mg/kg	$p(x_{pt})$	$CV(x_{pt})$ %	p_{CA}	p_{NQ} %	Recoveries %
-	-	-	-	-	-	23	83	-
27	19	1	4	33	21	34	0	70
30	25	1	6	25	24	26	4	83
38	32	2	9	32	28	34	0	84
58	23	1	5	20	22	20	0	40
86	41	3	11	19	27	19	0	48
90	62	4	16	22	26	26	12	69

Table 2. Summary of the statistical treatment of the data and recovery rates of gluten of the performed PT on cake (baked matrix)

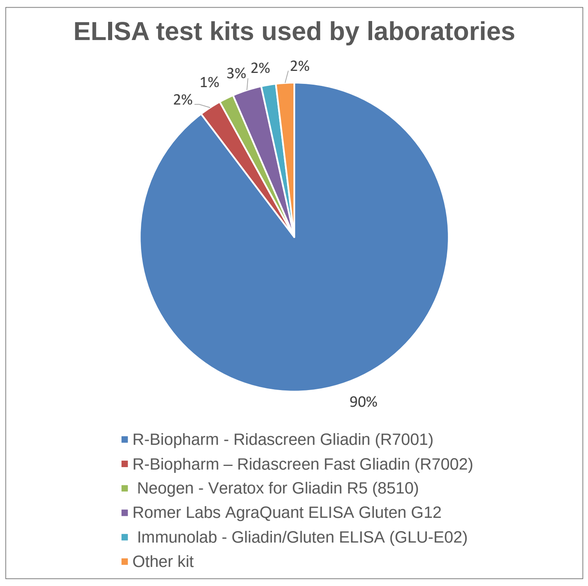

Figure 1. Distribution of Elisa test kits used by laboratories for detection and quantification of gluten

TABLE LEGEND	
X	Theoretical concentration in the matrix. The final concentrations of gluten in cake samples were calculated considering moisture loss during baking.
x_{pt}	Assigned value or conventionally true value, calculated by the robust algorithm A from ISO 13528 standard.
$u(x_{pt})$	Standard uncertainty of the assigned value; this value permits to quantify the confidence that can be given to the assigned value. It depends on the mathematical model applied (algorithm A) and is a function of the standard deviation and the number of results used for the estimation of the assigned value. It is calculated as indicated in § 5.6.2 of ISO 13528 standard.
$s(x_{pt})$	Robust standard deviation of the results, calculated by the robust algorithm A from ISO 13528 from all the results which participated to the estimation of the assigned value.
$p(x_{pt})$	Number of results taken into account for the estimation of the assigned value.
$CV(x_{pt})$	Coefficient of variation, this value permits to measure the dispersion of the results.
p_{CA}	Total number of returned results (including incoherent and qualitative ones)
p_{NQ}	Percentage of non quantitative results.

Statistical parameters and recovery rates of each test are given in Tables 1 and 2.
Assigned values were estimated for all trials except for both non spiked samples because more than 80% of the participants indicated a qualitative result (as detection or quantification limit). Uncertainties, that quantify the confidence to the assigned values, are good and vary from 1 to 14 mg/kg, according, among other factors, to the allergen concentration and number of results taken into account to estimate the value. Coefficients of variation, reflecting the dispersion of the results as a function of the assigned value, were satisfactory for these trials, ranging from 18% to 36% according to the test. This shows a good consistency of the results from one laboratory to another, whatever the matrix and the contamination level.
Recovery rates of gluten in infant flour samples were higher than 85% for each performed test. Rather the contrary, recovery rates of this allergen in processed cakes varied between 40% and 80%. This bias between baked and non-baked samples is described graphically in Figure 2. The data also show that there is no link between analyte concentrations and recovery rates.

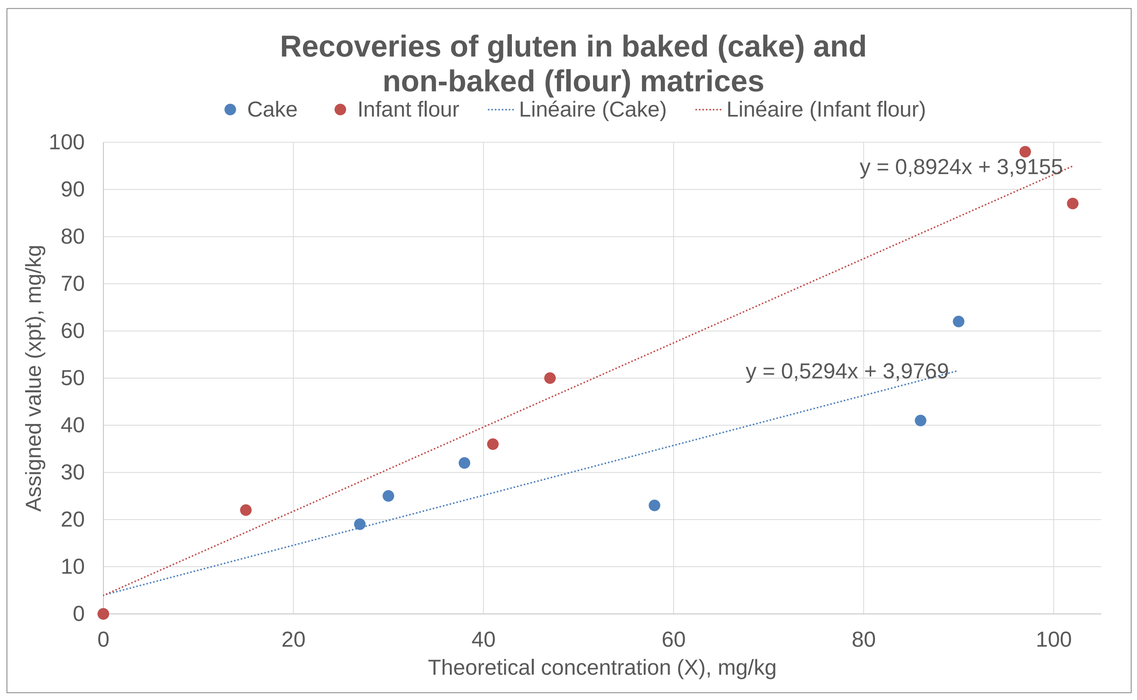

Figure 2. Recoveries of gluten in backed (cake) and non-backed (infant flour) matrices

Concerning ELISA kits used to perform the analysis, the majority of the laboratories use the R-Biopharm - Ridascreen Gliadin (R7001) one (Figure 1).

CONCLUSION

These results show that gluten proteins recovery rates are higher for infant flour samples than for cake ones. An average loss of 20% is observed in baked matrices (cake). This study confirms that thermal treatment is an important factor that can affect gluten quantification [2]. This can be a real problem within the framework of processed food quality control exposing celiac consumers at risk and laboratories need to be warned. Participating in proficiency tests can enable participants to optimize their analytical procedures to improve their performances for gluten quantification in thermally processed foods.

REFERENCES

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[2] Gomaa, A., Boye, J. I., 2013, Impact of thermal processing time and cookie size on the detection of casein, egg, gluten and soy allergens in food. Food research international, 52, 483-489.
[3] ISO 13528 - Statistical methods for use in proficiency testing by interlaboratory comparisons.