Proficiency-testing scheme using true samples for Gluten detection in processed food

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INTRODUCTION

Food allergies represent a significant health problem in industrialized countries. Detection and quantification of allergens in food are essential to guarantee compliance with food labelling and to ensure costumer protection. The number of laboratories performing allergens analyses has gradually increased in recent years: many analytical methods have been developed and some of them have become commercially available in kit formats [1]. However, the allergens detection in processed foods remains a challenge for the laboratories: the extraction of denatured or altered proteins tends in fact to be difficult due to their reduced solubility as compared to native proteins [2]. According to the requirements of ISO 17043 standard [3], laboratories participating in a proficiency testing scheme (PTS) must operate under routine conditions and analyze samples as close as possible to the real ones. To meet this requirement, BIPEA set up a PT intended to the detection and quantification of gluten in cakes. The samples were made by preparing cakes including in the recipe wheat flour in well controlled proportions before the cooking process. Intrinsically contaminated cakes were obtained, closer to the reality than samples to which gluten is artificially added after the cooking process.

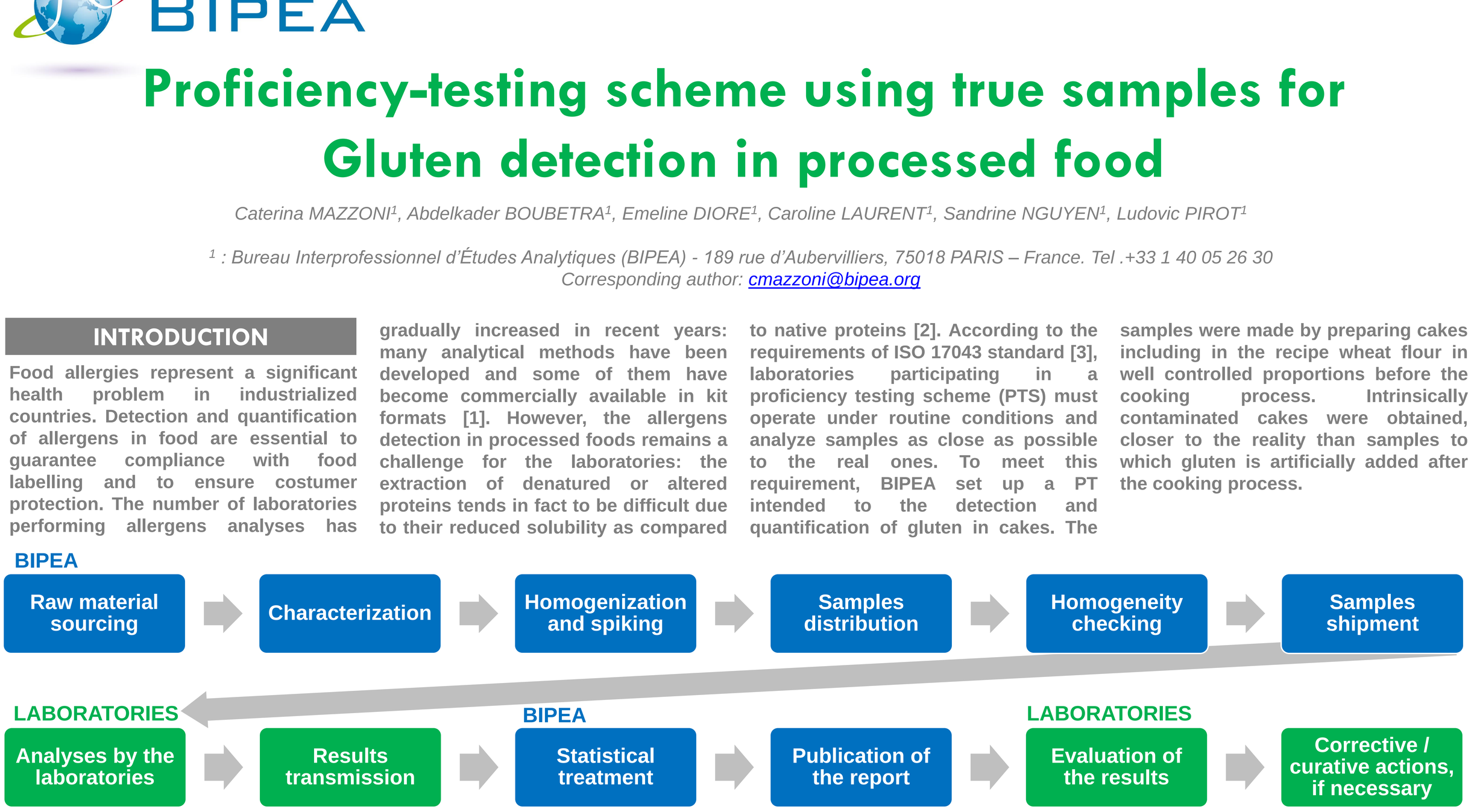

Figure 1 - Organisation of the PTS by BIPEA

SAMPLES PRODUCTION

The samples were made by preparing cakes using a mix of gluten free flours (rice flour, corn, potato and manioc starches, guar meal), sugar, eggs, butter, milk, sodium bicarbonate and potassium tartrate. The target allergen was added in well controlled proportions during the paste preparation step, before baking the cakes. The prepared cakes were ground into fine powders and divided into series of samples using a carousel.

SAMPLES SHIPMENT

Three samples of 300 g were sent to the 24 laboratories participating in the test: two out of them were prepared with different concentrations of gluten and one was a negative sample. The theoretical allergen concentrations in the two samples containing gluten are indicated in Table 1.
The period granted to the laboratories for analyzing and submitting their results was of 4 weeks.

HOMOGENEITY and STABILITY CHECKING

After the production and before the shipment, BIPEA proceeded to homogeneity and stability checking, according to requirements of the ANNEX B of the ISO 13528 standard [1]. The analyses were performed by an accredited laboratory using ELISA method.

For the **homogeneity** checking,10 samples were selected from the production following a regular step and were analyzed *in duplo* in random order. The results were studied through several statistical tests:

-Fisher test (variance analysis): observed F value < critical F value;

-Test of significant inhomogeneity: between sample variance < critical c value;

-Study of the ratio of between samples standard deviation/standard deviation for proficiency assessment: s_s/SDPA < 30%.

The **stability** was verified analyzing *in duplo* 3 samples stored at (5±3) °C for a period of 8 weeks. According to ISO 13528 standard [1], the samples can be considered stable if the absolute difference between the means at t_0 and t_1 is inferior or equal to the 0,3*standard deviation for proficiency assessment ($|\grave{y}_0 - \grave{y}_1| \leq 0.3*SDPA$).
The treatment of the analyses' results showed a sufficient homogeneity and stability of the samples for the analysis period granted to the laboratories (Figure 2).

STATISTICAL TREATMENT

The statistical treatments were conducted according to **ISO 13528 standard [4]**. The assigned values (X) were estimated using the robust means of the results. The proficiencies of each laboratory were evaluated thanks to tolerance values (TV) of twice the standard deviations.

The results (x) could be evaluated and classified through z-scores:

- $z \leq |2|$: satisfactory
- $|2| < z \leq |3|$: questionable
- $z > |3|$: unsatisfactory.

where :

$$z = \frac{x - X}{\frac{TV}{2}}$$

RESULTS and DISCUSSION

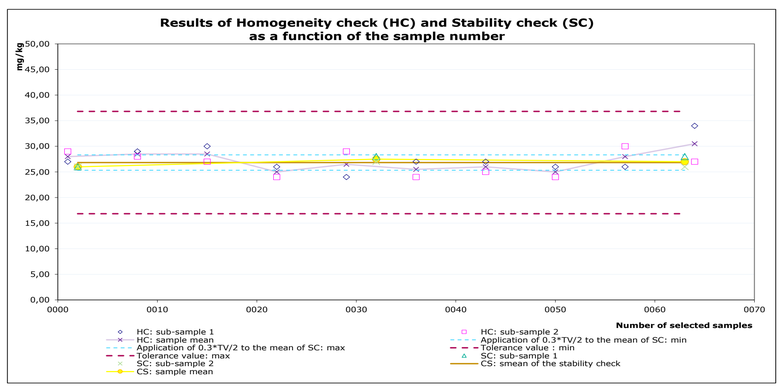

Fig. 2 – Homogeneity and stability checks as a function of the sample number (low concentration)

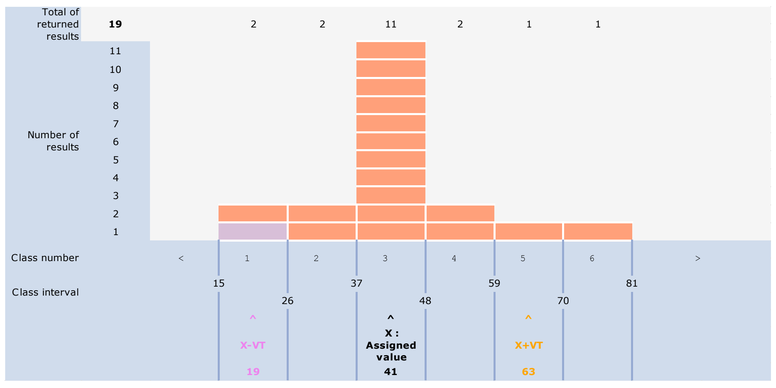

Fig. 3 – Spiked sample 2: distribution of the results obtained by the ELISA kit method (mg/kg)

Twenty-two laboratories on average participate in this interlaboratory test.
For the negative sample, 19 laboratories gave their results as quantification limit. Two laboratories detected and quantified the gluten in the sample (false positive).
Table 1 summarizes the obtained results for the two positive samples. For this proficiency test, the major part of the laboratories performed Elisa method; only two laboratories applied PCR method. Assigned (consensus) values were calculated from the participants' results using ELISA protocol and the performances of these laboratories have been evaluated individually and collectively according to ISO 17043 standard [3].
Figure 3 shows the distribution of the results as histogram for one of the positive samples (high concentration level). Assigned values and the tolerance intervals are indicated in the horizontal axes. The results represent unimodal statistical distribution for this sample with 3 laboratories outside of the tolerance interval. Same results were obtained for the second sample (low concentration level).

General information	**Spiked sample**	**1**	**2**
	Theoretical gluten concentration (mg/kg)	58	87
	Participants - p_{tot}	24	24
	PCR results	2	2
	ELISA KIT results	21	20
Assigned Value: ELISA KIT method	Assigned value (mg/kg)	23	41
	Standard uncertainty of the assigned value - u_x (mg/kg)	1	3
	Standard deviation - s^*_x (mg/kg)	5	11
	Number of results- p_x	20	19
	Coefficient of variation - CV_x (%)	22	27
Proficiency: ELISA KIT method	Standard deviation for proficiency assessment- SDPA (mg/kg)	5	11
	Tolerance value - TV = 2 x SDPA (mg/kg)	10	22
	Number of untrue results - p_D	3	3
Total population: ELISA KIT method	Robust mean- x^*_{tot} (mg/kg)	23	41
	Robust standard deviation- s*tot (mg/kg)	5	11
	Coefficient of variation - CV_{tot} (%)	22	27

Table 1 – Statistical treatment of the results (positive samples)

CONCLUSION

The results obtained for this interlaboratory test were good for the major part of the laboratories (for the two positive samples, only 3 results were out of the tolerance intervals). The recovery rates were satisfactory considering the baking step (between 40% and 47%). This test is part of an annual proficiency testing scheme, allowing a real long-term follow-up of the laboratories' results for the different allergens. The interlaboratory comparisons are a good tool of quality management and can be used to follow the performances of the laboratories, highlighting drifts or recurring analytical difficulties, which are a first step before the implementation of corrective/curative action.

REFERENCES

[1] Poms, R. E., Klein, C. L. and Anklam, E., 2004, Methods for allergen analysis in food: a review. *Food Additives and Contaminants*, 21, 1-31.
[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 17043** - Conformity assessment - General requirements for proficiency testing
[4] **ISO 13528** - Statistical methods for use in proficiency testing by interlaboratory comparisons.