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Validation of a Novel Method Based on Immunoturbidimetry for Determination of Gluten in Food and Beverage Matrices

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Introduction

Gluten-related disorders are the most common food intolerances which can affect up to 1% of the population. They come along with serious damage of the mucosa in the small intestine 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 food labeling are crucial. 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 analyzer.

Technical Basis

The BioSystems Gluten method represents a significant advancement over existing gluten quantification methodologies by employing an alternative antigen–antibody interaction approach based on immunoturbidimetry, integrated into a fully automated analytical platform. This method utilizes nanoparticle–conjugated (specific for the 33-mer fragment of gliadin) suspended in a liquid reagent that agglutinate in the presence of gluten, forming a network detectable via photometric measurement.

The system automates reaction steps—reagent and sample pipetting, incubation, and reading—within a single rotor-based incubation phase, which reduces errors during the execution of the analysis and allows obtaining results directly in mg/kg (mg/L) of gluten in 10 minutes (first result and subsequent results every 48 seconds). No washing steps or frequent calibrations are needed.

The extraction protocol is simplified to a single-step, single-temperature and reduced extraction time procedure. The use of a proprietary Extraction Solution (Ref. 31003) eliminates the need for hazardous chemicals or specialized equipment such as a fume hood. This Extraction Solution has demonstrated compatibility with complex matrices containing polyphenols—such as chocolate, wine, or legumes—without requiring additional sample pretreatment.

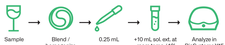
BioSystems has also developed a Gluten Spike Solution (Ref. 31002) to get internal controls. It has a concentration of 250 mg/L of gluten and is traceable to the PWG-gliadin standard (Prolamin Working Group). The Spike Solution can be used diluted in the Extraction Solution or directly added to the matrix in order to evaluate recoveries.

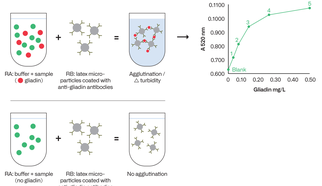
Kit components

Reagents (A and B) | Standards (31 to 55) | Gluten Spike Solution | Gluten Extraction Solution

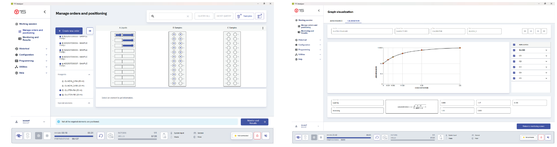
Solids extraction:

Liquids extraction:





BioSystems Y15 Analyzer

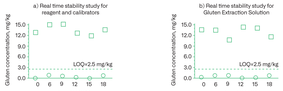


Schematics of the BioSystems Y15 Analyzer software for gluten analysis.

Stability Studies

Real time stability Study

The real time stability study was conducted using five blank test portions (corn flour) and five test portions of corn flour artificially contaminated with wheat flour at 10 mg/kg. For the study, reagents/calibrators and extraction solution aged in real time for 6, 9, 12, 15, and 18 months were used. In total, four different lots of reagent and standards, and three different lots of Gluten Extraction Solution, were tested throughout the study.



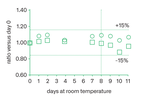
Gluten stability in the extracted sample

Gluten stability in extracted sample study was conducted using two matrixes with known gluten concentrations. The resulting extracts were stored at room temperature (20-22 °C) and the gluten concentration was analyzed up to 11 days.

The obtained results showed that the sample extract remains stable at room temperature (20-22 °C) when stored in a sealed tube with a screw cap for at least 8 days, with no deviations exceeding 15%.

Across all evaluated time points, gluten concentrations below the LOQ were observed in the blank (0 mg/kg) samples. For the 10 mg/kg gluten samples, an average recovery of 132% was obtained with respect to the target value (105% with respect to the obtained value on day 0), with an average repeatability of 10%.

The real time stability data showed that both the reagents/standards and the Gluten Extraction Solution maintained proper functionality and a shelf-life of 18 months can be assumed.



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Method Validation

The method was validated according to new AOAC Guidelines for Quantitative Gluten Methods (2024) [1], using non-processed and incurred matrices artificially contaminated with gluten from wheat, barley, and rye, at concentrations from 0 to 30 mg/kg gluten. The studies were performed mostly by the method developer, while the Independent laboratory performed the study for the rice cookies incurred with wheat, barley and rye.

Food Matrix Study

For each matrix and contamination level, twelve aliquots of each sample were weighed into screw-capped tubes, as described in the package insert. All replicates were blindly coded and randomized. The data collection approach was carried out with two analysts over two days using three BioSystems Gluten lots. Each test portion was analyzed in duplicate following the design 2b described in the Guidelines.

Food matrix study	matrix	Gluten contaminant source			Target contamination level
		Wheat flour	Barley flour	Rye flour	
Non-processed matrices	Corn flour	✓	✓	✓	0, 5, 10, 20 mg/kg gluten
	Rice flour	✓			
	Sausage	✓	✓		
	Wine	✓			
Incurred matrices	Cornbread	✓	✓	✓	0, 10, 20, 30 mg/kg gluten
	Rice cookies	✓	✓	✓	

The data obtained from this study were used to determine the intermediate precision, repeatability, recovery and product consistency. The data were also used for limit of detection (LOD) and limit of quantification (LOQ) estimation of the BioSystems Gluten test method.

Precision statistics were calculated based on the nested design of the Guidelines for Validation of Quantitative Gluten Methods (Annex D). The factors, in order of nesting, were Lot, Day/Analyst (confounded), and Test Portion/Extraction. Statistics were calculated using R and RStudio software with the VCA package available in R-CRAN. Intermediate precision, LOD and LOQ were calculated following the Guidelines.

BioSystems Gluten Results for non-processed matrices

Matrix	Contaminant	Target contamination level, mg/kg	Mean (n=24) mg/kg	Recovery (%)	Bias mg/kg	S_r mg/kg	RSD$_r$ (%)	S_R mg/kg	RSD$_R$ (%)
Corn flour	Wheat flour	0	0.242	-	0.242	0.15	63.2	0.16	64.9
		5	4.89	97.9	-0.111	0.78	16.1	0.83	17.3
		10	10.7	106.8	0.684	1.90	17.6	1.93	17.9
		20	19.5	97.7	-0.469	2.00	10.3	2.19	11.2
Corn flour	Barley flour	0	0.117	-	0.117	0.15	128.9	0.17	142.8
		5	5.01	100.1	0.107	1.04	19.9	1.04	19.9
		10	11.7	117	1.701	2.12	18.1	2.12	18.1
		20	22.3	111.7	2.333	2.42	10.8	2.88	12.9
Corn flour (a)	Barley flour	0	1.82	-	1.82	0.14	7.6	2.34	17.76
		5	7.70	154	2.70	1.22	15.9	1.66	21.5
		10	15.7	157.2	5.72	4.48	28.5	5.40	34.4
		20	34.6	172.9	14.6	9.50	27.5	11.65	33.7
Corn flour	Rye flour	0	0.315	-	0.315	0.19	59.7	0.20	64.1
		5	10.1	202.4	5.12	0.46	4.58	1.26	12.5
		10	21.7	216.7	11.7	2.23	10.4	2.44	11.4
		20	45.1	225.6	25.1	3.02	6.69	4.28	9.49
Rice flour	Wheat flour	0	1.58	-	1.58	0.13	8.27	0.14	9.19
		5	8.01	160.2	3.01	0.47	5.91	0.58	7.20
		10	12.05	120.5	2.05	1.17	9.68	1.65	13.7
		20	20.7	103.7	0.733	2.21	10.7	2.26	10.9
Wine	Wheat flour	0	0.163	-	0.163	0.09	57.0	0.11	68.6
		5	5.006	100.1	0.006	0.16	3.15	0.29	5.76
		10	9.61	96.1	-0.396	0.39	4.08	0.42	4.39
		20	20.5	102.5	0.504	0.74	3.61	1.18	5.77
Sausage	Wheat flour	0	0.237	-	0.237	0.10	41.2	0.17	71.3
		5	7.64	152.8	2.64	0.72	9.38	0.72	9.38
		10	11.8	118.5	1.85	1.08	9.15	1.95	16.5
		20	20.4	101.9	0.375	1.74	8.54	1.80	8.82
Sausage	Barley flour	0	0.237	-	0.237	0.10	41.2	0.17	71.3
		5	5.01	100.2	0.009	0.39	7.82	0.51	10.3
		10	11.3	113	1.305	1.22	10.8	1.34	11.9
		20	23.1	115.5	3.104	2.33	10.1	2.33	10.1

(a) conducted by the independent laboratory

BioSystems Gluten Results for incurred matrices

Matrix	Contaminant	Target contamination level, mg/kg	Mean (n=24) mg/kg	Recovery (%)	Bias mg/kg	S_r mg/kg	RSD$_r$ (%)	S_R mg/kg	RSD$_R$ (%)
Rice cookies	Wheat flour	0	0.184	-	0.19	0.03	18.1	0.18	98.9
		10	9.27	92.7	-0.73	0.69	7.46	1.15	12.4
		20	19.2	96.0	-0.81	1.30	6.77	2.54	13.2
		30	26.0	86.7	-4.00	0.96	3.70	2.35	9.03
Rice cookies (a)	Wheat flour	0	0.380	-	0.38	0.22	56.5	0.29	74.3
		10	13.5	134.5	3.45	0.74	5.50	1.13	8.40
		20	24.7	123.4	4.67	2.38	9.70	2.96	12.0
		30	37.3	124.2	7.25	2.66	7.10	6.65	17.9
Rice cookies	Barley flour	0	0.460	-	0.46	0.24	52.4	0.41	90.3
		10	9.02	90.2	-0.98	0.67	7.48	1.72	19.0
		20	21.3	106.5	1.30	4.47	21.0	5.35	25.1
		30	43.9	146.3	13.90	4.20	9.56	4.56	10.4
Rice cookies (a)	Barley flour	0	0.340	-	0.20	57.6	0.22	63.5	
		10	15.6	155.5	5.55	0.84	5.40	2.23	14.3
		20	33.4	167.1	13.4	5.00	15.0	5.57	16.7
		30	49.3	164.2	19.3	3.37	6.80	6.32	12.8
Rice cookies	Rye flour	0	0.550	-	0.55	0.19	32.4	0.23	42.1
		10	14.9	149.4	4.94	1.05	7.02	1.59	10.6
		20	31.4	156.9	11.4	3.09	9.83	3.99	12.7
		30	55.7	185.7	25.71	3.36	6.03	3.83	6.9
Rice cookies (a)	Rye flour	0	0.440	-	-	0.21	48.1	0.36	81.9
		10	17.4	174.3	7.43	0.80	4.60	2.71	15.5
		20	42.2	210.9	22.2	3.01	7.10	5.64	13.4
		30	56.1	187.1	26.1	8.37	14.9	9.48	16.9
Cornbread	Wheat flour	0	0.380	-	0.38	0.11	28.2	0.23	60.7
		10	11.1	111.4	1.14	1.13	10.1	1.28	11.5
		20	17.3	86.5	-2.69	0.82	4.84	2.12	12.4
		30	30.6	102.1	0.62	1.47	4.81	1.72	5.61

(a) conducted by the independent laboratory

An overall estimate of LOD and LOQ was made considering all results obtained by both the developer and the independent laboratory across all matrixes and contamination levels, resulting in an LOD of 1.21 mg/kg gluten and an LOQ of 2.40 mg/kg gluten, supporting the LOQ of 2.5 mg/kg of gluten claimed for this method.

Gluten Spike Solution Food Matrix Study

A complementary assay for food matrix validation was performed by fortifying a different group of matrices using the Gluten Spike Solution. This group of samples included:

- A processed meat product (cold cut)
- An alcoholic beverage rich in polyphenols (red wine)
- Bakery products (cookies)
- A powdered product with high polyphenol content (instant cocoa)
- A dairy product (curd)

The samples were fortified with the appropriate volume of Gluten Spike Solution to achieve target concentrations of 2.5, 5, 10, and 20 mg/kg of gluten.

The results demonstrate accurate outcomes for each studied attribute, with no significant matrix interference, and recovery rates ranging from 86-130%.

The RSDr values (1.4% - 8.3%), are lower than those observed with processed and non-processed samples, indicating that sample homogeneity is better when using a liquid solution like the Gluten Spike Solution compared to mixing a solid gluten source.

Gluten source	Blank matrix	Gluten level mg/kg	Mean (n=5) mg/kg	Recovery (%)	Bias mg/kg	S_r mg/kg	RSD$_r$ (%)
Gluten Spike Solution	Cold cut	0	0.292	-	0.29	0.05	-
		2.5	2.77	99.1	0.27	0.10	3.7
		5	5.08	95.4	0.08	0.23	4.6
		10	11.4	111.5	1.44	0.58	5.0
		20	20.1	99.1	0.12	0.53	2.6
	Instant Cocoa Powder	0	0.100	-	0.100	0.16	-
		2.5	2.26	86.4	-0.240	0.10	4.5
		5	4.47	87.4	-0.530	0.32	7.1
		10	8.75	86.5	-1.250	0.31	3.6
		20	17.7	87.9	-2.330	0.73	4.2
	Cookies	0	0.143	-	0.14	0.06	-
		2.5	2.50	94.1	0.00	0.10	4.1
		5	4.71	91.4	-0.29	0.06	1.4
		10	10.1	99.3	0.08	0.20	2.0
		20	19.2	95.3	-0.81	0.28	1.4
	Curd	0	0.793	-	0.793	0.11	-
		2.5	3.21	96.8	0.713	0.11	3.5
		5	6.54	107.0	1.141	0.27	4.5
		10	12.8	119.7	2.766	0.50	3.9
		20	26.7	129.8	6.746	2.21	8.3
	Red wine	0	0.139	-	0.139	0.06	-
		2.5	2.68	101.6	0.182	0.13	4.8
		5	4.96	96.3	-0.042	0.07	1.4
		10	10.6	104.5	0.590	0.43	4.1
		20	20.7	102.6	0.654	0.64	3.1

Quality Controls, Reference and Proficiency Testing Material Study

Quality control and reference materials from FAPAS and proficiency test samples from DLA, were used for the quantitative detection of gluten. The samples were prepared following the instructions provided by the respective reference material suppliers and analyzed by different technicians on separate days, in accordance with the BioSystems Gluten method protocol.

Table provides detailed information on the sample type, matrix, number of participants, assigned value, and the results obtained by BioSystems, including the measured gluten concentration (mg/kg), bias from the assigned value, and recovery rate (%).

The results obtained fell within the acceptance range established by the provider, with recovery rates ranging from 63% to 148% compared to AOAC OMA 2012.01, thereby meeting the criteria set by AOAC.

Additionally, the results obtained with FAPAS and DLA reference materials showed an accuracy between 76% and 103% relative to the assigned values, demonstrating the good accuracy and overall performance of the BioSystems Gluten method.

Material	AOAC OMA 2012.01		BioSystems Gluten Method		
FAPAS, Quality Control Material					
Reference sample	**Matrix**	**Assigned value, Xa (range for UI c2)**	**N° of data points Xa**	**mean (n=5) mg/kg**	**Bias mg/kg**	**Bias (%)**
T27247TBQC	Cake mix	19.3 (9.6 - 28.9)	100	12.10	-7.20	63
T27252AQC	Oat based foodstuff	16.6 (8.3 - 24.9)	61	19.3	1.70	110
T27260QC	Cooked biscuit	76 (38.0 - 114.0)	73	90.2	14.2	119
T27264BQC	Infant soya formula	24.8 (12.4 - 37.2)	52	36.8	12.00	148
T27271AQC	Cake mix	39.2 (19.6 - 58.8)	69	39.2	-0.060	97
T27275AQC	Cake mix	14.9 (7.5 - 22.4)	94	17.8	2.87	119
T27301BQC	Cake mix	16.0 (8.0 - 24.0)	78	17.0	0.960	106
T27314QC	Cooked Biscuit	56.3 (28.1 - 84.1)	58	55.1	-1.22	98
T27319AQC	Cake mix	13.9 (6.9 - 20.8)	88	16.6	2.70	119
T27331BQC	Cake mix	5.53 (2.76 - 8.29)	48	4.07	-1.46	74
FAPAS, Reference Material						
Reference sample	**Matrix**	**Reference value RV (U), mg/kg**	**N° of data points RV**	**mean (n=5) mg/kg**	**Bias mg/kg**	**Bias (%)**
TYG001RM	Cake mix	14.4 (1.7)	95	14.7	0.300	103
TGY002RM	Cake mix	28.8 (2.4)	103	28.00	-0.80	97
DLA, Reference Material						
Reference sample	**Matrix**	**Reference value RV (U), mg/kg**	**N° of data points RV**	**mean (n=2) mg/kg**	**Bias mg/kg**	**Bias (%)**
DLA ALM2 (2021)	Cocoa biscuit	0	-	0.0960	0.096	-
	Cocoa biscuit + wheat flour	2	4	1.94	-0.06	97
	Cocoa biscuit + wheat flour	10	10	8.39	-1.61	84
	Cocoa biscuit + wheat flour	20	10	18.2	-1.85	91
	Cocoa biscuit + wheat flour	50	10	38.1	-11.9	76
	Cocoa biscuit + wheat flour	100	10	81.6	-18.4	82

First kit validated following the new AOAC guidelines for gluten methods
AOAC Performance Tested Method℠ ***072503***

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Selectivity: Breadth Study

The BioSystems Gluten method effectively detects and quantifies gluten in flour derived from various cereal sources, including common and ancient wheat varieties. Recovery values for gluten, fell within the generally accepted range of 50–150% for most commodities.

Exceptions were noted for *Triticum compactum* (Tiroler Mittelfrüher Binkel) and *Triticum turanicum* (Kamut), which showed recoveries of 48% and 47%, respectively.

All samples of minor gluten source grains were kindly provided by the Department of Crop Sciences at the University of Natural Resources and Life Sciences (BOKU), Vienna.

Selectivity study: rice flour spiked at 10 mg/kg gluten from minor gluten sources.

Species (genotype)	Expected Gluten		BioSystems Gluten	
	mg/kg	mg/kg	RSDr	Recovery
Triticum aestivum (Arnold)	10.3	11.65	30%	114%
Triticum compactum (Tiroler Mittelfrüher Binkel)	10.3	5.00	55%	49%
Triticum durum (Sambadur)	10.2	5.41	26%	53%
Triticum monococcum (Monlis)	10.4	7.43	9%	72%
Triticum dicoccon (TRI 16444)	10.6	11.00	13%	104%
Triticum turanicum (Kamut)	10.8	5.11	42%	47%
Triticum spelta (Ebners Rotkorn)	10.4	7.10	19%	69%
xTriticosecale (Kortago)	10.5	8.89	18%	85%

Selectivity: Cross-reactivity and Interference Studies

Cross-reactivity study: the study evaluated matrices recommended by AOAC that do not contain gluten but are potentially cross-reactive, to determine whether they produce a measurable response exceeding the LOQ claimed by the method. Each commodity (purchased from a local retailer or online store) was tested once following the instructions detailed in the insert package.
All commodities were prescreened using AOAC OMA 2012.01 [2] to confirm the absence of gluten contamination.

All matrixes tested using the candidate method produced results <2.5 mg/kg (<LOQ) for the cross-reactivity study, except for the same matrixes that tested positive in the prescreening study with the AOAC OMA 2102.01. These samples were discarded from the interference study.

Interference study: the same commodities used in the cross-reactivity study were tested at 10 mg/kg of each gluten source (wheat, barley, and rye flours). Materials were tested as they normally used, except for the guar gum and xanthan gum that were diluted 1/10 in rice flour, due to their high viscosity. Each commodity was tested in a single replicate following the instructions detailed in the insert package.

All matrixes fell within the generally accepted range of 50–150% for most commodities. For matrixes contaminated with rye flour, the values obtained were always higher, reaching up to 180% of recovery values. This overestimation may be due to the assumption that prolamins and glutelins are present in the same proportion, but do not always fit. Several studies have shown that this conversion factor for determining gluten content is inappropriate, especially for rye [3].

Matrix	AOAC OMA 2012.01	Biosystems Gluten (mg/kg gluten)		
	Unspiked sample, mg/kg gluten	Unspiked sample	+ 10 mg/kg gluten from wheat flour	+ 10 mg/kg gluten from rye flour	+ 10 mg/kg gluten from barley flour
Almond flour	<LoQ	<LoQ	8.9	10.3	9.6
Amaranth flour	<LoQ	<LoQ	9.0	7.4	8.7
Arrowroot	<LoQ	<LoQ	8.0	11.9	8.1
Black bean flour	<LoQ	<LoQ	8.9	13.7	5.4
Beef meat	<LoQ	<LoQ	9.7	16.3	6.5
Buckwheat flour	<LoQ	<LoQ	9.5	9.6	9.2
Cashew	<LoQ	<LoQ	12.9	9.6	9.2
Chestnut flour	<LoQ	<LoQ	9.0	9.4	5.1
Chia seeds	<LoQ	<LoQ	8.6	14.0	5.0
Chicken meat	<LoQ	<LoQ	8.5	9.0	8.2
Chickpea flour	<LoQ	<LoQ	9.4	9.7	10.0
Cocoa	<LoQ	<LoQ	8.7	9.4	6.6
Coconut flour	<LoQ	<LoQ	8.6	9.0	7.6
Ground coffee	<LoQ	<LoQ	9.9	11.7	8.1
Condensed milk	<LoQ	<LoQ	9.0	10.9	8.1
Cumin	<LoQ	<LoQ	8.4	12.7	8.0
Dried fruits (raisins)	<LoQ	<LoQ	12.4	19.4	6.2
Dried fruits (red cranberries)	<LoQ	<LoQ	8.6	14.0	5.0
Egg powder, chicken	<LoQ	<LoQ	7.5	11.4	9.3
Fava bean, ground	<LoQ	<LoQ	6.0	8.8	7.4
Flax seed flour	<LoQ	<LoQ	8.0	10.0	9.5
Green pea flour	<LoQ	<LoQ	9.0	12.1	6.0
Guar gum (1:10 in rice flour)	<LoQ	<LoQ	9.1	18.3	7.1
Hazelnut flour (toasted)	<LoQ	<LoQ	7.6	7.7	7.7
Lentil flour	<LoQ	<LoQ	9.7	9.1	6.2
Lima bean flour	<LoQ	<LoQ	7.0	10.6	7.9
Lupin flour (brand 1)	7.0	6.0	-	-	-
Lupin flour (brand 2)	14.7	17.3	-	-	-
Milk powder, cow	<LoQ	<LoQ	6.5	5.9	3.3
Millet flour	<LoQ	<LoQ	9.8	11.9	8.0
Oat Flour	7.1	7.9	-	-	-
Oat flakes, ground	<LoQ	<LoQ	12.0	16.2	8.1
Parsley	<LoQ	<LoQ	11.6	8.7	7.9
Pea protein	<LoQ	<LoQ	13.0	12.4	10.1
Peanut (whole fruit ground)	<LoQ	<LoQ	8.1	11.1	9.1
Pork meat	<LoQ	<LoQ	9.5	14.5	9.0
Potato flour	<LoQ	<LoQ	11.0	18.0	6.7
Quinoa flour	<LoQ	<LoQ	10.0	17.0	6.9
Red lentil Fusilli (Pasta)	<LoQ	<LoQ	9.7	12.4	8.8
Rice semolina	<LoQ	<LoQ	9.4	11.1	8.3
Salmon	<LoQ	<LoQ	10.4	8.1	6.8
Sesame flour	<LoQ	<LoQ	10.5	20.8	11.5
Sorghum flour	<LoQ	<LoQ	9.1	14.2	9.6
Soy Flour	<LoQ	<LoQ	6.1	7.2	6.5
Soybean (textured)	<LoQ	<LoQ	7.0	9.9	8.1
Sweet rice flour	<LoQ	<LoQ	8.3	14.2	8.7
Tapioca flour	<LoQ	<LoQ	13.0	11.3	7.6
Teff flour	<LoQ	<LoQ	9.2	16.2	9.9
Walnuts	<LoQ	<LoQ	13.5	11.1	8.0
White bean	<LoQ	<LoQ	10.1	15.2	7.9
Xanthan gum (1:10 in rice flour)	<LoQ	<LoQ	9.3	15.9	6.5

AOAC 2012.01: LOQ: 5 mg/kg | BioSystems Gluten: LOQ: 2.5 mg/kg

Conclusion

The **BioSystems Gluten** assay is a novel, fully automated, quantitative method based on immunoturbidimetry for the specific detection of the 33-mer immunotoxic peptide fraction of gluten in food and beverages.

It effectively detects gluten in various processed and non-processed foods containing wheat, barley, and rye, with good recovery and low variability.

The BioSystems Gluten assay demonstrated a quantitative linear range of 2.5 to 40 mg/kg with a limit of quantification (LOQ) of 2.5 mg/kg. Limit of linearity can be extended into 200 mg/kg via automated sample predilution.

Selectivity and interference studies confirmed recovery values within the acceptance criteria established by AOAC, with no need for additional treatment of polyphenol-rich samples.

Moreover, the **BioSystems Gluten Spike Solution** also developed is a valuable tool for users, enabling the preparation of series controls to evaluate both the functionality of the extraction process and the determination with the reagent.

The BioSystems Gluten method, combined with the dedicated **Gluten Extraction Solution**, provides an innovative, specific, and reliable detection approach—similar to ELISA, but with several key advantages. It is fully automated on the BioSystems Y15 analyzer, allowing for high-throughput testing with minimal manual intervention, faster results, no washing steps, and no need for frequent calibration.

These features make it ideal for routine gluten testing in food industry quality control labs seeking efficient and cost-effective solutions. *AOAC Performance Tested Method℠* ***072503***.

	ELISA	BioSystems Gluten
Quantitative	YES	YES
Direct (targets protein)	YES	YES
Method of detection: Photometry	YES	YES
Affordable price	YES	YES
Multiparametric	NO	YES
Calibration in every run	YES	NO
Washing steps	YES	NO
Processing time	~80 tests in at least 2 hours	1st result in 10 min Following results every 48 s ~60 tests in 1 h
Fully automated	NO	YES

BioSystems Gluten	
Method	Immunoturbidimetry with latex nanoparticles
Antibody	Monoclonal against 33-mer fragment in gliadin (from wheat)
Adjustment	Four Parameter Logistic (4PL) Regression
Limit of Quantification	2.5 mg/kg (mg/L) gluten
Measurement interval	2.5 – 40 mg/kg (mg/L) gluten extendable to 200 mg/kg (mg/L) via automated sample predilution
Stability	18 months at 2-8 °C
Validated matrices	Corn flour, rice flour, sausage, wine, cornbread, rice cookies
Reagents preparation	Reagent, calibrators and extraction solution are ready to use

REFERENCES:
[1] AOAC Gluten Working Group (2024) Guidelines for Validation of Quantitative Gluten Methods, with Specific Examples for ELISA Assays [Internet]. Rockville, Maryland.
[2] AOAC INTERNATIONAL. (2012) AOAC Official Method 2012.01: Gliadin as a Measure of Gluten in Foods Containing Wheat, Rye, and Barley. Rockville, MD, USA.
[3] Xhaferaj M, Muskovics G, Schall E, Bugyi Z, Tömösközi S, Scherf KA (2023). Characterization of rye flours and their potential as reference material for gluten analysis. Food Chem 408, 135148.