

Immunochemical determination of gluten in malts and beers

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The gluten content in different varieties of barley and malts, and in different types of beers, was determined by a 'sandwich' enzyme immunoassay (RIDASCREEN® Gliadin kit). The gluten levels in barley wheat, rye and spelt malts ranged 18.8–45.0, 44.0–68.0, 41.6 and 21.2 g kg⁻¹, respectively. When various types of beer were compared, the gluten concentration increased as follows: alcohol-free beer (<3.0), lager beers (<3.0–8.7 mg l⁻¹), stouts (9.0–15.2 mg l⁻¹) and wheat beers (10.6–41.2 mg l⁻¹). When 10 Czech lager beers were analysed, using both sandwich and competitive ELISA, the results showed that the latter method provided values several times higher than the former. Gluten balance was carried out during the brewing process, starting from the raw materials and terminating at the final beer. Gluten levels decreased due to precipitation during the mashing process, primary and secondary fermentation and, lastly, as a result of adsorption during beer stabilization. The gluten content in beer is, thus, approximately three orders of magnitude lower than in the raw malt.

Keywords: *Gluten, gliadin, peptide, protein, beer, malt, barley, wheat, prolamin content, coeliac disease*

Introduction

Coeliac disease (CD) is a digestive disease that damages the small intestine and interferes with absorption of nutrients from food (Lewis 2005). People who suffer from CD cannot tolerate a protein called gluten, which is found in wheat, rye, barley and, possibly, oats. Gluten consists, generally, of prolamin and glutelin proteins. Wheat prolamin is known as gliadin and barley prolamin as hordein. When people with CD consume foods containing gluten, their immune system responds by damaging the small intestine. Specifically, tiny finger-like protrusions, called villi, on the lining of the small intestine are lost. Nutrients from food are absorbed into the bloodstream through these villi; thus, without them, a person becomes malnourished. Because the body's own immune system causes the damage, coeliac disease is considered an autoimmune disorder (Lewis 2005). However, it is also classified as a disease of malabsorption because nutrients are not absorbed. If a person responds to a gluten-free diet (GFD), the physician knows for certain that the diagnosis of coeliac disease is correct. A GFD means avoiding all foods that contain wheat

(including spelt and triticale), rye, barley and, possibly, oats (Poms et al. 2004). Whether there is a maximum tolerable daily intake of gluten, which can be estimated for treated coeliacs, is an important issue. One study suggests that 10 mg should be considered as the maximum tolerable daily intake of gluten in treated coeliacs (Catassi et al. 2005). On the other hand, another study (Biagi et al. 2004) reports that even 1 mg of gluten per day prevents mucosal recovery. As each person suffering from CD has different tolerance levels to gluten ingestion, general, safe daily intake limits for gluten cannot be set.

With an annual consumption of almost 160 l of beer per capita, the Czech Republic is the world's number one beer-drinking nation. Beer constitutes an important part of the diet for a significant section of the Czech population. Beer contains only about 0.3% proteins or polypeptides, the principal source of which is malt. Malt is produced mostly only from wheat or barley. These cereals, however, also comprise prolamins that are associated with CD. Knowledge about the distribution and final levels of these proteins or their 'toxic' fragments in beer is essential to ensure the safe consumption of this drink

by coeliacs (Ellis et al. 1990, 1994; Kanerva et al. 2005; Hernando et al. 2005). Comprehensive quantitative data on the gluten content of beers is still lacking, and the same situation applies for gluten balance during the brewing process. This study has been undertaken to ascertain whether beer contains any gluten that could be toxic to coeliacs.

Materials and methods

Determination of gluten

Two sandwich enzyme-linked immunosorbent assay (ELISA) commercial kits were used for gluten determination, the RIDASCREEN® Gliadin kit, based on the monoclonal antibody R5 (Valdés et al. 2003; Méndez et al. 2005) (R-Biopharm AG, Darmstadt, Germany; Product no. R 7001), and the Gliadin ELISA kit, based on two monoclonal antibodies (Gabrovská et al. 2003, 2004, 2006) (Immunotech, Prague, Czech Republic; Prod. no. IM3717), with detection limits for gluten of 3 and 20 mg kg⁻¹ respectively. The manufacturers' instructions for proper use were followed. As the RIDASCREEN® Gliadin kit is effective for intact but not hydrolysed prolamins, hydrolysed peptides in beers were also analysed with a laboratory-made competitive ELISA kit, based on R5 antibody (Ferre et al. 2004; Hernando et al. 2005). This system (Méndez et al. 2005) has a detection limit of 6 ppm gluten, a reproducibility of 7.7%, repeatability of 4.7% and nearly quantitative recovery (90–96%). Gliadin standard (PWG gliadin standard – candidate reference material – IRMM-480) was used for calibration (Van Eckert 2002).

Sample preparation

Five grams of grains or malts were ground to a powder. A 1.0 g amount of representative flour was extracted with 10 ml of 60% (v/v) ethanol solution by shaking for 30 min, followed by centrifugation at 2500 g for 30 min (Valdés et al. 2003). One ml of beer or brewing semi-products (after removal of carbon dioxide) was mixed with 9 ml of ethanol solution (60% v/v). Beers with low prolamins concentrations were concentrated by freeze-drying.

Analyses of wheat, barleys, barley malts and malts from other cereals

Eleven commercial malts made from barley, wheat, spelt and rye were analysed for gluten content. These commercial malts were purchased from Weyermann Specialty Malting Company (Bamberg, Germany). Five barley commercial malts were prepared from five different barley varieties by the same malting technological process used in the Nymburk malt

house (Malteries Soufflet ČR, Plc., Czech Republic). All samples were analysed for gluten content by the RIDASCREEN® Gliadin kit, except for wheat and wheat malt samples, which were analysed by the Gliadin ELISA kit.

Technological trials: Brewing the beer

Beer for gluten balance trials was prepared in a brewery pilot plant (50 l of wort) at the Department of Fermentation Chemistry and Bioengineering of the Institute of Chemical Technology Prague. Mashing was a double mash process with a mashing-in temperature of 50°C. Only barley malt of the Pilsen-type was used for mashing. Wort was separated by the classical lautering process. Hop boiling time was 90 min. Only dried natural Czech Saaz hops were used. Final wort bitterness was 35 BU (EBC bitterness units, i.e. mg of bitter substances per litre of wort). After hot trub separation, fermentation was carried out with bottom-fermenting yeast strain 95, purchased from Research Institute of Brewing and Malting, Plc. (Prague, Czech Republic), using 14×10^6 viable cells per ml. The primary fermentation process took place at 12°C. A cold maturation was carried out for 21 days at 1°C. After kieselguhr filtration, beer was stabilised by polyvinylpyrrolidone (PVPP; 30 g hl⁻¹) and by silica gel (Stabiquick strong; 30 g hl⁻¹).

Analyses of beers

Nineteen commercial beers, which represent all principle brands available on the Czech market, were analysed for gluten content. All beers were analysed for gluten content by the RIDASCREEN® Gliadin kit, while some beers with very low gluten content (detected by RIDASCREEN® Gliadin kit) were also analysed by the competitive ELISA kit based on the R5 antibody. One commercial beer was additionally treated for removal of proteins by silica gel – Stabiquick strong and Stabifix super (2 g l⁻¹) from Stabifix Braurei-Technik (Munich, Germany). This trial was performed for confirmation if there was a possibility of reducing the level of gluten in the final beer.

Results and discussion

Barley (wheat), hop, yeast and water are the basic raw materials for brewing beer. In beer, gluten or by-products of its hydrolysis can come only from barley (wheat). Malt is produced from barley and wheat by a malting process, which consists of steeping, germination and kilning. The activity of amylase enzymes and proteolytic modification in grains increases during the malting process. Wheat and

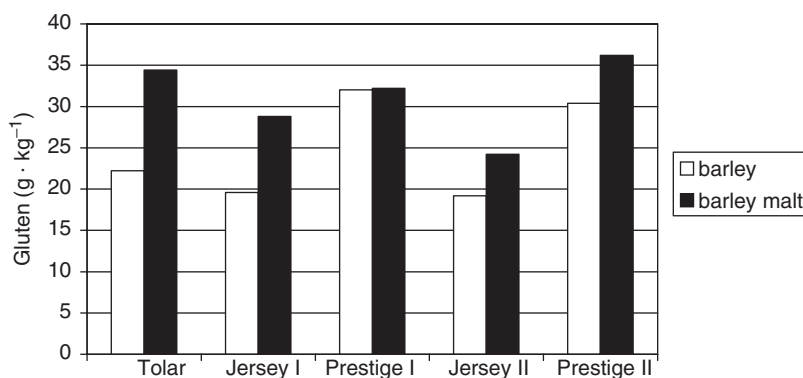


Figure 1. Gluten content in barley varieties and corresponding malts. Varieties I and II originated from different culture area (all samples were analysed by RIDASCREEN® Gliadin kit).

malt, prepared from the wheat, were analysed for gluten content. Wheat contained 50.4 ± 1.8 g gluten per kg and the corresponding malt 68.0 ± 4.0 g gluten per kg. These gluten levels were determined by the Gliadin ELISA kit, in which the antibody used (against wheat gliadin) has only $\sim 20\%$ cross-reactivity with barley prolamins (Gabrovská et al. 2004, 2006). The higher level of gluten in malt compared to original grain is fully confirmed by analysis of gluten in three barley varieties from two different growing areas and the corresponding malt samples produced under the same malting conditions (Figure 1). Gluten analysis of these samples was performed by the RIDASCREEN® Gliadin kit. In this case, the monoclonal antibody detects the gliadin fractions from wheat and the corresponding prolamins from barley and rye (Valdés et al. 2003) equally. Content of gluten in cereals and malts are lower than the theoretical levels (Belitz et al. 2004), due to the analytical technique used. This result was also found in a previous study (Ellis et al. 1990). Table I shows gluten content in commercial cereal malts. The highest content was in wheat pale malt, while the content of gluten in several kinds of barley malt varied from 18.8 to 45.0 g kg⁻¹. In spelt and rye malt, the gluten content was in approximately the same range.

Gluten balance was examined from raw materials to final beer during the brewing process (Table II). Malt, used for brewing beer, was the 100% gluten source. Malt extract is transferred into solution during the mashing process in the brewing house when most of the proteins are precipitated and only some split into simple polypeptides (Lewis 2005). The majority of precipitated proteins remain in the spent grain after the lautering process; only a small proportion of gluten (1.75%) passed from malt to sweet wort. Negligible gluten depletion was noted in hopped wort after boiling (1.70%). The pH decreased during the primary and secondary

Table I. Mean gluten content in types of malt.

Malt type	Gluten (g kg ⁻¹) ^{a,c}
Caraaroma (barley)	42.0 ± 0.2
Melanoid (barley)	20.4 ± 0.2
Cararred (barley)	20.6 ± 0.2
Carafa (barley)	45.0 ± 0.2
Pilsner (barley)	18.8 ± 0.6
Smoked (barley)	20.8 ± 1.0
Munich (barley)	20.2 ± 0.2
Wheat chocolate (Wheat) ^b	44.0 ± 1.6
Wheat pale (Wheat) ^b	68.0 ± 4.0
Spelt chocolate (Spelt)	21.2 ± 0.4
Rye chocolate (Rye)	41.6 ± 1.4

^aMean ± standard deviation ($n = 3$). ^bSample analysed by Gliadin ELISA kit. ^cAll samples, except wheat, were analysed by RIDASCREEN® Gliadin kit.

Table II. Gluten balance during brewing process.

Product	Gluten ^b (mg kg ⁻¹)	(%) ^a
Malt	18 780	100
Sweet wort	49.4	1.75
Wort	48.0	1.70
Beer	6.0	0.21
Stabilised beer	<3.0	<0.11

^aGluten content in malt represents 100%. ^bAll samples were analysed by RIDASCREEN® Gliadin kit.

fermentation processes, causing precipitation of some polypeptides and their adsorption onto yeast cell surfaces (only 0.21% of the original gluten content remains in beer). After the filtration process, beer is colloidal stabilised by removal of proteins and polyphenolic substances by adsorption on polyvinylpyrrolidone (PVPP) and kiesegel (silica gel), resulting in <0.11% of original content of gluten (Table II).

As shown in Table III, gluten levels of commercial beers on the Czech market are very variable. Gluten concentration increases from alcohol-free beer ($<3.0 \text{ mg l}^{-1}$), lager beers ($<3.0\text{--}8.7 \text{ mg l}^{-1}$), stouts ($9.0\text{--}15.2 \text{ mg l}^{-1}$), to wheat beers ($10.6\text{--}41.2 \text{ mg l}^{-1}$). The gluten content of some Czech lager beers, analysed by the sandwich RIDASCREEN® Gliadin kit and the competitive ELISA kit (both based on R5 antibody), are shown in Table IV. It is evident that not all Czech lager beers contain $<20 \text{ mg}$ gluten per litre. Since 1996, the legislative threshold for gluten-free foods from nature is 20 mg kg^{-1} gluten

Table III. Gluten content of beers from the Czech market.

Beer	Gluten (mg l^{-1}) ^{a,b}
A – Czech alcohol-free beer	<3.0
B – Czech lager beer	<3.0
C – Czech lager beer	<3.0
D – Czech lager beer	6.6 ± 0.2
E – Czech lager beer	8.7 ± 0.4
F – Czech lager beer	4.2 ± 0.4
G – Czech lager beer	4.4 ± 0.4
H – Czech lager beer	<3.0
I – Czech lager beer	<3.0
J – Czech lager beer	<3.0
K – Czech lager beer	5.2 ± 0.4
L – Czech lager beer	3.0 ± 0.2
M – Czech lager beer	<3.0
N – Belgium Belle Vue Kriek	10.6 ± 0.4
O – Czech wheat beer	23.6 ± 1.8
P – Belgian Hoegaarden wheat beer	39.0 ± 0.4
Q – Czech wheat beer	41.2 ± 6.0
R – Irish Guinness stout	15.2 ± 1.8
S – Irish Murphy's stout	9.0 ± 0.6

^aMean $\pm$ standard deviation ($n=3$). ^bAll samples were analysed by RIDASCREEN® Gliadin kit.

Table IV. Gluten content of some Czech lager beers (analysed by two methods).

Czech lager beer samples	Gluten (mg kg^{-1})	
	RIDASCREEN® sandwich ELISA	R5 competitive ELISA
A	<3.0	24.5
B	<3.0	28.3
C	<3.0	58.6
D	6.6	102.1
E	8.7	36.0
F	4.2	22.0
G	4.4	54.7
H	<3.0	11.0
I	<3.0	<6.0
J	<3.0	11.0
J ^a	<3.0	<6.0
J ^b	<3.0	<6.0

^aTreated with Stabiquick strong (2 g l^{-1}). ^bTreated with Stabifix super (2 g l^{-1}).

(10 mg kg^{-1} gliadin) (Deutsch 2005). The R5 antibody is capable of recognising several, small repetitive celiac-toxic epitopes, QQPFP, LQPFP, QLPYP, QLPTP, QQSFP, QQTFP, PQPFP, QQPYP and PQPFP, in prolamins molecules (Osman et al. 2001; Kahlenberg et al. 2006). The sandwich R5-ELISA needs at least two specific celiac-toxic epitopes. Prolamins present in beer are partially hydrolysed in fragments with one or more celiac-toxic epitope. Consequently, these small fragments, containing a single celiac-toxic epitope, cannot be measured by conventional sandwich R5-ELISA – the hydrolysed prolamins are always underestimated. Competitive R5-ELISA is more efficient than sandwich ELISA in quantifying hydrolysed prolamins, as demonstrated in the analysis in Table IV, where the competitive R5-ELISA yields a higher prolamins content than sandwich ELISA. Competitive R5-ELISA is a reliable assay for determination of gluten-free beers based on wheat or barley malt extract, as demonstrated in barley malt beers approved by the Finnish Coeliac Society for consumption by coeliacs (Hernando et al. 2005). Prolamins present in beer are partially hydrolysed and these small fragments always contain proline (Dale et al. 1989; Siebert 1999; Siebert and Lynn 2005). The haze-forming activity of a polypeptide in beer depends greatly on its proline content (Siebert 1999). Haze-forming polyphenols have at least two binding groups, each of which has at least two hydroxyl groups on an aromatic ring for binding the proline part of protein (Siebert 1999). As proline is structurally similar to pyrrolidone, the PVPP can bind polyphenols (Siebert and Lynn 2005; Leiper et al. 2005). Thus, measurement of prolamins concentration is an excellent tool for predicting colloidal beer stability (Siebert 1999; Siebert and Lynn 2005).

Conclusions

Two commercial sandwich enzyme-linked immunosorbent assays (Gliadin ELISA and RIDASCREEN® Gliadin kits) and a competitive ELISA kit were used for gluten determination in barley, wheat, malts and beers. Gliadin ELISA kit is applicable only for intact prolamins from wheat and wheat malts. The RIDASCREEN® Gliadin kit can be used for intact prolamins from wheat, barley and rye, while the competitive R5-ELISA is ideal for testing hydrolysed wheat, barley and rye prolamins in beer.

Gluten levels in barley wheat, rye and spelt malts ranged $18.8\text{--}45.0$, $44.0\text{--}68.0$, 41.6 and 21.2 g kg^{-1} , respectively. The concentration of gluten decreased from 100% in malt to less than 0.11% in stabilized

beer during the mashing process, primary and secondary fermentation and stabilization process.

Gluten concentrations were found to increase from alcohol-free beer ($<3.0 \text{ mg l}^{-1}$), lager beers ($<3.0\text{--}8.7 \text{ mg l}^{-1}$), stouts ($9.0\text{--}15.2 \text{ mg l}^{-1}$) to wheat beers ($10.6\text{--}41.2 \text{ mg l}^{-1}$). Only 84% of the 19 beers sampled (analysed by RIDASCREEN® Gliadin kit) was safe for consumption by coeliacs as regards the legislative limit (20 mg l^{-1}). The number of 'safe' beers dramatically decreased to 30%, if the samples were analysed by competitive ELISA. Nevertheless, there is no safe beer produced from barley or wheat regarding the maximum tolerable daily intake (10 mg) and different tolerance levels of gluten for coeliac disease sufferers.

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