Gluten Fragment Detection with a Competitive ELISA

SIGRID HAAS-LAUTERBACH, ULRIKE IMMER, and MAREIKE RICHTER
R-Biopharm, An der neuen Bergstraße 17, D-64297 Darmstadt, Germany
PETER KOEHLER
German Research Center for Food Chemistry, Lise-Meitner-Strasse 34, 85354 Freising, Germany

The second generation of a competitive ELISA for prolamin quantification based on the R5 antibody was studied for method performance and suitability to detect partially hydrolyzed prolamins in food. To be able to convert signal intensities to gluten concentrations, as required by the Codex Alimentarius Standard, a new calibrator consisting of a peptic-tryptic digest of wheat, rye, and barley prolamins was used for the first time. LOD and LOQ of the assay were 1.36 and 5.0 mg prolamin/kg food, respectively. Analysis of beer samples and a hydrolyzed wheat product showed that the assay provided significantly higher prolamin concentrations, compared to the sandwich ELISA based on the same antibody, which is only suitable for the detection of intact prolamins. Spiking experiments with defined concentrations of partially hydrolyzed prolamins gave recoveries ranging from 92 to 136%.

Wheat proteins are related to immunoglobulin E (IgE)-based allergic reactions on one side and to celiac disease as a chronic autoimmune disorder on the other side. Genetically predisposed celiac disease, often beginning in early childhood, is known as a non-IgE-mediated hypersensitivity to the storage proteins of wheat, rye, barley, and possibly oats, which have been termed gluten. Within the gluten proteins, prolamins, soluble in aqueous alcohols, are distinguished from the alcohol/water-insoluble glutelins. Gluten proteins have been given specific names, e.g., gliadins and glutelins from wheat, secalins from rye, hordeins from barley, and avenins from oats. The toxicity of oats is still under discussion (1, 2). About 2% of the celiac patients are intolerant to oats and have to avoid it in their gluten-free diet.

The mechanisms of IgE-mediated allergic reaction and celiac disease are different, but the same proteins may cause the two diverse reactions and, in the end, two groups of patients are affected. About 67% of the IgE-mediated allergic reactions against wheat are related to the albumin/globulin fraction (3), and only a part of these patients react to prolamins, mainly to α-gliadins. Celiac patients are sensitive to prolamins (e.g., α/β/γ- and α-gliadins and related prolamins from rye and barley), as well as to glutelins, such as low molecular weight (LMW) aggregated α/β/γ- and α-gliadins (4), and high MW subunits of glutenin (5) from wheat and homologous glutelin subunits from rye and barley. Glutelins can be described as aggregated prolamins because, in contrast to monomeric prolamins, these proteins have two or more cysteine residues involved in intermolecular disulfide bonds. In particular, the amino acid composition of LMW subunits of glutenin is similar to the α/β-gliadins (6).

The prolamins of celiac toxic cereals are rich in proline (P) and glutamine (Q), which enhance resistance toward hydrolysis by gastrointestinal peptidases during digestion. It was shown that peptides consisting of a minimum of nine amino acids (especially from the N-terminal part of the protein) are active by stimulating lymphocytes (7). Potentially, celiac-toxic peptides bind to human leucocyte antigens HLA-DQ2 or -DQ8 molecules, activate T-cells, and trigger a sequence of reactions. T-cell activation is enhanced by tissue transglutaminase, which deamidates specific glutenine side chains of peptides derived from celiac-toxic proteins (8).

Different legislation around the world regulates allergen labeling of processed food. Within the United States, the Food Allergen Labeling and Consumer Protection Act defines the so-called “Big 8”—milk, egg, fish, crustacean, tree nuts, wheat, peanut, and soybeans—as the major causes of allergic reactions to food. The European Union Directive 2007/68/EC (9) also includes wheat, as well as other gluten-containing cereals, in its list of 14 food allergens. For both regulations, the allergens have to be declared if present in food, although no threshold levels are given.

The Codex Alimentarius (10) and the Commission Regulation (EC) No. 41/2009, on the other hand (11), define the term “gluten” and “gluten-free” and establish rules for the presence of limited amounts of gluten in dietary food products for patients with celiac disease (naturally gluten-free food: 20 mg gluten/kg; low gluten-containing food: up to 100 mg gluten/kg). Since 2006, the Codex Alimentarius additionally recommends the use of the “R5 Mendez method” (ELISA) as a type I method to detect gluten in food. This was based on results of the work of Mendez et al. (12) and a collaborative study conducted in 2001 (13). The R5 method determines the prolamin content of food, which has to be converted to the gluten content. In the Codex, a conversion factor of 2 is suggested, since the “prolamin content of gluten is generally taken as 50%.” This factor is an approximation, since it may vary depending on cereal species or processing of food, i.e., in starches, the conversion factor may be higher than in most other cereal products (14).

Besides the prolamin (gliadin) assay based on the sandwich format, which has been developed to quantify intact proteins and fragments with at least two binding epitopes, a prolamin (gliadin) competitive format was established to detect partially hydrolyzed prolamins containing peptides with only one binding
epitope. The competitive format, based on the R5 antibody, should be used for food that has undergone fermentation and/ or enzymatic hydrolysis, such as beer, starch based syrups, and sourdoughs. The first generation assay was calibrated to a potentially toxic peptide containing the epitope QQPFP, even knowing that the result, expressed as peptide equivalents, could not be recalculated to prolamin. Now a subsequent second-generation competitive assay is released using a mixture of hydrolyzed prolamins from wheat, rye, and barley as a new calibrator.

The aim of this paper is to show the next step forward using this second-generation gliadin competitive assay. It will be shown that the RIDASCREEN® Gliadin competitive second-generation assay (R-Biopharm, Darmstadt, Germany) is able to measure potentially toxic prolamin fragments in beer and starch syrup by using a novel calibrator containing partially hydrolyzed prolamins. The results can be expressed as prolamin concentration in food, allowing a correlation to the threshold value of the Codex Alimentarius.

**Experimental**

**Preparation of the Calibrator**

For the second-generation competitive assay, peptic-tryptic hydrolyzates of prolamins from different cereals were used as calibrators, which were prepared according to Gesendorfer et al. (15). Briefly, the prolamin fractions of rye (cultivar Nikita) and barley (cultivar Barke) were isolated from flour by means of a modified Osborne fractionation. A highly purified and well-characterized gliadin preparation provided by the Working Group on Prolamin Analysis and Toxicity (WGPAT; 16) was used as the prolamin fraction from wheat. After characterization of the protein composition by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and RP-HPLC, each prolamin fraction was digested by pepsin and trypsin to obtain partially hydrolyzed prolamin fractions from wheat, rye, and barley. The nitrogen (N) content of the prolamins, as well as of the respective prolamin digests, was determined by combustion analysis with a Dumas analyzer (FP-328 combustion instrument Leco, Kirchheim, Germany). The protein and peptide content was calculated by multiplying the N-content by factor 5.7. Thus, the peptide content could be easily converted to the prolamin content.

Table 1. Characteristics of the competitive RIDASCREEN® Gliadin second-generation ELISA

<table>
<thead>
<tr>
<th></th>
<th>Calibration range, ng/mL gliadin</th>
<th>LOD, mg prolamin/kg</th>
<th>LOQ, mg prolamin/kg</th>
<th>Cross-reactivity</th>
<th>Intra-assay CV, %</th>
<th>Interassay CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0, 10, 30, 90, 270</td>
<td>1.36</td>
<td>5.0</td>
<td>No reaction to maize, rice, oats, millet, buckwheat, or soy</td>
<td>5.6</td>
<td>2.7</td>
</tr>
</tbody>
</table>

The prolamins fractions and the digested fractions were evaluated in the R5 gliadin sandwich and the R5 gliadin competitive format (R-Biopharm). Both assay formats were able to quantify the intact prolamin preparations (15). As expected, the peptic-tryptic digests showed lower responses in the sandwich format but gave higher responses in the competitive assay. To prepare the final calibrator, the digests of wheat, rye, and barley prolamins were dissolved in water (same peptide concentration), mixed in a 1 : 1 : 1 (v/v/v) ratio and lyophilized. The dry mixture of hydrolyzed wheat, rye, and barley prolamins was dissolved in 60% (v/v) ethanol (1 mg/mL), diluted in a 0.1 M phosphate buffered saline-Tween buffer, and used as the calibrator in the competitive test format (final concentration of prolamin equivalents 10 to 270 ng/mL). A factor of 2 was used to convert prolamin into gluten concentrations.

**ELISA Procedure**

The wells of a microtiter plate were coated with a low and constant amount of gliadin (ethanolic wheat flour suspension) as the antigen. The calibrators or the extracts (100 µL/well) of food samples, as well as 100 µL/well peroxidase (POD)-labeled anti-gliadin antibodies (monoclonal R5 antibodies), were added to the gliadin coated microtiter plate at the same time and incubated for 30 min at 22°C. The POD-labeled antibodies (enzyme conjugate) were bound to gliadin on the plate or to dissolved prolamin peptides (competition) forming antigen-antibody complexes. During a washing step (detergent containing PBS-buffer, pH 7.4) the prolamin-enzyme conjugate in solution was discarded, whereas the plate-bound gliadin conjugate remained. Substrate/chromogen [red stained saturated tetramethylbenzidine (TMB)/peroxide solution] (100 µL/well) was added to the wells and incubated. Bound enzyme conjugate converted the chromogen into a blue product. The addition of 100 µL/well stop solution (1 N H2SO4) led to a color change from blue to yellow. The measurement was made spectrophotometrically at 450 nm. The absorption was inversely proportional to the prolamin amount in the sample.

**Sample Extraction Procedure**

Solid samples were homogenized, and 1 g was extracted with 10 mL 60% (v/v) ethanol for 10 min at 22°C under rotation. In the case of beer samples, 1 mL sample was extracted with 9 mL 60% ethanol, pH 8.5, containing 10% (w/v) fish gelatin in order to enable a sufficient extraction of prolamin fragments masked by hop constituents.

**Spiking Procedure**

A stock solution of the hydrolyzed prolamin mixture was prepared in 60% (v/v) ethanol (concentration = 1 mg/mL). Appropriate amounts of the solution were used for spiking to

Table 2. Calibrator composition of the first (former version) and the second (new version) generation of the RIDASCREEN® Gliadin competitive ELISA

<table>
<thead>
<tr>
<th>Gliadin competitive</th>
<th>First generation</th>
<th>Second generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator</td>
<td>Synthetic peptide, including QQPFP</td>
<td>Mixture of peptic-tryptic wheat, rye, barley, and prolamin hydrolyzate</td>
</tr>
<tr>
<td>Result expressed as</td>
<td>µg peptide equivalent/g food</td>
<td>mg prolamin/kg</td>
</tr>
</tbody>
</table>
1 g or 1 mL of the appropriate matrix. Afterwards, the spiked samples were extracted with 60% ethanol as described in the sample extraction procedure. Due to the fact that beer or syrup are the main samples of interest in the competitive format, these matrixes were used in spiking experiments.

Results and Discussion

The need for gluten-free labeling requires methods that are able to measure the prolamin content of food with high sensitivity. To follow the Codex threshold for gluten-free food, ELISA methods are the state of the art today, combining recognition of celiac-toxic amino acid sequences of prolamins with a simple method of determination. In the case of gluten, not only intact molecules, but also peptide fragments of different length, induce T-cell mediated reactions. Therefore, it is important to measure small peptides too.

A 33-amino acid peptide from α/β-gliadin with the sequence LQLQPFPQPQLPYPQPQLPYPQPQPF has been shown to be resistant to gastric and pancreatic hydrolysis and acts as a strong stimulator to intestinal T-cells. It has been shown to exhibit celiac disease toxicity in vivo and in vitro (17). This peptide and partial amino acid sequences of it were used to check their reactivity with the R5 antibody (18, 19). This antibody is able to recognize peptide motifs as small as the pentamer QQPFP and related structures like QXPW/FP. These amino acid motifs are potentially toxic for celiac patients. They are present as multiple motifs in γ- and ω-gliadins, as well as in lower abundance in α/β-gliadins and in LMW glutenin subunits (contain only one motif). The latter become soluble in aqueous alcohols under reducing conditions. The sequence motif also occurs in rye and barley prolamins. This is the reason why the R5 antibody is internationally recognized as the most fitting for determining the content of celiac-toxic prolamins in foodstuffs.

Some cereal-based foods contain fermented or partially hydrolyzed gluten, e.g., beer, sourdough, or syrup based on wheat starch. Gluten fragments in these foods cannot be detected by a classical sandwich ELISA method for determination of gliadins (20). In general, the competitive ELISA is able to detect these small peptide fragments of prolamins in highly processed food.

The first-generation competitive assay was calibrated to a potentially toxic peptide containing the epitope QQPFP. The result was expressed as peptide equivalents and had no direct relation to the Codex limit values. A revised second-generation competitive assay contains a new calibrator that consists of a hydrolyzed prolamin mixture from wheat, rye, and barley.

Data on the performance of the second-generation RIDASCREEN® Gliadin competitive ELISA is shown in Table 3, and can be found in the validation report of R-Biopharm (21). The LOD was determined by extracting five zero-level matrixes (syrup, rice flour, chestnut flour, starch, and beer) 10 times, measuring each extract in duplicate. From these calculated concentrations of all measurements the mean value was obtained, and the 3-fold SD was added. The resulting value was 1.36 mg prolamin/kg.

To determine the LOQ, a contaminated corn pasta sample with a concentration of 12 mg gliadin/kg was mixed with a noncontaminated corn pasta sample to obtain a final concentration of 5 mg gliadin/kg. The existence of gliadin in the contaminated sample was confirmed by PCR analysis. The

### Table 3. Prolamin concentration of various beer samples as determined by the sandwich and second-generation competitive R5 ELISA

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Gliadin sandwich, mg prolamin/kg</th>
<th>Gliadin competitive, mg prolamin/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beers labeled “gluten-free”</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austrian beer, millet based</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Italian beer, rice based</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>German beer, barley based, specially produced&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Spanish beer 1</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Finnish beer 1</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td><strong>Barley-based beers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finnish beer 2</td>
<td>&lt;LOQ</td>
<td>9.7</td>
</tr>
<tr>
<td>American beer 1</td>
<td>&lt;LOQ</td>
<td>6.4</td>
</tr>
<tr>
<td>German beer 2</td>
<td>12.2</td>
<td>6.9</td>
</tr>
<tr>
<td>German beer 3</td>
<td>&lt;LOQ</td>
<td>7.5</td>
</tr>
<tr>
<td>Mexican beer 1</td>
<td>&lt;LOQ</td>
<td>11.0</td>
</tr>
<tr>
<td>American beer 2</td>
<td>&lt;LOQ</td>
<td>16.1</td>
</tr>
<tr>
<td>American beer 3</td>
<td>&lt;LOQ</td>
<td>18.0</td>
</tr>
<tr>
<td>German beer 4</td>
<td>3.2</td>
<td>18.9</td>
</tr>
<tr>
<td>German beer 5</td>
<td>10.5</td>
<td>23.9</td>
</tr>
<tr>
<td>Cherry-Cassis beer</td>
<td>8.5</td>
<td>24.1</td>
</tr>
<tr>
<td>Chinese beer 1</td>
<td>3.7</td>
<td>24.4</td>
</tr>
<tr>
<td>German beer 6</td>
<td>6.8</td>
<td>25.3</td>
</tr>
<tr>
<td>German beer 7</td>
<td>7.2</td>
<td>25.7</td>
</tr>
<tr>
<td>Apple-herbs beer</td>
<td>8.6</td>
<td>27.5</td>
</tr>
<tr>
<td>Czech beer 1</td>
<td>9.9</td>
<td>32.4</td>
</tr>
<tr>
<td>Barley beer</td>
<td>14.4</td>
<td>36.0</td>
</tr>
<tr>
<td>German beer 8</td>
<td>12.2</td>
<td>38.0</td>
</tr>
<tr>
<td>German beer 9</td>
<td>9.9</td>
<td>38.8</td>
</tr>
<tr>
<td>German beer 10</td>
<td>12.6</td>
<td>45.3</td>
</tr>
<tr>
<td>German beer 11</td>
<td>14.8</td>
<td>47.5</td>
</tr>
<tr>
<td>German beer 12</td>
<td>11.8</td>
<td>50.0</td>
</tr>
<tr>
<td>German beer 13</td>
<td>16.9</td>
<td>51.3</td>
</tr>
<tr>
<td>German beer 14</td>
<td>14.3</td>
<td>52.3</td>
</tr>
<tr>
<td>German beer 15</td>
<td>21.7</td>
<td>57.8</td>
</tr>
<tr>
<td>German beer 16</td>
<td>15.7</td>
<td>61.0</td>
</tr>
<tr>
<td>Irish beer 1</td>
<td>16.4</td>
<td>73.3</td>
</tr>
<tr>
<td>German beer 17</td>
<td>51.0</td>
<td>268.9</td>
</tr>
<tr>
<td>German beer 18</td>
<td>1504.0</td>
<td>1542.7</td>
</tr>
<tr>
<td>LOQ</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>LOD</td>
<td>1.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Enzymatically treated.
mixed sample was extracted, 10 replicates were analyzed, and the mean was 5.52 ± 0.23 mg gliadin/kg. Therefore, it could be shown that the recovery of a sample at the LOQ is near 100%, and that the variation of results at the LOQ level is comparable to the intra-assay and interassay variance of samples, which were found to be 5.6 and 2.7%, respectively.

In addition to the antibody, the calibrator material plays an important role. The first generation of RIDASCREEN® Gliadin competitive ELISA used the peptide including the sequence QQPFP as the calibrator due to the fact that small potentially toxic peptides are left after digestion of food, and to show that the competitive format measures additionally smaller fractions in comparison to the sandwich ELISA. Different results of the same sample can be stated comparing both formats. However, the peptide equivalents obtained with this competitive format could not be converted to gluten concentration to be able to compare them with the Codex threshold values. This problem was solved by using a new calibrator consisting of a mixture of partially hydrolyzed prolamins from wheat, rye, and barley (15) in the second-generation competitive ELISA (Table 2), which now allows expression of the results in mg prolamin/kg food. This result can be converted into gluten by multiplying by the factor 2 (i.e., 2 × mg prolamin/kg), as required by the Codex Alimentarius.

Different beer samples from the market were analyzed with the second-generation competitive format in comparison to the sandwich ELISA (Table 3). Due to the fact that the competitive ELISA also detected small prolamin fragments, higher prolamin concentrations were obtained in comparison to the sandwich ELISA. In four beer samples labeled gluten-free, no prolamins could be detected by either ELISA format. It was shown that some beer samples, measured below the LOQ in the sandwich format, were clearly above the LOQ in the competitive format. In general, the competitive assay provided higher prolamin concentrations than the sandwich assay. These results have been generated from one experiment. A lot of beer samples have been reanalyzed over the time, showing similar results.

For recovery experiments three of the negative beer samples (concentrations below the LOD) were spiked with different levels of the peptic-tryptic hydrolyzate of wheat, rye, and barley prolamins. As shown in Table 4, recoveries in a range from 92 to 136% (mean recovery of 109%) were obtained.

Gluten-free, corn-based starch and starch syrup samples from the market were analyzed by the second-generation competitive ELISA. These samples were spiked with the prolamin hydrolyzate to measure the recovery in the competitive format. The results are shown in Table 5. The results are related to the spike control (without matrix). A mean overall recovery of 100.1% (100.4% for the starch and 99.7% for syrup) was obtained. Repeated experiments showed similar results.

### Table 4. Recovery of partially hydrolyzed prolamin in beer after spiking of gluten-free beer with a peptic-tryptic digest of wheat, rye, and barley prolamins as determined by the RIDASCREEN® Gliadin competitive second-generation ELISA

<table>
<thead>
<tr>
<th>Spike level of gluten-free beer, mg prolamin/kg</th>
<th>Concentration, mg prolamin/kg</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer without spike &lt;LOQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.8</td>
<td>136</td>
</tr>
<tr>
<td>10</td>
<td>9.7</td>
<td>97</td>
</tr>
<tr>
<td>20</td>
<td>21.0</td>
<td>105</td>
</tr>
<tr>
<td>30</td>
<td>29.7</td>
<td>99</td>
</tr>
<tr>
<td>50</td>
<td>45.9</td>
<td>92</td>
</tr>
<tr>
<td>90</td>
<td>88.7</td>
<td>99</td>
</tr>
<tr>
<td>100</td>
<td>113.6</td>
<td>114</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rice beer</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer without spike &lt; LOQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>103</td>
</tr>
<tr>
<td>10</td>
<td>11.3</td>
<td>96</td>
</tr>
<tr>
<td>30</td>
<td>35.7</td>
<td>119</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specially produced barley-based beer&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer without spike &lt;LOQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12.3</td>
<td>127</td>
</tr>
<tr>
<td>35</td>
<td>28.6</td>
<td>104</td>
</tr>
<tr>
<td>120</td>
<td>109.4</td>
<td>125</td>
</tr>
</tbody>
</table>

<sup>a</sup> Enzymatically treated.

### Table 5. Recoveries of partially hydrolyzed prolamin in corn starch and syrup samples after spiking with peptic-tryptic digest of wheat, rye, and barley prolamins as determined by the RIDASCREEN® Gliadin competitive second-generation ELISA

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Spike control&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Corn starch</th>
<th>Corn syrup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target, mg prolamin/kg</td>
<td>Result, mg prolamin/kg</td>
<td>Result, mg prolamin/kg</td>
</tr>
<tr>
<td>3</td>
<td>3.9</td>
<td>5.4</td>
<td>138.5</td>
</tr>
<tr>
<td>10</td>
<td>9.7</td>
<td>9.0</td>
<td>92.8</td>
</tr>
<tr>
<td>30</td>
<td>33.9</td>
<td>35.9</td>
<td>119.7</td>
</tr>
<tr>
<td>35</td>
<td>27.5</td>
<td>22.5</td>
<td>81.8</td>
</tr>
<tr>
<td>120</td>
<td>122.7</td>
<td>102.1</td>
<td>83.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Extraction procedure without matrix.

<sup>b</sup> Recovery was related to the result of each spike control.
Table 6. Gliadin concentration of a wheat hydrolyzate, as determined by the sandwich and the second-generation competitive format ELISAs

<table>
<thead>
<tr>
<th>Declaration</th>
<th>Extraction</th>
<th>Gliadin sandwich, mg gliadin/kg</th>
<th>Gliadin competitive, mg gliadin/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat hydrolyzate (80% protein)</td>
<td>Cocktail</td>
<td>68.3</td>
<td>—</td>
</tr>
<tr>
<td>Wheat hydrolyzate (80% protein)</td>
<td>60% Ethanol</td>
<td>71.0</td>
<td>2850</td>
</tr>
<tr>
<td>LOQ</td>
<td></td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>LOD</td>
<td></td>
<td>1.5</td>
<td>1.36</td>
</tr>
</tbody>
</table>

Table 6 contains data of the analysis of an industrially produced wheat-based noncharacterized hydrolyzed sample, measured by both assay formats. Both assays provided clearly different results. This showed that small gluten fragments included in the sample could be quantified by the competitive assay, whereas they were not detectable in the sandwich assay. Thus, the sandwich format is not suitable to quantify gluten in processed samples containing small gluten fragments. In the sandwich assay, peptide fragments often form complexes with the antibodies coated on the microtiter plate. Due to the lack of a second binding site, no complex can be formed with antibody conjugates in solution, afterwards leading to false negative results.

Conclusions

It was shown that the competitive R5 RIDASCREEN® ELISA in combination with the new calibrator containing partially hydrolyzed prolamins is a fast and accurate method that allows a reliable quantitative determination of prolams in cereal-based foods, especially in food containing partially hydrolyzed gluten. In contrast to the first-generation assay, which was calibrated by using a peptide containing the sequence QQFP, the second-generation assay provides gliadin concentrations directly related to the threshold values of gluten in gluten-free foods given by the Codex Alimentarius Standard. Collaborative studies for processed (RIDASCREEN® Gliadin), as well as fermented/hydrolyzed food (RIDASCREEN® Gliadin competitive), are currently being conducted under the shared leadership of the American Association for Clinical Chemistry (AACC International) and the WGPAT.

References