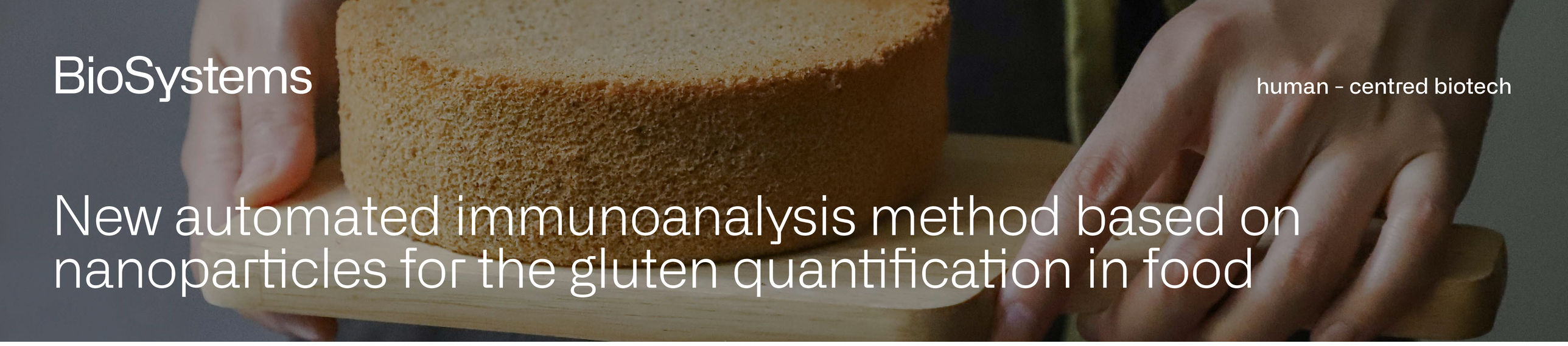

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New automated immunoanalysis method based on nanoparticles for the gluten quantification in food

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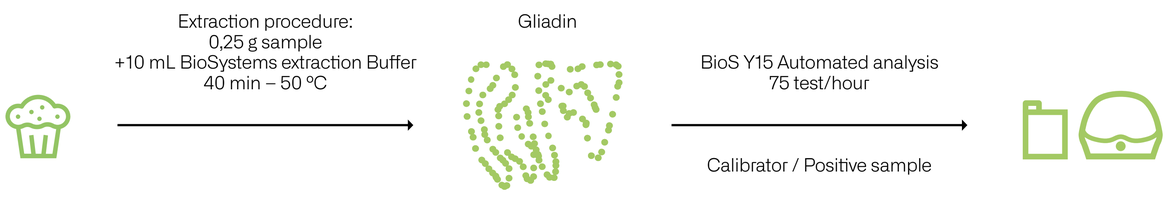
Introduction

Celiac Disease is one of the most common food intolerances which can affect up to 1% of the population. It comes along with serious damage of the mucosa in the small intestine and is caused by the storage proteins—called "gluten"—of wheat, barley, and rye. Sensitive individuals need to stick to a strict gluten-free diet so, the correct quantification and labeling of foods are crucial for following a gluten-free diet. There are different analysis methods commercially available to determine the presence of gluten, being the lateral-flow assays and ELISA the most used. Both are based on the antigen-antibody reaction. Rapid tests are user-friendly but lack quantification capabilities, while ELISA offers quantitative results but is prone to errors due to its less automated process. This work presents a new method for the quantification of gluten, based on an automated turbidimetric immunoassay on a BioSystems Y15 spectrophotometric analyser.

Technical basis

The **BioSystems Gluten test** is based on the immunoturbidimetry technique and uses latex nanoparticles coated with a monoclonal antibody developed by BioSystems. The antibody recognises specifically the **33-mer fragment** of gliadin, known for its high toxicity in people with celiac disease.

When the coated nanoparticles react with the gliadin present in sample cause an increase in the turbidity of the medium that is monitored by photometry. The increase in absorbance is proportional to the concentration of gluten in the sample.



To release the gliadin present in the sample, the **BioSystems extraction buffer** has been developed. It consists in an innovative and environmentally friendly buffer and free from hazardous substances. The sample's extract is added to the automatic BioSystems Y15, which reduces errors during the execution of the analysis and allows obtaining results directly in mg/kg (or mg/L) of gluten in **10 minutes.**

Results - Product performance

The data have been obtained on a BioSystems Y15 analyser. The validation has been conducted according to the criteria established by the Association of Official Analytical Collaboration (AOAC) International.

Measurement range - Linearity

The measurement range of the kit is from 2,5 to 40 mg/kg of gluten (1,25 to 20 mg/kg of gliadin).

	Results			
Assigned value (mg/kg)	Mean (5 repl.)	Sr	RSDr (%)	Bias (mg/kg)
0	0.3	0.04	-	
4	3.52	0.11	3.0	-0.48
8	7.47	0.29	3.9	-0.53
12	11.87	0.33	2.8	-0.13
16	16.03	0.33	2.0	0.03
20	19.41	0.50	2.6	-0.59
24	25.06	0.80	3.2	1.06
32	33.63	1.85	5.5	1.63
40	38.15	1.35	3.5	-1.85

Recovery, precision, limit of detection (LoD) and limit of quantification (LoQ)

The precision and recovery studies have been carried out with four matrices contaminated with 4 levels of gluten (0, 5, 10 and 20 mg/kg) from different sources (wheat, barley and rye). The table below summarizes the obtained results and the LoD and LoQ.

			Results						
Matrix	Contaminant	Target contamination level	Mean concentration obtained (mg/kg)	Recovery (%)	Bias	Sr	RSDr (%)	LoD	LoQ
Corn flour	Wheat flour	0	0.3	-	0.26	0.18	69.0	0.9	2.1
		5	5.9	118	0.90	0.63	10.7		
		10	10.7	107	0.74	1.17	10.9		
		20	19.5	97	-0.54	1.64	8.43		
Rice flour	Wheat flour	0	1.6	-	1.57	0.17	10.8	2.10	3.3
		5	7.9	158	2.91	0.73	9.25		
		10	13.1	131	3.11	1.96	14.9		
		20	20.6	103	0.56	2.40	11.7		
Wine	Wheat flour	0	0.1	-	0.11	0.10	87.0	0.4	1.1
		5	5.0	100	0.01	0.17	3.46		
		10	9.6	96	-0.41	0.32	3.39		
		20	20.8	104	0.83	1.66	7.99		
Sausage	Wheat flour	0	0.3	-	0.30	0.22	73.5	1.0	2.5
		5	4.1	82	-0.89	0.33	7.98		
		10	8.4	84	-1.60	1.31	15.6		
		20	19.9	100	-0.08	2.08	10.5		
Corn fluor	Barley flour	0	0.6	-	0.63	0.08	12.3	0.9	1.4
		5	5.5	109	0.45	0.85	15.6		
		10	10.8	108	0.83	1.47	13.6		
		20	22.1	111	2.15	2.54	11.5		
Sausage	Barley flour	0	0.2	-	0.19	0.09	49.6	0.5	1.1
		5	4.9	99	-0.05	0.27	5.5		
		10	11.0	110	1.00	1.25	11.4		
		20	24.7	123	4.65	3.45	14.0		
Corn flour	Rye flour	0	0.1	-	0.14	0.06	42.7	0.3	0.7
		5	9.9	199	4.94	0.76	7.64		
		10	21.1	211	11.13	1.27	6.01		
		20	43.8	219	23.76	3.22	7.37		

Reference materials, selectivity and method comparison

Different Quality Control samples (FAPAS®) with a known gluten concentration have been evaluated with the BioSystems Gluten Test. The new method shows an excellent capacity for quantification of gluten in the evaluated samples. The results are shown in the table below.

		Quantitative results (mg/kg)			
		R-Biopharm	BioSystems Gluten Test		
Reference	Matrix	Assigned value (range for |z| ≤2)	Mean (N=5)	Sr	RSDr (%)
27247B	Cake mix	19.3 (9.6-28.9)	12.1	0.13	1.06
27252A	Oat based foodstuff	16.6 (8.3-24.9)	18.3	1.16	6.34
27262	Cooked biscuit	76 (38-114)	90.2	5,.4	5.70
27264B	Infant soya formula	24.8 (12.4-37.2)	37.8	0.46	1.22
27271A	Cake mix	39.2 (19.6-58.8)	38.2	1.15	3.00
27275A	Cake mix	14.9 (7.5-22.4)	17.8	0.57	3.19
T27301BQC	Cake mix	16 (8.0-24.0)	17.0	1.33	7.86
T27314QC	Cooked Biscuit	38.8 (19.4-58.1)	55.1	1.52	2.75
T27331AQC	Cake mix	13.9 (6.9-20.8)	16.6	1.03	6.23
T27331BQC	Cake mix	5.53 (2.76-8.29)	4.1	0.22	5.40

In order to confirm the ability of the new method to correctly quantify gluten in different matrices existing on the market, more than 200 different samples with gluten and gluten-free have been evaluated and compared with the AOAC reference method (Ridascreen® Gliadin, R-Biopharm).

	Results (mg/kg)	
	ELISA R-biopharm	BioSystems
Chickpeas Fusilli	<LoQ	<LoQ
Chips cheese & onion	26.9	25.5
Peanut butter Gluten Free	<LoQ	<LoQ
Cereals: corn balls and honey	143	113.7
Soy Sauce Gluten Free	7.4	<LoQ
Ground fennel	30	38.3
Broth for paella	19	13.9
Elver substitute	<LoQ	<LoQ
Sunflower seed with salt-water	<LoQ	<LoQ
Rinse water	<LoQ	<LoQ
Rinse water + 12 mg/kg gluten	12.0	16.6
Ham croquette	<LoQ	<LoQ
Ham croquette + 20 mg/kg gluten	20.0	23.6
Marinated loin with garlic	<LoQ	<LoQ
Mussel in tomato sauce + 40 mg/kg gluten	40.0	34.0
Boar's head sausage	<LoQ	<LoQ
Boar's head sausage + 12 mg/kg gluten	12.0	12.9

In addition, some gluten-free samples, spiked at different levels of gluten, have been evaluated obtaining excellent recovery gluten values. Some of the obtained results are shown on the table.

A selectivity study to ensure that the BioSystems Gluten method does not cross-react and produce false-positive results when tested on common commodities has been carried out (AOAC guidelines). At the same time, the study demonstrates the new method's ability to detect target compounds, gluten and gliadin, without interference from the common commodities when the matrices were spiked at 10 mg/kg using wheat flour. As summarizes on the table, the method performed as expected with the selected food matrices ad recoveries fall within the 70-120% range.

	Result gluten (mg/kg)		
	ELISA R-biopharm	BioSystems	
		un-spiked	10 mg/kg
Almond	<LoQ	<LoQ	8.6
Pork meat	<LoQ	<LoQ	7.0
Chicken meat	<LoQ	<LoQ	7.6
Guar gum (1:10)	<LoQ	<LoQ	8.2
Parsley flakes	<LoQ	<LoQ	11.6
White bean flour	<LoQ	<LoQ	11.3
Peanut	<LoQ	<LoQ	7.3
Coconut flour	<LoQ	<LoQ	8.6
Cumin	<LoQ	<LoQ	8.4
Ground coffee	<LoQ	<LoQ	9.8
Skimmed Milk	<LoQ	<LoQ	10.4
Instant Chocolate Gluten Free	<LoQ	<LoQ	10.9
Condensed milk	<LoQ	<LoQ	8.7
sesame	<LoQ	<LoQ	9.7
Dried red cranberries	<LoQ	<LoQ	8.4
Textured soybean	<LoQ	<LoQ	7.1
Teff flour	<LoQ	<LoQ	8.5

Conclusions

The BioSystems Gluten test together with the developed extraction solution is a useful tool for automated, fast, precise, and simple determination of gluten. Automation of the measurement in the BioSystems Y15 analyser improves the precision and accuracy of the results, eliminating possible measurement errors by the user, and allows for great flexibility in the analysis of samples, from a few to high throughput of 75 samples per hour.

References

Official Methods of Analysis (2016) 20th Edition, AOAC INTERNATIONAL, Rockville, MD, Appendix F/K.