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A PRELIMINARY STUDY ABOUT GLUTEN LEVELS IN SARDINIAN CRAFT BEERS

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ABSTRACT

The aim of this study was to determine the gluten content of a representative sampling of Sardinian craft beers. Twenty-five craft beers produced by seven Sardinian microbreweries were analyzed. All beers were produced without micro-filtration, pasteurization or preservative additions provided by Italian law. The competitive enzyme immunoassay (Ridascreen competitive ELISA kit) was used for gluten quantification. The levels of gluten found ranged from 39 mg/L in a Pilsner beer to 2400 mg/L in a Weizen. No gluten-free beer was found and eight (32%) of the twenty-five beers analyzed contained less than 100 mg/L of gluten and could be labeled as "very low gluten" according to the Commission Regulation (EC) No 41/2009.
Furthermore a significant positive correlation between gluten content and foam stability was found.

Keywords: Craft beer; Beer; ELISA; Antibody R5; Celiac disease; Gluten-free beer; Foam stability

INTRODUCTION

It is well known that beer is one of the most consumed alcoholic beverages all over the world. Its average annual consumption is 74 and 86 liters per capita in Europe and Northern America respectively **(Hager *et al.*, 2014)**. In Italy it is around 29.2 liters per capita whereas in Sardinia, an Italian region in the center of the Mediterranean Sea, it is almost double **(Assobirra, 2011)**.
Currently, beer consumption is appreciably shifting towards the less explored world of craft beer **(Assobirra, 2015)**. Craft beer is mainly chosen according to different flavor preferences and is mainly drunk by habitual beer drinkers in places traditionally connected to its consumption such as pubs and restaurants **(Aquilani *et al.*, 2015)**. It is perceived to be of higher quality than industrial beer. Moreover, its antioxidant capacity is higher **(Sanna and Pretti, 2015; Tubaro, 2009)**.
As sanctioned by the Italian law**(Legge 28 luglio 2016, n. 154, 2016)**, craft beer must be produced by microbreweries, small and independent companies which produce limited amounts of beer (less than 200.000 hectoliters per year) and must be neither micro-filtered nor pasteurized. Furthermore craft beers are often bottled with yeast and sugar to naturally produce carbon dioxide within the bottle in a process called bottle refermentation **(Briggs, 2004)**.
The experience of microbreweries in Sardinia started quite early, if compared with the rest of Italy. It was in 1993 when the micro-brewery Adis Scopel (Guspini, Sardinia, Italy) proposed its beers to the local market. In 2016 twenty-two microbreweries in operation could be counted in the region, evenly distributed throughout a territory that has a little more than 1.6 million inhabitants.
Since for brewing usually gluten-containing barley malts are used, some amount of gluten is expected in the final product and it may be toxic for celiac patients. Celiac Disease (CD) is an autoimmune disorder characterized by a chronic inflammation of the small intestine due to a permanent intolerance to gluten proteins (or to their fragments produced during digestion) found in wheat, barley and rye **(Selimoglu*et al.*, 2010)**. Gluten proteins are composed of prolamins and glutelins located in the starchy endosperm of the cereal. The most troublesome components of gluten are the prolamins; such as gliadin from wheat, hordein from barley, secalin from rye and avenin from oats **(Van Landschoot, 2011)**. The functional changes in celiac patients include reduction in food digestion, decreased absorption of macro and micronutrients and increased secretion of water and solutes **(Rostom*et al.*, 2006)**.
Combining the cases of NCGS (Non-Celiac Gluten Sensitivity) with those of CD, the incidence rises alarmingly to 6 people out of 100 worldwide **(Genetics home reference, 2015)**. According to the Annual Report of the Italian Ministry of Health, CD prevalence in Italy is 0.28%, but it is thought to be underestimated (Figure 1). Sardinia is the Italian region with the highest prevalence of CD **(Italian Ministry of Health, 2014)**.
CD seems to be highly correlated with other autoimmune diseases **(Gujral et al., 2012)**, so its relatively high rate in Sardinian population is not surprising. The Mediterranean island of Sardinia is in fact well known for being a genetic isolate with a particularly high prevalence of autoimmune diseases **(Sardu et al., 2012)**. As observed all over the world, CD prevalence is also increasing in Sardinia (Figure 1) with the total number of diagnosed CD patients passing from 3169 in 2007 to 6145 in 2014 **(Italian Ministry of Health, 2008, 2015).** For these reasons, gluten content characterization of food and beverage appears impellent.

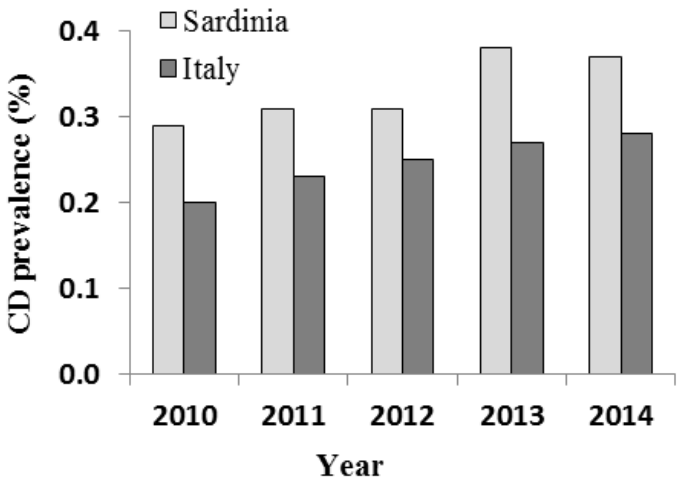

Figure 1 Increasing of Celiac Desease (CD) prevalence in Italy and Sardinia from 2010 to 2014. Data from the annual reports on Celiac Disease of the Italian Ministry of Health (2011, 2013, 2014, 2015).

Elimination of sources of gluten from the diet is the only effective remedy to lead a normal life. Therefore, it is very important to know the effective daily dose of gluten intake, without neglecting the hydrolyzed forms present in fermented products such as beer, and a correct characterization and proper labeling of products becomes essential.

In 2009 the Codex Alimentarius Commission asserted that gluten-free foods cannot contain wheat, rye, barley, oats or their crossbred varieties, unless they have been specially processed to reduce the gluten level below 20 ppm **(Codex Alimentarius Commission, 2008)**.

Several commercial test kits for gluten quantification are available and the majority are based on ELISA (enzyme linked immunosorbent assay). The official standard method for gluten determination according to the Codex Alimentarius is an ELISA which uses the R5 antibody. Nowadays, second generation competitive ELISA based on the Mendez R5 antibody is considered the most reliable method to detect partially hydrolyzed prolamins in beer **(Panda *et al.*, 2015; Hager *et al.*, 2014; Haas-Lauterbach*et al.*, 2013)**.

Considering the massive amount of beer consumed in Sardinia, the number of craft breweries and the increase of diagnosed CD patients on the island, we made a screening of the most representative craft beer styles produced in Sardinia to quantify their actual gluten content. In order to assess the productive range of the island, eighteen different beer styles were selected with an alcohol content in the range from 3.59 %V/V to 8.15% V/V and different grist composition.

MATERIAL AND METHODS

Beer sampling

Twenty-five bottled beers produced in Sardinia by seven local micro-breweries were analyzed: seventeen all-barley malt (one Altbier, two Amber Ale, one America IPA, one Bitter, one Dubbel, one Golden Ale, one Helles, one Imperial Stout, one Italian Grape Ale, two Pale Ale, three Pilsner, one Rauchbier and one Strong Ale) and eight wheat content beer (three Blanche, one Saison, one Stout, two Weizen and one White IPA). For gluten determination and beer analysis three bottles of each brand and beer style were analyzed.

Determination of gluten contents

Analysis of gluten content in beer was performed by a competitive immune enzymatic method because it is the method of choice for the determination of peptide fragments in beers and other food products containing hydrolyzed proteins **(Codex Alimentarius Commission, 2008)**.Analysis was carried out by the RIDASCREEN Gliadin competitive assay (Art. No. R7021, R-Biopharm, Darmstadt, Germany) according to the manufacturer's instruction. The monoclonal antibody R5 recognizes several small repetitive celiac-toxic epitopes (QQPFP, LQPFP, QLPYP, QLPTP, QQSFP, QQTFP, PQPFP, QQPYP and PQPFP). Since the epitope QQPFP is present in wheat gliadin, barley hordein and rye secalin, antibody R5 recognizes fractions in all of these grains **(Haas-Lauterbach*et al.*, 2012)**.

Bottles were shaked for 3 minutes in order to suspend the pellet on the bottom and 100 ml of beer was poured in a beaker and degassed; 1 ml sample was used for gluten determination. Gliadin concentrations were converted into gluten concentrations by multiplying the test results by a factor of 2 **(Codex Alimentarius Commission, 2008)**. The beer containing wheat was diluted to a final dilution factor of 2500; the all-barley malt beers were diluted to a final dilution factor of 500 as described in the assay. A commercially available gluten-free beer made from barley malt (Peroni gluten-free, Peroni, Roma, Italia) was used as negative control, and the kit prolamine-free solution was used as blank.

Beer analysis

The original extract (% w/w), apparent extract (% w/w) and alcohol content (% V/V) were measured with a DMA 4500 density meter (model PBA-B Generation M, Anton Paar, Graz, Austria). Foam stability was measured with a NIBEM-TPH foam stability tester (Haffmans, Zeist, The Netherlands) according to the official Analytica EBC method 9.42.1 **(European Brewery Convention, 1998)**. Analysis was conducted at 20°C, using carbon dioxide to generate a standard glass full of foam. The Nibem tester measures the foam collapse time over a distance of 30mm. Turbidity analysis was performed according to the official Analytica-EBC method 9.29 **(European Brewery Convention, 1998)** using a Turbidity meter (model HI-93703 Hanna Instruments) and was expressed in EBC units.

RESULTS AND DISCUSSION

Determination of gluten contents in Sardinian craft beer samples

Gluten level of industrial beers has been analyzed by competitive ELISA in precedent studies **(Guerdrum and Bamforth, 2011; Van Landschoot, 2011; Dostálek*et al.*, 2006)**, but gluten content of Sardinian craft beers was here analyzed for the first time. Table 1 shows the measured gluten levels and data analysis of twenty-five craft beers.

Table 1 Gluten levels, original extract, apparent extract, alcohol content, turbidity and foam stability in craft beers analyzed

Beer style	Gluten (mg/L)	Original extract (% w/w)	Apparent extract (% w/w)	Alcohol content (% v/v)	Turbidity (EBC units)	Foam stability (s/3 cm)
Altbier *	163±19	14.08±0.02	3.13±0.01	5.91±0.01	1.4±0.1	286±12
Amber ale *	97±1	17.16±0.24	3.92±0.06	7.30±0.09	2.0±0.4	242±2
Amber ale *	90±0	11.78±0.02	3.29±0.01	4.52±0.01	2.4±0.2	251±5
IPA *	153±6	15.90±0.01	3.32±0.01	6.87±0.01	1.2±0.5	269±31
Bitter *	61±1	11.42±0.03	3.12±0.01	4.43±0.01	11.5±0.2	265±4
Blanche #	162±8	15.44±0.22	4.32±0.02	5.93±0.12	6.6±0.5	270±8
Blanche #	459±120	12.52±0.01	3.21±0.01	4.97±0.01	1.2±0.2	203±1
Blanche #	490±14	12.01±0.03	2.88±0.02	4.85±0.04	10.3±0.1	282±15
Dubbel *	177±47	18.46±0.16	6.70±0.01	6.59±0.06	24.6±0.6	286±9
Golden ale *	53±18	12.28±0.01	2.59±0.01	5.16±0.01	3.1±0.3	251±1
Hells *	124±43	12.44±0.02	3.14±0.01	4.96±0.01	22.6±1.1	217±14
IGA *	227±49	20.18±0.01	5.74±0.01	8.15±0.04	2.3±0.6	268±7
Pale ale *	112±19	13.07±0.17	3.00±0.05	5.37±0.11	61±2.5	244±5
Pale ale *	198±0	12.87±0.08	2.68±0.02	5.44±0.04	2.0±0.2	286±8
Pilsner *	108±10	12.33±0.01	2.75±0.01	5.06±0.01	1.8±0.2	231±11
Pilsner *	50±7.0	11.30±0.04	2.38±0.02	4.73±0.02	2.1±0.6	204±6
Pilsner *	39±7	13.13±0.02	3.36±0.02	5.24±0.01	2.1±0.5	217±10
Rauchbier *	80±6	13.02±0.01	3.65±0.01	5.00±0.03	4.8±0.9	236±15
Saison #	170±3	14.09±0.03	3.48±0.01	5.74±0.01	5.4±0.1	289±1
Stout *	182±38	17.11±0.01	4.45±0.01	6.94±0.01	5.3±0.2	299±16
Stout #	153±13	11.16±0.01	4.62±0.01	3.59±0.01	55.1±0.1	168±3
Strong ale *	49±3	17.53±0.03	4.06±0.01	7.42±0.02	1.8±0.3	236±1
Weizen#	500±0	12.27±0.01	2.13±0.01	5.40±0.01	4.3±0.1	250±4
Weizen#	2400±437	12.56±0.01	2.65±0.01	5.29±0.01	3.6±0.6	164±12
White IPA #	224±22	11.22±0.02	2.32±0.02	4.71±0.02	1.6±0.1	289±7
Gluten free	<10**	10.55±0.01	1.52±0.01	5.29±0.01	1.1±0.1	176±8

Values are averages of three measurements ± the standard deviation. *All barley-malt beers. #Wheat or wheat malt in grist composition. ** Results beyond the quantification limit of the ELISA used

As expected, beer including wheat or wheat malt in the total grist, contained higher levels of gluten (from 150 to 2400 ppm) compared with beers without wheat or malted wheat in the grist (39 to 220 ppm). None of the craft beers analyzed was gluten-free. The lowest level found was 39 mg/L in a Pilsner beer and the highest was 2400 mg/L in a Weizen. In eight beer samples (32%) we found a level of gluten below 100 mg/L. These beers could be labeled as "very low gluten" according to the Codex Alimentarius Commission Standard **(Codex Alimentarius Commission, 2008)**. In a previous study **(Guerdrum and Bamforth, 2011)**, a range of commercially available industrial beers was analyzed using the same Ridascreen Gliadin Competitive ELISA kit. 36% of those beers turned out to be gluten-free and all of them were "low in gluten" with the exception of a wheat beer. As reported above, unlike industrial beers, the craft beers analyzed were produced without a stabilization process, pasteurization or micro-filtration and they were bottle-fermented. Besides, industrial beers are often stabilized ~~beer~~ before packaging by kieselguhr filtration, polyvinylpolypyrrolidone filtration (PVPP) or silica gel. Large-scale breweries often follow a general formula and maize or rice products are often added to cut beer manufacture costs **(Briggs, 2004)**. Maize and rice are gluten-free ingredients and contribute to keep low gluten level in finished beer. Stabilization processes and different grist composition may result in relatively low gliadin levels in industrial beers.

Gluten content relationship with foam retention, turbidity and original extract

A correlation analysis among gluten content and all-barley malt beer analysisis shown in Figure 2. Gluten composition is well known for having influence on chemical-physical characteristics of beer foam **(Bamforth and Kanauchi, 2003; Steiner *et al*., 2010).** Moreover different authors reported evidence that some hordein species improve quality and persistence of beer foam **(Sheehan and Skerritt, 1997; Vaag*et al*., 1999, Evans *et al*., 2003)**. The significant correlation between gluten content and foam stability found in this study (Figure 2a) was not surprising. As already described by Bamforth and Kanauchi **(2003)** this may be due to the interaction between hop acids and some members of the hordein storage protein family deriving from malt.

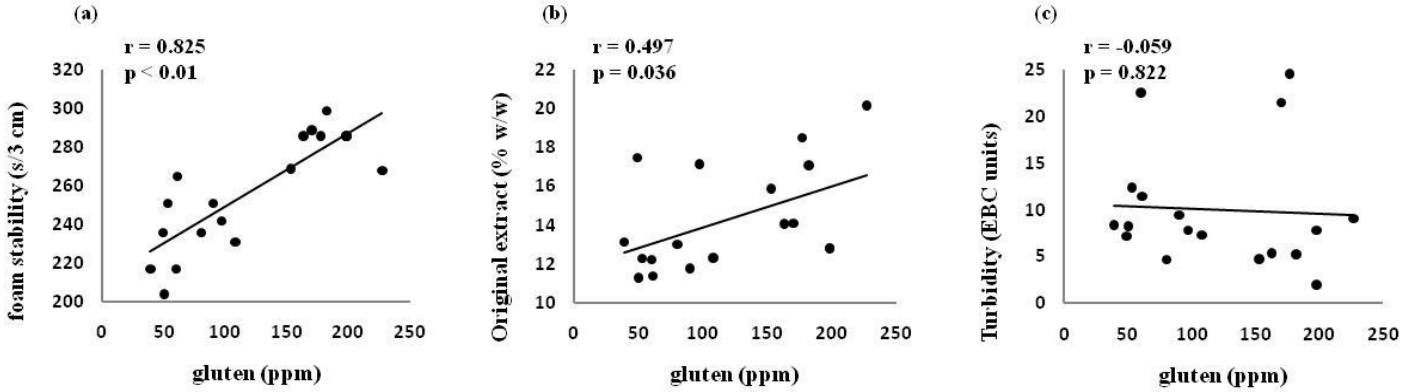

Figure 2 Relationships between gluten content and foam stability (a), original extract (b) and turbidity (c) among all-barley malt beers. Correlation was performed using Pearson correlation coefficient.

According to **Steiner *et al*., (2010)**, the same hordein storage proteins are also responsible for turbidity in beer. Nonetheless, we found no correlation between the two (Figure 2c). This could be explained by interaction of several variables more frequently occurring in craft beers than in industrial beer such as presence of yeasts (deriving from the bottle fermentation). Furthermore yeasts flocculation ability (the ability of yeasts to settle on the bottom at the end of refermentation) and cell number, can vary from beer to beer and cause turbidity independently from gluten content **(Boulton, 2001).**

It appears more difficult to explicate the lack of correlation (Figure 2b) that was found between gluten content and original extract (the amount of sugar in wort before fermentation) which directly derives from a higher amount of malt used to produce beer and therefore more gluten potential. A possible explanation could be the high variability observed in craft beer production, both among microbreweries and within the same plant, due to the lack of automation in the production process which makes it entirely dependent on manpower skills.

CONCLUSION

Although literature shows a relative low gluten content for commercial beers, with a high percentage of unaware gluten-free beers, a surprising high gluten level was found in Sardinian craft beers. Even if 37% of the analyzed beers could be labeled as "very low gluten", we found no gluten-free beers among our samples. Considering the high rate of gluten intolerance in Sardinia population,and the high etherogenity level of individual sensitivity, gluten sensitive persons should be careful when drinking craft beer. Moreover, correlation analysis highlight the relevance of gluten content on foam stability of craft beers.

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