Development of the first Spanish beer based on barley malt suitable for coeliacs: Estrella Damm Apta Para Celiacos

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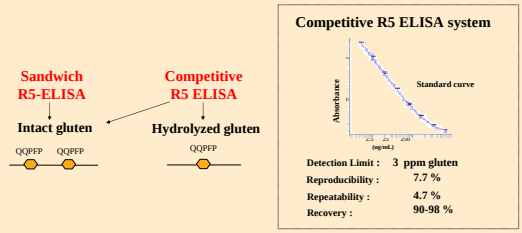
Introduction

People who suffer from coeliac disease cannot include wheat, barley or rye in the diet. Therefore the coeliac population cannot drink beers based on wheat and barley. One of the main problems has been the difficulty to measure hydrolyzed prolamins in foods samples, as in the case of prolamins in malt and residual prolamins in beers, which are partially or completely hydrolyzed as a result of proteolytic activity during the malting process. Levels of hydrolyzed fragments containing a unique QQPFP epitope will be underestimated by sandwich R5-ELISA since this system needs at least two QQPFP epitopes.

Thus, we have developed a competitive ELISA, based on R5 antibody, capable to quantify hydrolyzed prolamins in beers, with a sensitivity of 3 ppm. The aim of this study is to extend the capability of the competitive R5-ELISA to manufacture a commercial beer based on barley malt and to study the gluten balance during the brewing process in order to guarantee a level of gluten safe for the consumption by coeliacs in collaboration with Damm Group S.A.

Material and Methods

Prolamins were extracted from the samples with 60% ethanol. Sandwich and competitive R5-ELISA have been used to quantify hydrolyzed prolamins in beers, barley grains, malt extracts and worts. R5-Western Blot and MALDI-TOF Mass Spectrometry has been used to confirm the extent of hydrolysis.



Results and Discussion

The efficiency of the competitive R5-ELISA comparing to the sandwich is higher in quantifying hydrolysed prolamins as demonstrated in the analysis of commercial beers based on barley malt. While in some beers the levels of prolamins by sandwich R5-ELISA are lower than 20 ppm, the competitive R5-ELISA always yielded higher prolamins values with factors ranging from 1.9 to 17-fold. Besides, data indicate that levels of prolamins in beers from different countries and/or companies are unpredictable. Western blot confirms the presence/absence of hydrolysed prolamins in some of the beers analysed. The region of intact and hydrolysed immunoreactive bands are indicated by arrows and values in ppm of gluten were quantified by competitive R5 ELISA (Figure 1).

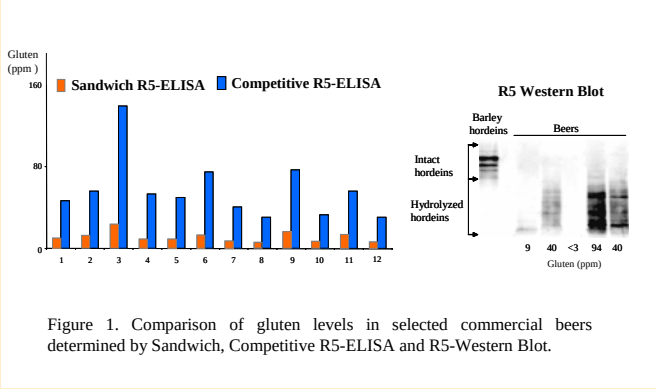


Figure 1. Comparison of gluten levels in selected commercial beers determined by Sandwich, Competitive R5-ELISA and R5-Western Blot.

The analysis of gluten during the brewing process in a pilot plant shows that barley grains and malts present a massive gluten content while in worts these levels are lower comparing with barley grains and malts (Figure 2).

The qualitative analysis of hordeins in barley grains, malts and worts, performed by MALDI-TOF Mass Spectrometry technique, show a significantly decrease in hordeins mass signal in a similar way as when the analysis is performed by R5-Western blot. The hordeins in grains and in malts are detected by Western blot by a presence of typical immunoreactive bands detected with R5 antibody in the range which corresponds to intact hordeins (30 to 40 kDa) In worts is observed a change in this pattern appearing lower mass molecular weight bands due to the apparition of low mass molecular weight fragments (hydrolysed hordeins) resulting from hordein hydrolysis (Figure 2).

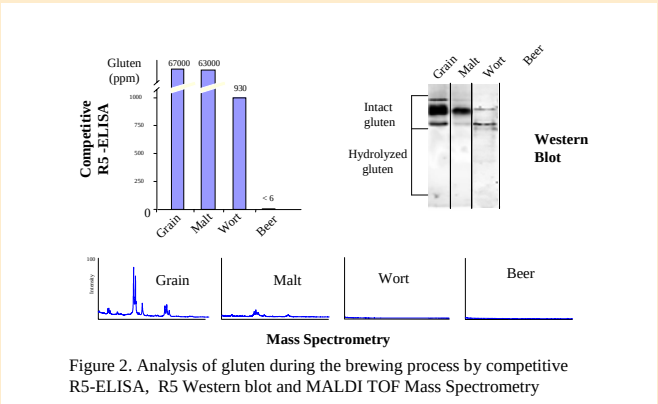


Figure 2. Analysis of gluten during the brewing process by competitive R5-ELISA, R5 Western blot and MALDI TOF Mass Spectrometry

In the framework of a collaborative project with S.A. Damm, 179 bottled beers from different batches along 2005-2006, obtained from different brews (A-F) were analysed by competitive R5-ELISA (Figure 3, top). Analysis of most of these beers demonstrate levels of gluten below 6 ppm (maximum gluten level guaranteed by S.A. Damm). In brew F, 11 bottled beers were analysed in order to probe that every one presented gluten values below 6 ppm. Western Blot of selected beers from different brews demonstrate the absence of both intact and hydrolyzed hordeins and a contaminated beer with 9 ppm has been included as a control in this analysis (Figure 3, bottom). Therefore this brewing process has been used for the commercialization of the first Spanish beer based on barley malt called Estrella Damm Apta para celíacos "suitable for coeliacs" (Estrella Damm APC). This beer has been approved by the Spanish Coeliac Society for consumption by coeliacs.

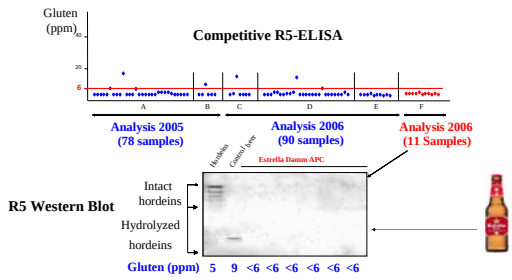


Figure3. (Top) Analysis by competitive R5-ELISA of bottled beers from different brews (A-F). (Bottom) Western Blot of selected beers from different brews.

In order to guarantee levels of gluten below 6 ppm different samples from the filtered beer tank prepared for the production of such Estrella Damm "suitable for coeliacs" were analysed by competitive R5-ELISA. Besides, after packing every hour of the commercial bottled beers were sampled and analysed for testing gluten levels below 6 ppm and then were certified by the National Center of Biotechnology (CNB) (Figure 4).

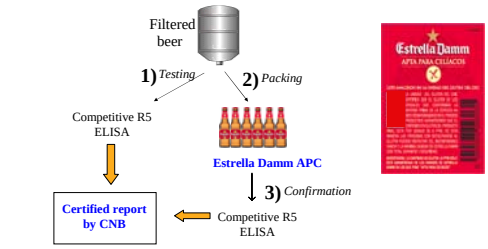


Figure 4. Testing and confirmation of filtered beer to guarantee gluten level below 6 ppm by competitive R5 ELISA

Conclusions

The competitive R5-ELISA is the most adequate technique to quantify hydrolyzed gluten in beers. Those beers presenting gluten values under 6 ppm could be labelled as "gluten-free" and suitable for coeliacs according the actual legislation in most countries.

References

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