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Monitoring of gluten in Czech commercial beers

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Abstract: Between 2014 and 2019, a total of 361 commercially available Czech beer samples (103 brands) were analysed for gluten content using a competitive enzyme immunoassay. The gluten levels in different types of beers ranged from < 10 mg L^{-1} to 3 380.0 mg L^{-1}. The percentage of samples that can claim the gluten-free status (< 20 mg L^{-1}) were in categories gluten-free (99%), radler (84.6%), non-alcoholic beer (44.2%), lager beer group I (16.6%), lager beer group II (16.3%), special beer (25%) and wheat beer (0%). The important finding from this work was that 2 out of 196 beer samples with a gluten-free label contained more gluten than allowed under the guidelines established by the European Union and the Codex Alimentarius Standard. From a consumer point of view, it is also significant that in addition to gluten-free samples, 15.4% of radlers, 55.8% of non-alcoholic beers, 71.4 to 75.5% of all lagers and 66.6% of special beer samples could be labelled as very low gluten (21–100 mg L^{-1}).

Keywords: beer; ELISA; coeliac (celiac) disease; food safety

Coeliac (celiac) disease (CD) is one of the most common autoimmune diseases, when people who suffer from it do not tolerate gluten proteins (Rodrigo 2006). The overall prevalence of CD in western populations is between 0.5 and 1.7% (Gujral et al. 2012). Gluten ingestion in coeliac patients causes a variety of gastrointestinal and non-gastrointestinal symptoms and biochemical abnormalities that ameliorate after gluten withdrawal (Ciacci et al. 2007). Gluten is a group of proteins composed of gliadins (formerly called prolamins) and glutelins that occur in wheat (*Triticum* sp.) and other closely related cereals, such as barley (*Hordeum* sp.), rye (*Secale* sp.) and oats (*Avena* sp.). Their trivial names are based on the names of the respective plants and for barley these are hordein and hordenin (Ciacci et al. 2007).

Most beers are brewed from barley or wheat and thus they are considered to be unsuitable for people suffering from CD (Bamforth 2009; Mikuliková et al. 2013). However, this view does not include the possible modification of proteins and their removal during the malting and brewing processes. In addition, the levels of adjuncts used, such as maize, rice and other gluten-free cereals, can also have a significant impact on the final gluten content (Bamforth 2009; Guerdrum & Bamforth 2011).

The Codex Alimentarius (adopted by Codex Alimentarius Commision CAC) as well as the European Union define a limit for gluten-free beverages that cannot exceed the 20 mg L^{-1} gluten threshold. The Crossed Grain Trademark (CGT) is registered and protected across the European Union and provides buyers with a quick reference point whilst outshopping. It is a quality and safety guarantee of food that meets the criteria of the Association of European Coeliac Societies (AOECS) Standard (for Gluten-Free Foods technical requirements for licensing the Crossed Grain Symbol) from 2016.

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Products labelled as very low gluten can have a gluten content above 21 and up to 100 mg L^{-1} (Popping & Diaz-Amigo 2018; Watson et al. 2018). The assortment of beers suitable for gluten-free diets has therefore increased considerably.

Methods used for the analysis of cereal proteins such as gliadin (prolamin) are based on the principles of electrophoresis, liquid chromatography and immunochemical reactions (Mikuliková et al. 2013; Manfredi et al. 2015). The pioneer paper focused on determination of gluten in beers used an immunochemical approach (Dostálek et al. 2006). The immunochemical ELISA assay is the recommended method based on the monoclonal R5-antibody (mAb R5), which recognises the pentapeptide QQPFP (glutamine-glutamin-proline-phenylalanine-proline) and related sequences (Kahlenberg et al. 2006; Watson et al. 2018). AOECS and the Association of Official Analytical Chemists (AOAC) approved this method for the analysis of beers that can be declared gluten-free (Watson et al. 2019). The goal of this study was to carry out a monitoring of gluten content in different Czech beers using a commercially available RIDASCREEN® Gliadin competitive kit. The main focus was on long-term monitoring of beers declared to be gluten-free in terms of safety for gluten-sensitive beer consumers.

MATERIAL AND METHODS

Instruments and chemicals. An EL8808IU ELISA reader (BioTek Instruments, USA) was used to measure gluten in beer. The absorption was assessed using RIDASOFT® Win software (R-Biopharm AG, Germany). The following were used: Fish gelatine (Sigma-Aldrich, Canada), ethanol for UV (Lach-Ner, s.r.o., Czech Republic), sodium hydroxide (Lach-Ner, s.r.o., Czech Republic), distilled water, RIDASCREEN® Gliadin competitive kit (Art. No. R7021; R-Biopharm AG, Germany).

Beer samples. A total of 361 samples of commercially available Czech beers were analysed for their gluten content. The monitored beers were divided into groups (number of samples/number of brands): Gluten-free beer (196/4), radler (13/13), non-alcoholic beer (43/17), lager beer group I (42/27), lager beer group II (49/28), special beer (12/11), and wheat beers (6/3), see Table 1.

Determination of gluten content by ELISA. The determination was carried out according to the product information folder. The lower limit of detection and the limit of quantification for gluten according to this method were 4.6 and 10 mg kg^{-1}, respectively. The range of variation for the analysis of beer samples in this work corresponds to those in the information folder (RIDASCREEN® Gliadin competitive Art. No. R7021; available in German at: https://food.r-biopharm.com/wp-content/uploads/sites/2/2016/10/R7021-Gliadin-competitive-16-09-21.pdf). The gluten content was calculated based on an assumed 1: 1 ratio between gliadin and glutelin. Each sample was analysed in duplicate.

RESULTS AND DISCUSSION

The commercially available test kit for analysing fermented products such as beer that may contain small peptide fragments of prolamins from wheat, barley, rye and oats, is based on R5 monoclonal antibody recognition of gliadin (prolamin) sequences, among them the potentially toxic sequence QQPFP (Guerdrum & Bamforth 2012). The selectivity of this antibody for the QQPFP epitope thus allows the detection of gliadin in beer (Guerdrum & Bamforth 2011).

Table 1. The gluten content of beer samples divided into groups

Group	ABV[a] (%)	Average (mg L^{-1})	Max[b] (mg L^{-1})	Min[c] (mg L^{-1})	Total number of samples	Number of gluten-free samples[d]	Number of very low gluten samples[e]
Gluten-free beer	0.5–5.7	10.4	65.2	< 10.0	196	194	2
Radler	1.5–2.5	12.8	23.0	< 10.0	13	11	2
Non-alcoholic beer	< 0.5	27.5	55.6	< 10.0	43	19	24
Lager beer group I	3.5–4.5	51.6	127.6	< 10.0	42	7	30
Lager beer group II	4.5–5.0	54.8	216.0	< 10.0	49	8	37
Special beer	> 5.5	51.8	258.0	< 10.0	12	3	8
Wheat beer	4.7–5.0	2198.3	3380.0	1618.0	6	0	0

[a]alcohol by volume; [b]maximum measured value; [c]minimum measured value; [d]gluten: < 20 mg L^{-1}; [e]gluten: 21–100 mg L^{-1}

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Between 2014 and 2019, 196 beer samples (4 brands) declared as gluten-free and originating from the Czech Republic were analysed for gluten in order to assess the risks associated with their consumption for people suffering from CD. The gluten-free beer samples were below the limit of quantification (< 10 mg L^{-1}) in 99% of cases. However, two samples of gluten-free beers analysed were identified as containing higher gluten levels, which was 23.6 mg L^{-1} in one case and 65.2 mg L^{-1} in the other.

The 20 mg L^{-1} threshold for a gluten-free claim is in practice achieved by using gluten-free cereals such as maize, rice and sorghum and the pseudocereal quinoa (De Meo et al. 2011). In addition, there are several other technical solutions for reducing the levels of gluten in beer, including protein precipitation and enzymatic hydrolysis of gluten (Hager et al. 2014; Watson et al. 2019). It can be only speculated as to what production and/or quality control problem led to exceeding the gluten threshold in the case of two samples incorrectly marked as gluten-free. However, the 65.2 mg L^{-1} gluten level in the declared gluten-free beer is already a level that can cause significant damage to the intestinal morphology of coeliac patients in three months (Catassi et al. 2007).

Based on our results, some radlers and alcohol-free beers can be considered as alternatives to gluten-free beer. In general, radler is a beer diluted with fruit juice. The average gluten content in 13 radler samples was 12.8 mg L^{-1}, with the highest value of 23.0 mg L^{-1}. These results show that 84.6% of the radler samples can be labelled as gluten-free, while the remaining products as those with very low gluten content.

More than 44% of the non-alcoholic beer samples had gluten contents below the 20 mg L^{-1} threshold, which is appropriate for a gluten-free claim. In the remaining samples, the maximum gluten value was only 55.6 mg L^{-1}. These non-alcoholic beers can be classified as products with very low gluten. This is because non-alcoholic beers are produced with limited fermentation using worts of low original gravity (4–7.5% wt.) and a lower grain bill (Brányik et al. 2012).

The beer samples divided according to their alcohol by volume (ABV) into lager beer groups I, II and special beers had the maximum detected gluten values of 127.6, 216.0 and 258.0 mg L^{-1}, respectively. These maximum values are consistent with the increasing original gravity of beers, which can be deduced from their increasing alcohol content. In our studies, 16.3 to 16.6% of samples from lager beer groups I and II complied with a gluten-free claim, while among special beers, 25% of samples met the criteria. Unfortunately, many breweries do not analyse the gluten content of their beers, so they do not usually take advantage of this information during their marketing campaigns.

Wheat beers were the least suitable for a gluten-free diet. Their average gluten content (6 samples) had an average of 2 198.3 mg L^{-1}. Another study analysed 9 wheat beers and found the gluten content to be at least 270 mg L^{-1} (Watson et al. 2018).

CONCLUSION

People suffering from the coeliac disease, a lifelong disease caused by gluten intolerance, must exclude or reduce wheat, barley, rye and oats from their diet. Reliable information for consumers is a prerequisite for safe consumption of foods. The five-year monitoring of Czech commercial beers revealed that the occurrence of beers falsely labelled as gluten-free was 1%. In contrast, a significant number of radlers, non-alcoholic beers and even lagers and special beers comply with the threshold for a gluten-free claim, without this being indicated on the product label.

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