Immunochemical determination of gluten in malts and beers

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Immunochemical determination of gluten in malts and beers

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Abstract

The gluten content in the different varieties of barley, malts, and different types of beers were determined by means of "sandwich" enzyme immunoassay (RIDASCREEN[®] Gliadin kit). The gluten levels in barley wheat, rye and spelt malts ranged from 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). Ten Czech lager beers were analysed using both sandwich and competitive ELISA and the results were compared. The latter method provided several times higher values than former one. Gluten balance was carried out during the brewing process starting from raw materials and terminating at the final beer. The amount of protein decreased due to precipitation of protein 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 that 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 from prolamin and glutelin proteins. Wheat prolamin is known as gliadin and barley prolamin as hordein. When people

with CD eat 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. Without villi, a person becomes malnourished. Because the body's own immune system causes the damage, coeliac disease is considered as 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 the gluten-free diet (GFD), the physician will know 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 some maximum tolerable daily intake of gluten which can be estimated for treated coeliacs is an important issue. There is a study which 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, there is another study (Biagi et al. 2004) which reports that even a milligram of gluten a day keeps the mucosal recovery away. As each person suffering from CD has different tolerance to gluten ingestion, we cannot fix general safe daily intake limit for gluten.

With an annual consumption of almost 160 litres of beer per capita, the Czech Republic is the world's number one beer 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 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; Ellis et al. 1994; Kanerva et al. 2005;

Hernando et al. 2005). Comprehensive quantitative data on the gluten content of beers is still lacking, the same situation is for gluten balance during the brewing process. This study has been undertaken to find out 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, prod. no. R 7001) and Gliadin ELISA kit based on two monoclonal antibodies (Gabrovská et al. 2003; Gabrovská et al. 2004; Gabrovská et al. 2006) (Immunotech a Beckman Coulter Company, Prague, Czech Republic, prod. No. IM3717) with a detection limit for gluten of 3 and 20 mg kg⁻¹ respectively. The instructions manufacturers for their proper use were followed. As RIDASCREEN[®] Gliadin kit is effective for intact prolamins but not for hydrolysed ones, hydrolysed peptides in beers were also analysed by a home-made competitive ELISA kit based on R5 antibody (Ferre et al. 2004; Hernando et al. 2005). This system (Méndez, García and Ferre 2005) has a detection limit 6 ppm of gluten, reproducibility 7.7 %, repeatability 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 grinded to powder. A 1.0 g amount of representative flour was extracted with 10 ml of 60 % (v/v) ethanol solution by

shaking for 30 minutes, followed by centrifugation at 2500 g for 30 minutes (Valdés et al. 2003). One ml of beer or brewing semiproducts (after removal of carbon dioxide) was mixed with 9 ml of ethanol solution (60% v/v). Beers with low prolamin 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 in Nymburk malt house (Malteries Soufflet ČR, Plc., Czech Republic). All samples were analysed for gluten content by RIDASCREEN® Gliadin kit except of wheat and wheat malt samples, which were analysed by Gliadin ELISA kit.

Technological trials – brewing the beer

Beer for gluten balance trial was prepared in brewery pilot plant (50 litres of wort) in the Department of Fermentation Chemistry and Bioengineering in the Institute of Chemical Technology Prague. Mashing process was double mash process with mashing-in temperature of 50 °C. Only barley malt of Pilsen type was used for mashing. Wort was separated by a classical lautering process. Hop boiling time was 90 minutes. Dried natural Czech Saaz hop was used only. Final wort bitterness was 35 BU (EBC bitterness units – 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 $14 \cdot 10^6$ 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

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3 filtration beer was stabilised by PVPP (30 g hl⁻¹) and by silica gel (Stabiquick strong –
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5 30 g hl⁻¹).
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8 9 *Analyses of beers*

10
11 Nineteen commercial beers which represent all principle brands of beers available on
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13 the Czech market were analysed for gluten content. All beers were analysed for gluten
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15 content by RIDASCREEN® Gliadin kit and only some beers with very low gluten
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17 content detected by RIDASCREEN® Gliadin kit were also analysed by competitive
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19 ELISA kit based on R5 antibody. One commercial beer was additionally treated for
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21 removal of proteins by silica gel – Stabiquick strong and Stabifix super (2 g·l⁻¹) from
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23 Stabifix Braurei-Technik (München, Germany). This trial was done for confirmation,
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25 if there is a possibility to reduce the level of gluten in final beer
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31 **Results and discussion**

32
33 Barley (wheat), hop, yeast and water are basic raw materials for brewing the beer.
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35 Gluten or products of their hydrolysis detected in beer can come only from barley
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37 (wheat). Malt for production of beer is produced from barley and wheat by malting
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39 process, which consists of steeping, germination and kilning. The activities of
40
41 amylase enzymes and proteolytic modification in grains increase during the malting
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43 process. Wheat and malt prepared from the wheat were analysed for gluten content.
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45 Wheat contained 50.4 ± 1.8 grams of gluten per kg and corresponding malt 68.0 ± 4.0
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47 grams of gluten per kg. These gluten levels were determined by Gliadin ELISA kit in
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49 which the used antibody (against wheat gliadin) has cross-reactivity with barley
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51 prolamins only about 20% (Gabrovská et al. 2004; Gabrovská et al. 2006). The higher
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53 level of gluten in malt compared to original grain is fully confirmed by analysis of
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55 gluten in three barley varieties from two different growing areas and the
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57 corresponding malt samples produced under the same malting conditions (Figure 1).
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Gluten analysis of these samples was performed by RIDASCREEN[®] Gliadin kit. In this case, the used monoclonal antibody detects the gliadin fractions from wheat and corresponding prolamins from barley and rye (Valdés et al. 2003) equally. Content of gluten in cereals and malts are lower then the theoretical ones (Belitz et al. 2004) due to used analytical technique. These results were found also in previous work (Ellis at al. 1990). Table I shows gluten content in commercial cereal malts. The highest content of gluten was ascertained in wheat pale malt. The content of gluten in several kinds of barley malt varied from 18.8 to 45.0 g kg⁻¹. The gluten content in spelt and rye malt ranged approximately over the same interval.

[Insert Figure 1 about here]

[Insert Table I about here]

The gluten balance was examined from raw materials to final beer during the brewing process (Table II). Total gluten content in malt, used for brewing beer, was 100 % gluten source. Malt extract was transferred into solution during the mashing process in the brewing house. During the mashing process in the brewing house most of proteins were precipitated and only some proteins were spit into simple polypeptides (Lewis 2005). The majority of precipitated proteins remained in the spent grain after lautering process. Only a small part of gluten (1.75 %) passed from malt to sweet wort. Negligible consequent gluten depletion was noted in hopped wort after hop boiling (1.70 %). The pH decreased during the primary and secondary

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3 fermentation processes. It caused precipitation of some polypeptides and their
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5 adsorption onto the yeast cell surface (only 0.21 % of the original gluten content
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7 remains in beer). After the filtration process beer is colloidally stabilised by removal
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9 of proteins and polyphenolic substances by adsorption on polyvinylpolypyrrolidone
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11 (PVPP) and kieselgel (silica gel). This process (less than 0.11 % of original content of
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13 gluten) resulted in decreasing of gluten content (Table II).
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[Insert Table II about here]

28 As shown in Table III, gluten level of commercial beers from the Czech market
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30 are very variable. The gluten concentration increases from alcohol free beer (<3.0),
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32 lager beers (<3.0 - 8.7 mg l^{-1}), stouts (9.0 - 15.2 mg l^{-1}), to wheat beers (10.6 - 41.2 mg l^{-1}).
33
34 The gluten content of some Czech lager beers analysed by sandwich
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36 RIDASCREEN® Gliadin kit and by competitive ELISA kit based on R5 antibody are
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38 shown in Table IV. The both kits are based on R5 antibody. It is evident that not all
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40 Czech lager beers contain less than 20 mg of gluten per litre. Since 1996, the
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42 legislation threshold for foods gluten-free by nature is 20 mg kg^{-1} of gluten (10 mg kg^{-1}
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44 of gliadin) (Deutsch 2005). The R5 antibody is capable of recognising several and
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46 small repetitive celiac-toxic epitopes QQPFP, LQPFP, QLPYP, QLPTP, QQSFP,
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48 QQTFP, PQPFP, QQPYP and PQPFP in prolamin molecules (Osman et al. 2001;
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50 Kahlenberg et al. 2006). The sandwich R5-ELISA needs at least two specific celiac-
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52 toxic epitopes. Prolamins presented in beer are partially hydrolysed in fragments with
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54 one or a few celiac-toxic epitopes. Consequently these small fragments containing a
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56 single celiac-toxic epitope cannot be measured by the conventional sandwich R5-
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ELISA, hydrolysed prolamins are always underestimated. The efficiency of the competitive R5-ELISA over the sandwich is superior in quantifying hydrolysed prolamins as demonstrated in the analysis of beers in Table IV. The competitive R5-ELISA yields prolamins contents higher than the sandwich ELISA. The competitive R5-ELISA is a reliable assay for determination of gluten-free beers based on wheat or barley malt extract as had been demonstrated in some beers based on barley malt and 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 of 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). Measurement of prolamins concentration thus can represent excellent tool for prediction of colloidal beer stability (Siebert 1999; Siebert and Lynn 2005).

[Insert Table III about here]

[Insert Table IV about here]

Conclusions

Two commercial sandwich enzyme-linked immunosorbent assays (Gliadin ELISA kit and RIDASCREEN® Gliadin kit) and 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. 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.

The gluten level ranges in barley wheat, rye and spelt malts were found to be 18.8-45.0, 44.0-68.0, 41.6, and 21.2 g kg⁻¹, respectively. The amount of these proteins, 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), lager beers (<3.0-8.7 mg·l⁻¹), stouts (9.0-15.2 mg·l⁻¹), to wheat beers (10.6-41.2 mg·l⁻¹). Only 84 % of nineteen beer samples (analysed by RIDASCREEN® Gliadin kit) was safe for consumption of coeliacs from point of view legislation limit (20 mg·l⁻¹). The number of safe beer samples 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 of gluten (10 mg) and different tolerance of the patients suffering coeliac disease to gluten level.

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Figure 1. Content of gluten in some barley varieties and corresponding barley malts. Varieties I and II origin from different culture area (all samples were analysed by RIDASCREEN[®] Gliadin kit).

Table I. Mean content of gluten in some malt types

Malt type	Gluten (g kg ⁻¹) ^{ac}
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

^a±standard deviation (n=3); ^bSample analysed by Gliadin ELISA kit

^cAll samples except of wheat samples were analysed by RIDASCREEN[®] Gliadin kit

Table II. Balance of gluten in course of the brewing process

Raw material, semiproducts, product	Gluten ^b (mg kg ⁻¹)	(%) ^a
Malt	18780	100
Sweet wort	49.4	1.75
Wort	48.0	1.70
Beer	6.0	0.21
Stabilised beer	<3.0	<0.11

^aContent of gluten in malt represents 100%

^bAll samples were analysed by RIDASCREEN[®] Gliadin kit.

Table III. Content of gluten in beers from the Czech market

Beer	Gluten (mg l ⁻¹) ^{ab}
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

^a±standard deviation (n=3);

^bAll samples were analysed by RIDASCREEN® Gliadin kit.

Table IV. Content of gluten in 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 – treated by Stabiquick strong (2g l ⁻¹)	< 3.0	< 6.0
J – treated by Stabifix super (2g l ⁻¹)	< 3.0	< 6.0

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