International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

Original article

Effects of silica gel on reduction in gluten during several beer brewing stages

Elisa I. Benítez,[1,2]* María R. Acquisgrana,[1] Nélida M. Peruchena,[2] Gladis L. Sosa[1] & Jorge E. Lozano[3]

1 Laboratorio de Química Teórica y Experimental (QuiTEx), Universidad Tecnológica Nacional Facultad Regional Resistencia, French 414, Resistencia, Argentina
2 IQUIBA-NEA (UNNE-CONICET), Avenida Libertad 5460, Corrientes, Argentina
3 PLAPIQUI (UNS-CONICET), Camino La Carrindanga Km.7, Bahía Blanca, 8000, Argentina

(Received 2 October 2015; Accepted in revised form 14 December 2015)

Summary Beer is an alcoholic beverage made with barley as a basic raw material, and therefore, it is not suitable for people with coeliac disease. During the brewing process, the elimination of the haze-active precursor is performed at the stabilisation stage. Some breweries use silica gel (SG) as stabilisation precursor. This work presents the studies conducted to identify the stage where the addition of SG is more convenient in order to reduce gluten, and the effect on the yeast nutritional value due its incorporation. The incorporation of SG during fermentation allows the reduction in more gluten than the others brewery stages studied, without changing significantly the yeast nutritional value of beer. Furthermore, the use of SG promotes the reduction in polysaccharides which obstruct filtration.

Keywords Barley, beverages, gluten, proteins.

Introduction

Beer is the alcoholic beverage most widely consumed throughout the world. It is a barley-based alcoholic beverage, and therefore, it is not suitable for people with coeliac disease (Hager *et al.*, 2014). The disease affects more than 1% of the population (Catassi *et al.*, 2015). The clinical gluten sensitivity differs considerably among patients (Elli *et al.*, 2015). The Codex Alimentarius Standard states that for food to be declared 'free from gluten', it must contain less than 20 mg kg^{-1}, and from 20 to 100 mg kg^{-1}, it could be mentioned as 'very low gluten content'. (Codex Alimentarius Commission, 2014).

Not all the polypeptides and proteins present in beer cause coeliac disease, only those belonging to the group called prolamines. In the case of barley, prolamines are hordeins (Taylor *et al.*, 2015). Hordeins are glycoproteins rich in proline, which in beer are involved in the formation of the postpackaging haze due to their interaction with the haze-active polyphenols (Gassara *et al.*, 2015).

Two fundamental types of proteins are identified in beer: those causing foam, which must be retained, and those responsible for haze formation, which should be reduced. On the brewery, the elimination of the haze-active precursor is performed during the stage named stabilisation in the refining of beer. Some breweries use silica gel (SG) as stabilisation precursor. Proteins involved in the foam formation of beer contain a very small proportion of proline and thus are not affected by the silica (Taylor *et al.*, 2015).

Silica gel has a very large surface area containing a network of pores. The surface of SG is covered in silanol (SiOH) groups which form interactions with proline residues in haze-active proteins (Taylor *et al.*, 2015). The mechanism of action of the SG is via hydrogen bonding of protein carbonyl groups to hydroxyl groups on SG (Ryder & Power, 2006).

Beer contains 300–800 mg L^{-1} of protein material. Proteins undergo changes in their content and composition during the malting and brewing processes. A large proportion is degraded during malting and mashing by the action of proteolytic enzymes on polypeptides and free amino acids (Steiner *et al.*, 2011). Another fraction is lost during boiling and wort cooling due to the interaction with polyphenols and precipitation (Colgrave *et al.*, 2013). Then, the use of SG during different brewing stages could be an advantage because more degraded proteins, involved in gluten formation, could be removed than during the stabilisation stage.

This work presents the studies conducted to identify the stage where the addition of SG is more convenient

*Correspondent: Fax: +54 362 4432683; e-mail: ebenitez@frre.utn.edu.ar

doi:10.1111/ijfs.13053

in order to reduce gluten and the effect on yeast nutritional value due its incorporation. This study was based on the use of enough concentration of SG to obtain a 'low-gluten' beer with a lower concentration of 20 mg kg^{-1} and which does not cause significant losses in yeast nutritional qualities. Furthermore, a significantly higher percentage of the population – more than 1–2% affected by coeliac disease – tend to consume gluten-free or gluten-reduced foods for the sake of a healthier lifestyle (Hager *et al.*, 2014; Taylor *et al.*, 2015). Therefore, the availability of beer with low gluten level would be a great advantage for these people.

Although beer could be claimed as 'gluten-free' due to the level of gluten obtained, it is not appropriate to use the term to be controversial (Hager *et al.*, 2014).

Materials and methods

Description of the experimental procedure

To evaluate the efficiency of SG for the removal of gluten without causing a reduction in the level of free amino nitrogen (FAN), proteins, polyphenols, polysaccharides and antioxidant capacity (AC), the following test was conducted. The first test consisted of adding SG in a final concentration of 500 mg kg^{-1} during the basic brewing stages: mashing, boiling and fermentation. The second test aimed to identify which of these stages removed the largest quantity of gluten, and the third test was performed to assess removal efficiency in the stabilisation steps that are typically carried out to produce beer. Therefore, the following paragraphs present a description of the overall process, the identification of the critical stages and finally the description of the stabilisation stages.

The brewery process

Mashing was carried out in a 5-L container. The procedure started by mixing 0.95 kg of barley malt from Argentina (Cargill Malt Division) with water at 62 °C for 90 min. The water/malt radio was of 4:1. Subsequently, wort was boiled for 1 h with the addition of more water to complete a final volume of 5 L and hops. This bitter wort was then settled down for 30 min before it was cooling down to 12 °C. The specific gravity before fermentation was 1060. The lager yeast (Saflager S-23; Fermentis, Marcq-en-Baroeul, France) was pitched at the rate of 6.3 $g.L^{-1}$ (C_y). Fermentation was carried out at 12 °C for a period of 15 days, followed by a maturation period of 7 days and a cold rest at 3 °C for another 2 days (Benítez *et al.*, 2013; Lataza Rovaletti *et al.*, 2014). The preparation of beer was carried out in duplicate.

Use of silica gel in the brewery process

This treatment consisted in adding SG at a concentration of 500 mg kg^{-1} during mashing, making a new addition with the same concentration during boiling and making a final addition at the beginning of fermentation. The addition of SG at the beginning of fermentation was made before yeast inoculation. During the mashing process when certain malt components are solubilised in water, most of the proteins are precipitated and only some are further hydrolysed into simple polypeptides (Hager *et al.*, 2014). This treatment was performed to study whether an excess of SG may cause a reduction in yeast growth due to a decrease in the polypeptides, and to verify whether the concentrations of the main beer components that provide its characteristics such as proteins, polyphenols and polysaccharides, were reduced. Microbial growth recording was performed by microscopic cell counting chamber. A growth curve was constructed and the number of initial cells (N_0), the maximum growth rate (μ), microbial replication time (t_r), cell death time (t_d) and the number of maximal cells (N_{max}) for samples with and without SG addition were determined (Table 1). Usually, for the exponential phase, the number of cells is given by eqn (1) (Ginovart *et al.*, 2011):

$$\mathrm{Ln}N = \mathrm{Ln}N_0 + \mu \cdot t \quad (1)$$

Yeast viability was determined by staining with methylene blue (Hutzler *et al.*, 2015). Figure 1 describes yeast growth. In this test, the refining stage was not carried out.

Table 1 Representative data from yeast growth: number of initial cells (N_0), specific growth rate (μ), microbial replication time (t_r), cell death time (t_d), R^2 for samples during the fermentation stage, gliadin and FAN concentration after fermentation with and without the addition of SG

	Control	SG
$N_0 \times 10^7$ (CFU mL^{-1})	1.1 ± 0.1 a	0.8 ± 0.04 b
$N_{max} \times 10^7$ (CFU mL^{-1})	12.9 ± 0.2 a	10.6 ± 0.3 b
μ (day^{-1})	0.86 ± 0.01 a	0.83 ± 0.05 a
t_r (h)	19.45	20.01
t_d (h)	116 ± 1	116 ± 1
Gliadin (mg kg^{-1})	88 ± 1	17 ± 1
FAN (mg L^{-1})	168 ± 3	162 ± 2
Protein (g L^{-1})	0.86 ± 0.04 a	0.76 ± 0.03 b
TPP (g L^{-1})	0.502 ± 0.007 a	0.414 ± 0.02 b
AC (g L^{-1})	0.154 ± 0.02 a	0.150 ± 0.03 a
TPS (g L^{-1})	61 ± 7 a	47 ± 3 b

Data are mean values ± SD. Means in same row with different lowercase letters are significantly different ($P < 0.05$).

Identification of the critical stage

This test was divided into three parts. The first SG addition was made during mashing, and then, brewing was carried out as described in the brewery process of brewing. The second addition was made during boiling and next the brewing process continued. The last addition was made at the beginning of fermentation. For each of these tests, three concentrations of SG were used: 500, 750 and 1000 mg kg^{-1}.

Finally, each prepared sample was divided into two samples, one without filtration and the other one for the filtration test. For the filtration test, a Büchner funnel (Ø = 50 mm) with a filter bed consisting of a precoat of 1 g diatomaceous earth (DE) (Standard Super-Cel, mean porosity = 3.5 μm, permeability = 2.8×10^{-13} m^2, Refil, Argentina) over a filter paper Whatman N°3 under vacuum (−50 kPa) was used. All samples were prepared in triplicate (Benítez *et al.*, 2013).

During the conventional filtration process in beer production, proteins, polyphenols and polysaccharides which may affect prolamin concentration are removed. Although the existing prolamin in beer is hordein, the methodology applied expresses its presence using gliadin as a standard (Hager *et al.*, 2014), which is the wheat prolamin. Then, in this work the hordein concentration is expressed as 'gliadin equivalent'.

Stabilisation

This test was conducted to assess the removal of hordein with SG in the refining process of beer, and was compared with polyvinylpolypyrrolidone (PVPP) stabilisation process which removes polyphenol instead of proteins. For the stabilisation treatment, a sample of beer (100 mL), previously DE filtrated, was treated at 4 ± 1 °C with either PVPP (Polyclar 10; Tudela, Argentina), or SG (DARACLAR®, Grace Argentina S.A.) at a concentration of 500 mg kg^{-1} with a contact time of two hours prior to filtration through Whatman N° 1 filter paper (Lataza Rovaletti *et al.*, 2014).

Measures

Proteins (P) were estimated using the Bradford method (Bradford, 1976), total polyphenols (TPP) were estimated using the Folin–Ciocalteu method (Singleton *et al.*, 1999), free amino acids (FAN) were assessed with the ninhydrin-based microwell assay (Abernathy *et al.*, 2009), and total polysaccharides (TPS) were

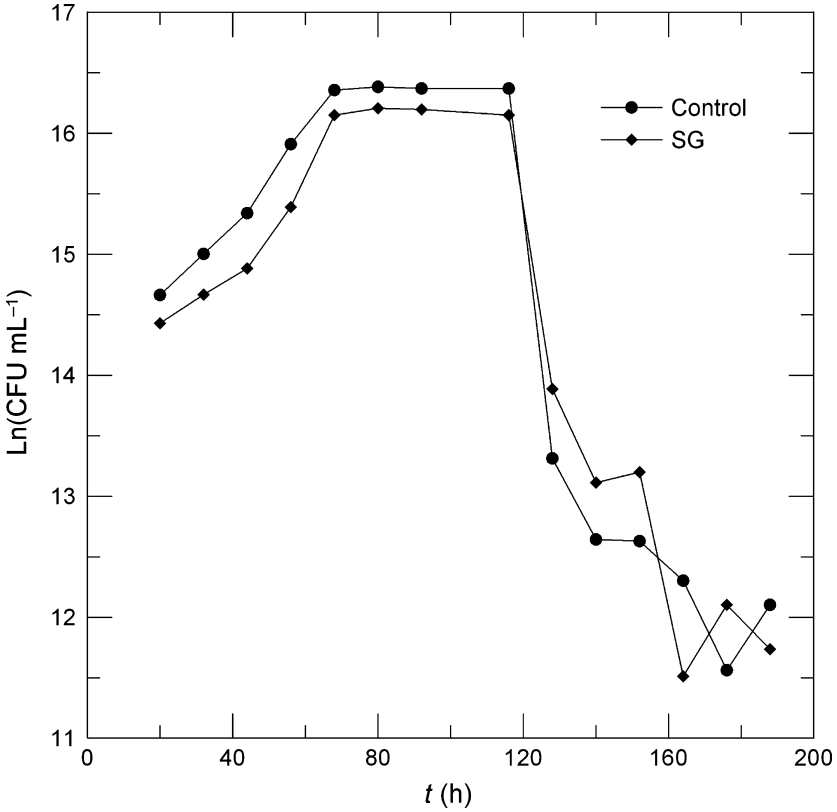

Figure 1 Yeast growth development during fermentation (control and silica gel samples).

determined with the phenol–sulphuric method of Segarra *et al.* (1995). The antioxidant capacity (AC) was estimated with the CUPRAC method (Özyürek *et al.*, 2011). The determination of 'gliadin equivalent' was carried out by the RIDASCREEN Gliadin competitive assay (Immer & Haas-Lauterbach, 2009). All determinations were made at least in duplicate.

Analysis of particles and aggregates

A dilute sample and a sample without dilution of beer after the filtration and SG addition were investigated through scanning electron microscopy (SEM) analysis with a SEM microscope (LEO, EVO 40, Cambridge, Ing.). Further details of the methodology are given in Lataza Rovaletti *et al.* (2014).

Twenty different SEM images of SG aggregates were subjected to the FERImage program which calculates fractal dimension (D_f) by means of a variogram and a Fourier power spectrum (Bianchi & Bonetto, 2001). The methodology described was previously used with aggregates of apple juice (Benítez *et al.*, 2010) and beer (Benítez *et al.*, 2013).

Statistical analysis

Data points were presented as the mean of the measured values. Data were subjected to variance analysis and the Tukey test at the 0.05 level of significance (Infostat 2002).

Results and discussion

Use of silica gel in the brewery process

Yeast growth during the brewery process with and without SG (control sample) addition was followed. The concentration of the number of cells that was pitched at the beginning of the fermentation for control and SG samples was calculated with (Benítez *et al.*, 2013):

$$N_y = 2.29 \times 10^6 \cdot C_y \qquad (2)$$

where N_y represents the number of yeast mL^{-1} and C_y represents the concentration of yeast expressed in g L^{-1}, giving a value of $N_y = 14.4 \times 10^6$ (CFU mL^{-1}). This last value was higher than that of N_0 calculated with eqn (1) for both samples, and this is probably due to the process of adaptation of the yeast to the wort nutrients. It was observed that N_0 and N_{max} for the sample with SG addition were slightly lower that the control sample, without SG (Table 1). No significant differences were observed for μ ($P < 0.05$). A general rule suggests that for a proper state of yeast health and purity, a $N_0 = 1 \times 10^7$ cells is added per millilitre of wort at a specific gravity of 1048 (Bamforth, 2003). Then, the data obtained from eqn (1) was enough to ensure that fermentation was properly performed because after only 20 h (t_r for the SG sample) the number of cells doubled. Therefore, the use of SG in the fermentation stage, with the concentration used in this work, does not significantly change yeast growth. Cell death time (t_d) for both samples was the same, indicating that the use of SG did not modify the nutrient limitation during fermentation.

Figures 2–4 show the reduction of the component with important yeast nutritional value during the fermentation stage, for the brewery process. The presence of protein and specially its degradation products, polypeptides, peptides and free amino nitrogen (FAN), are necessary for the cellular metabolism of the yeast. They influence yeast growth, the foam and haze properties of derived beer (Colgrave *et al.*, 2013). FAN and protein reduction during fermentation are observed in Fig. 2. FAN was reduced by 6% for the SG sample regarding the control sample, at the end of fermentation, but remained over a value of 162 mg L^{-1} at all times (Table 1). A minimum FAN level is required to maintain a healthy yeast and a good fermentation (Lei *et al.*, 2013). Therefore, the FAN level in both samples, control and SG, was enough to ensure a good fermentation.

Usually, the concentration of protein is reduced during fermentation, mainly due to the protein–polyphenol interaction. For the control sample, protein reduction was 12.2% and for SG 18.6% during fermentation (Fig. 2). Comparing the concentration of protein, at the end of fermentation, the reduction was 11.6% for the SG regarding the control sample. SG is known not to bind foam proteins (Taylor *et al.*, 2015), and the difference between both samples could be attributed to the polypeptides involved in the gluten of beer. The gliadin equivalent concentration found for the control sample was 88 ± 1 and 17.6 ± 1 mg kg^{-1} for the SG sample, with a reduction of 79.5% (Table 1). Furthermore, the level of gliadin equivalent in the SG sample was enough to claim the beer as reduced in gluten.

The initial concentration of TPS at the beginning of fermentation was 104 ± 5 g L^{-1} for the control sample and 82 ± 3 g L^{-1} for the SG sample, and the reduction at the end was 41.3% for the control sample and 43.3% for the SG sample (regarding the initial value of each sample), without significant differences ($P < 0.05$) (Fig. 3). The initial difference between samples may be due to the fact that the proteins involved in haze formation are bound to polysaccharides or organised in a matrix (Lataza Rovaletti *et al.*, 2014), and for the SG sample, the use of the absorbent

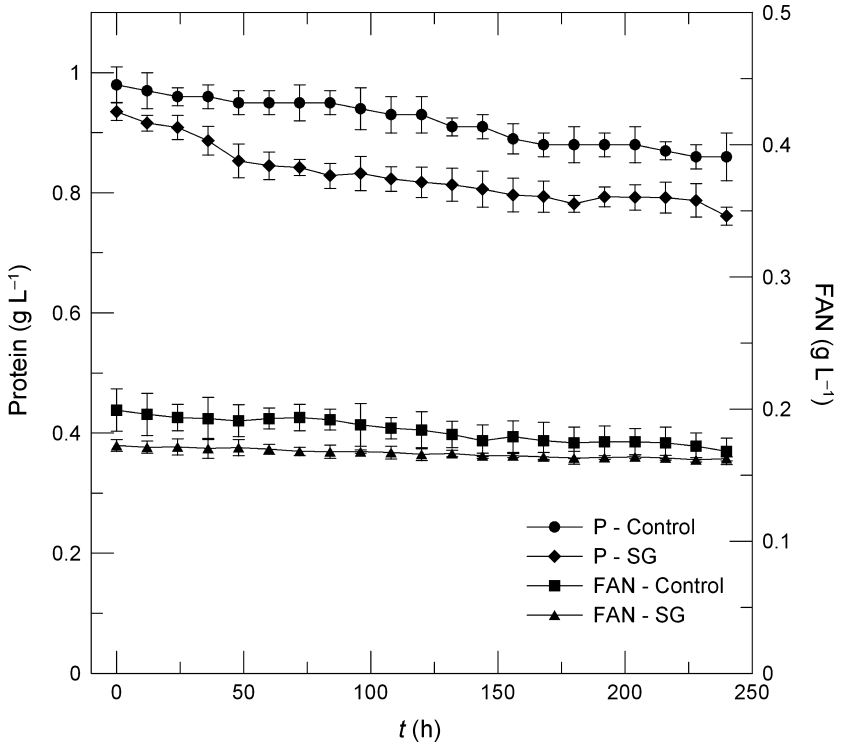

Figure 2 P and free amino nitrogen variation during the brewery process of fermentation with sequential addition of silica gel.

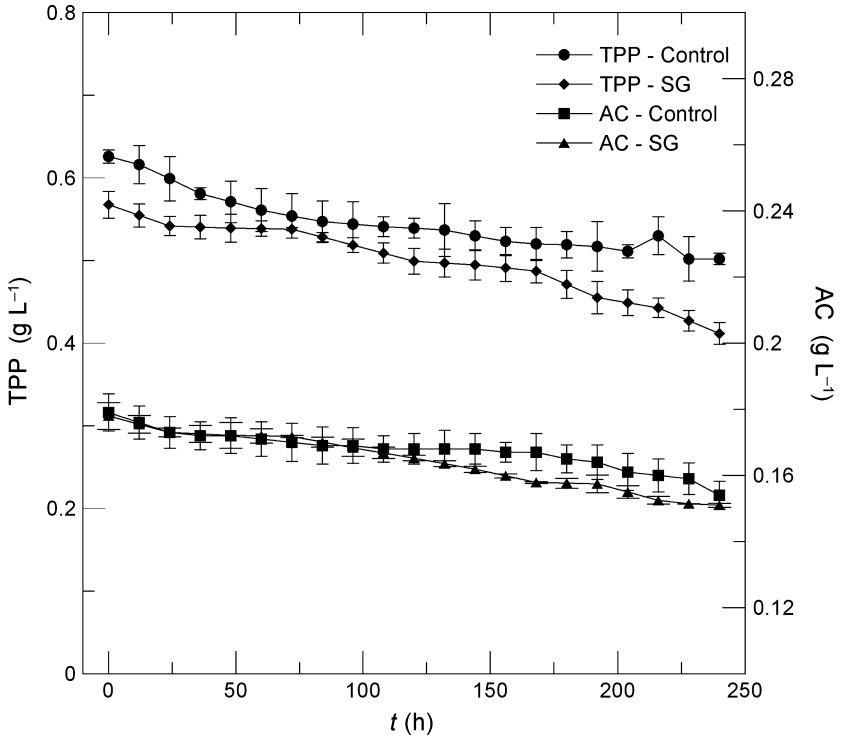

Figure 3 Total polyphenols and AC variation during the brewery process of fermentation with sequential addition of silica gel.

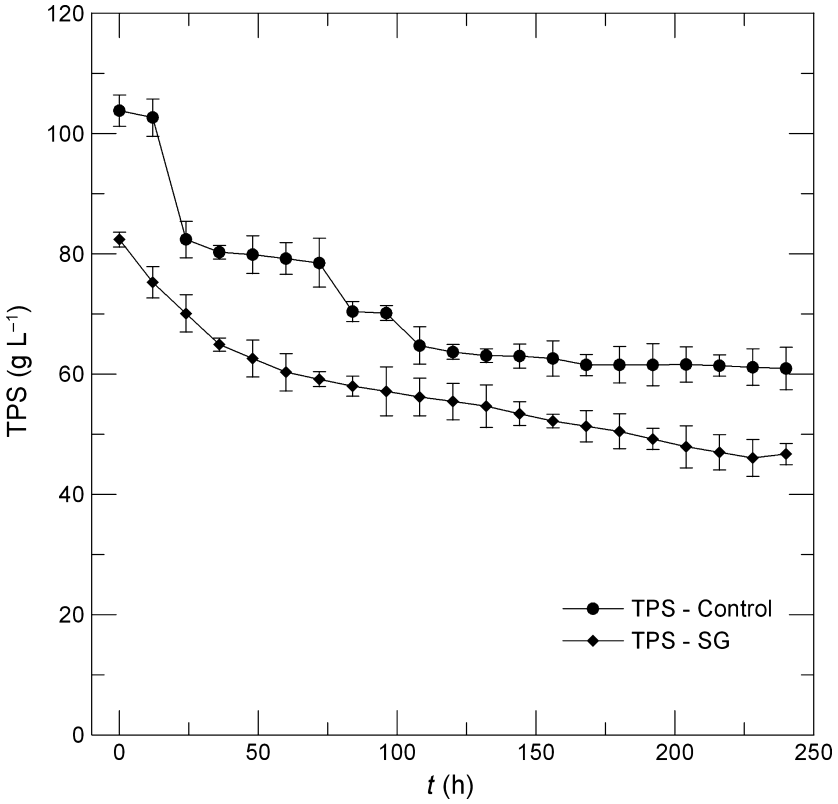

Figure 4 Total polysaccharides variation during the brewery process of fermentation with sequential addition of silica gel.

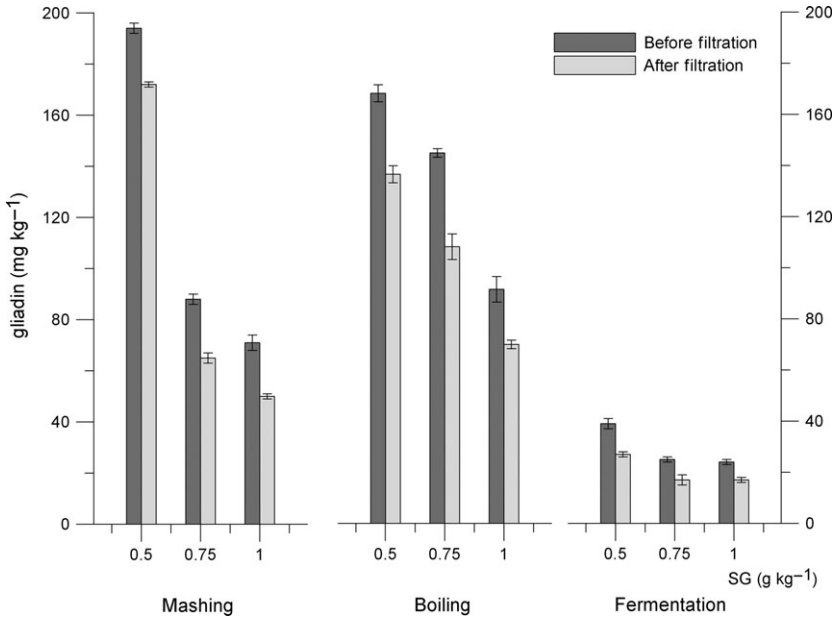

Figure 5 Gliadin level of samples treated with different concentrations of silica gel during mashing, boiling and fermentation, with and without final filtration.

enhances the formation of the protein–polysaccharide complex. The difference in TPS level between samples is 23.0% for the SG sample regarding the control sample at the end of fermentation (Table 1).

The use of SG at the beginning of fermentation promotes the reduction in TPS, which obstructs the regular filtration process. The use of SG could be an advantage as compared with the use of enzymes which

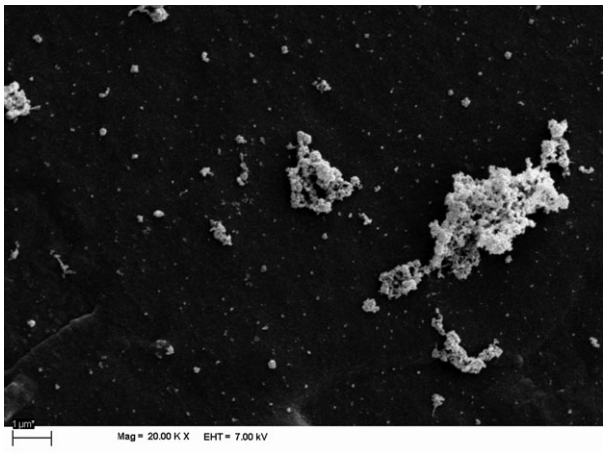

Figure 6 SEM micrograph of particles of a sample of beer after filtration with silica gel addition. Magnification: 20.00 KX. Scale bar: 2 µm = 135 pixels.

usually reduced the level of TPS but obstructs the subsequent stabilisation process (Sensidoni *et al*., 2011).

During fermentation, TPP concentration is reduced for both samples (Fig. 4) and this is due to protein–polyphenols interaction and polyphenol–polyphenol interaction. The last interaction causes the polymerisation of the polyphenols and their precipitation (Watrelot *et al*., 2015). The reduction in the TPP level between samples at the end of fermentation is 18.0% for the SG sample regarding the control sample (Table 1). It is known that SG reduces proteins and proteins linked to polyphenols (Morosanova, 2012). Therefore, the reduction observed may be attributed to this last interaction.

The AC in beer is mainly related to the presence of polyphenols (Zhao, 2014). Consequently, the reduction is attributed to the polyphenol decrease already mentioned. Nevertheless, no significant differences were observed between samples (Fig. 4). All the studies conducted in the brewery process lead to the conclusion that an excess of SG during the mashing, boiling and fermentation of the brewing process does not significantly modify the yeast growth and the yeast nutritional value of beer and leads to a significant reduction of gluten allowing to claim beer as 'reduced in gluten'. Although beer could be claimed as 'gluten-free' due to the level of gluten obtained, and considering that the stabilisation process which would reduce this value has not been carried out, it is not appropriate to use the term to be controversial and because beer was produced from malt which contains gluten.

Identification of the critical stage

In this study, the identification of the critical stage for prolamin reduction was tested.

During the conventional filtration of beer, proteins, polyphenols and polysaccharides were reduced and probably the level of prolamin decreased as well as a result of the treatment. In the present work, the simultaneous reduction due to filtration and the addition of SG during the previous brewing stage was studied.

During the three stages studied – mashing, boiling and fermentation, a reduction of gliadin equivalent was observed (Fig. 5). The reduction in this level was more significant in the later brewing stages, where SG was added, and this is probably due to a more advanced proteolytic action. The prolamin reduction in each stage increased as SG concentration increased. It is important to highlight that the concentrations of 0.75 and 1 g kg^{-1} during fermentation were effective to reduce the level of gluten to a value lower than 20 mg kg^{-1}. This could be explained observing SG capacity to remove haze particles (Taylor *et al*., 2015) and to form aggregates (Ryder & Power, 2006). With proteolysis, the fragment of proteins and small polypeptides could interact with SG and form aggregates that could separate during filtration. This can be seen in Fig. 3. After the conventional filtration, the small particles of an average diameter smaller than 0.6 µm (Benítez *et al*., 2013) are isolated and with SG they form colloidal chain-shaped aggregates (Fig. 6). The average fractal dimension value obtained by statistical analysis of the SEM images resulted $D_f = 2.45 \pm 0.05$, and close to 2.5, indicating rapid flocculation or diffusion limited aggregation by incorporation of individual particles, and not by aggregate–aggregate interaction (Benítez *et al*., 2013). The ability of SG to form aggregates allows agglomeration,

Table 2 Variation in composition during the stabilisation of beer

	TPP (mg L^{-1})	Protein (mg L^{-1})	FAN (mg L^{-1})	TPS (g L^{-1})	AC (mg L^{-1})	Gliadin (mg kg^{-1})
Before stabilisation	499 ± 4 a	490 ± 5 a	169 ± 4 a	55 ± 8 a	150 ± 4 a	60 ± 4 a
PVPP	407 ± 3 b	340 ± 3 b	164 ± 4 b	34 ± 4 b	150 ± 4 a	63 ± 5 a
SG	440 ± 3 c	320 ± 4 c	164 ± 2 b	36 ± 6 b	150 ± 3 a	43 ± 1 b

The data are mean values ± SD ($N = 3$). Means in same column with equal lowercase letters are not significantly different ($P < 0.05$).

increase in the size of aggregates and retention during filtration.

Stabilisation

Table 2 contains the variation in composition resulting from the use of the two common stabilisation methods: SG and PVPP. The behaviour observed was expected, SG removed more proteins and PVPP removed more TPP. Nevertheless, both treatments reduce both components and this is due to the fact that some proteins and polyphenols interact to form haze-active particles, and both stabilisation agents interact with the haze formed.

Furthermore, there was a significant loss of polysaccharides during the stabilisation process. This is probably because both polyphenols and protein interacted or were bound to polysaccharides (Lataza Rovaletti *et al*., 2014), and this may be the reason why reduction was similar for both treatments. FAN level was slightly reduced in both treatment, and this reduction was consistent with protein reduction. Nevertheless, the lower reduction could be due to the fact that not all FAN present could cause haze, only FAN containing proline (Hager *et al*., 2014). The AC was not significantly reduced during both stabilisation treatments. Polyphenols, which polymerised probably in the previous stage of the brewing process, interact with PVPP or SG, and during these previous stages, their AC was involved, as it can be seen in Fig. 2c. At the beginning of stabilisation, probably the polyphenols that were not polymerised which conserved their AC did not undergo a further reduction.

The gliadin equivalent level was reduced with the SG stabilisation method, but less than with the addition of SG during fermentation. No reduction in gliadin equivalent with the PVPP was observed.

Conclusion

It is important to note that in every stage of the brewing process, a natural reduction in prolamin was observed and the SG increased this reduction in a proportion over 20%. The fermentation stage is of particular interest, because an adequate concentration of SG allows the beverage to be regarded as reduced in gluten, prior to the regular stabilisation process. Probably, a combination of SG during fermentation and stabilisation is more effective in gliadin equivalent reduction. Further studies will be conducted. Nevertheless, the incorporation of SG during fermentation allows the reduction in proteins that are less processed and have less proteolytic action, without changing the yeast nutritional value of beer significantly.

To conclude, the use of SG promotes the reduction in TPS, which obstructs the filtration process. The use of SG could be an advantage as compared with the use of enzyme which usually reduces the level of TPS but obstructs the subsequent stabilisation process.

Acknowledgment

We thank the Facultad Regional Resistencia-Universidad Tecnológica Nacional and the Consejo Nacional de Investigaciones científicas y Técnicas (CONICET) for their financial support.

References

Abernathy, D.G., Spedding, G. & Starcher, B. (2009). Analysis of protein and total usable nitrogen in beer and wine using a microwell ninhydrin assay. *Journal of the Institute of Brewing*, **115**, 122–127.

Bamforth, C. (2003). *Beer: Tap into the Art and Science of Brewing* (2nd edn). New York: Oxford University Press.

Benítez, E.I., Lozano, J.E. & Genovese, D.B. (2010). Fractal dimension and mechanism of aggregation of apple juice particles. *Food Science and Technology International*, **16**, 179–186.

Benítez, E., Martinez Amezaga, N., Sosa, G., Peruchena, N. & Lozano, J. (2013). Turbidimetric behavior of colloidal particles in beer before filtration process. *Food and Bioprocess Technology*, **6**, 1082–1090.

Bianchi, F.D. & Bonetto, R.D. (2001). FERImage: an interactive program for fractal dimension, d_{per} and d_{min} calculation. *Scanning*, **23**, 193–197.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248–554.

Catassi, C., Gatti, S. & Lionetti, E. (2015). World perspective and celiac disease epidemiology. *Digestive Diseases*, **33**, 141–146.

Codex Alimentarius, 2014. CODEX STAN 118 – 1981. Available at: http://www.codexalimentarius.org/standards/list-standards/es/?no_cache = 1. (Consulted in 12/2014).

Colgrave, M., Goswami, H., Crispin, A., Howitt, C.A. & Tanner, G.J. (2013). Proteomics as a tool to understand the complexity of beer. *Food Research International*, **54**, 1001–1012.

Elli, L., Branchi, F., Tomba, C. *et al*. (2015). Diagnosis of gluten related disorders: celiac disease, wheat allergy and non-celiac gluten sensitivity. *World Journal of Gastroenterology*, **21**, 7110–7119.

Gassara, F., Antzak, C., Ajila, C.M., Sarma, S.J., Brar, S.K. & Verma, M. (2015). Chitin and chitosan as natural flocculants for beer clarification. *Journal of Food Engineering*, **166**, 80–85.

Ginovart, M., Prats, C., Portell, X. & Silbert, M. (2011). Exploring the lag phase and growth initiation of a yeast culture by means of an individual-based model. *Food Microbiology*, **28**, 810–817.

Hager, A.S., Taylor, J.P., Waters, D.M. & Arendt, E.K. (2014). Gluten free beer – A review. *Trends in Food Science and Technology*, **36**, 44–54.

Hutzler, M., Koob, J., Riedl, R., Schneiderbanger, H., Mueller-Auffermann, K. & Jacob, F. (2015). Yeast identification and characterization. In: *Brewing Microbiology* (edited by A. Hill) Pp. 65–104. Cambridge, UK: Woodhead Publishing.

Immer, U. & Haas-Lauterbach, S. (2009). Detection of gluten and related proteins in foods and beverages and safety issues related to gluten-free foods – specific detection with the R5 antibody in a ready-to-use test kit for industry. In: *The Science of Gluten-Free Foods and Beverages* (edited by E.K. Arendt & F. Dal Bello). Pp. 41–52. St. Paul, MN: AACC International.

Infostat. InfoStat versión 1.1. Universidad Nacional de Córdoba, Argentina (2002).

Lataza Rovaletti, M.M., Benítez, E.I., Martinez Amezaga, N.M., Peruchena, N.M., Sosa, G.L. & Lozano, J.E. (2014). Polysaccharides

influence on the interaction between tannic acid and haze active proteins in beer. *Food Research International*, **62**, 779–785.
Lei, H., Zhao, H. & Zhao, M. (2013). Proteases supplementation to high gravity worts enhances fermentation performance of brewer's yeast. *Biochemical Engineering Journal*, **77**, 1–6.
Morosanova, E. (2012). Silica and silica–titania sol–gel materials: synthesis and analytical application. *Talanta*, **102**, 114–122.
Özyürek, M., Güçlü, K. & Apak, R. (2011). The main and modified CUPRAC methods of antioxidant measurement. *Trends in Analytical Chemistry*, **30**, 652–664.
Ryder, D. & Power, J. (2006). Miscellaneous ingredients in aid of the process. In: *Handbook of Brewing* (edited by G.G. Stewart, I. Russell & F.G. Priest). Pp. 333–381. New York, USA: CRC Press.
Segarra, I., Lao, E., López-Tamames, E. & De la Torre-Boronat, M.C. (1995). Spectrophotometric methods for the analysis of polysaccharides levels in winemaking products. *American Journal of Enology and Viticulture*, **46**, 564–570.
Sensidoni, M., Marconi, O., Perretti, G., Freeman, G. & Fantozzi, P. (2011). Monitoring of beer filtration using photon correlation spectroscopy. *Journal of the Institute of Brewing*, **117**, 639–646.
Singleton, V.L., Orthofer, R. & Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteau reagent. *Methods in Enzymology*, **299**, 152–178.
Steiner, E., Arendt, E.K., Gastl, M. & Becker, T. (2011). Influence of the malting parameters on the haze formation of beer after filtration. *European Food Research and Technology*, **233**, 587–597.
Taylor, J.P., Jacob, F. & Arendt, E.K. (2015). Fundamental study on the impact of silica gel and tannic acid on hordein levels in beer. *Innovative Food Science and Emerging Technologies*, **31**, 177–184.
Watrelot, A.A., Renard, C. & Le Bourvellec, C. (2015). Comparison of microcalorimetry and haze formation to quantify the association of B-type procyanidins to poly-L-proline and bovine serum albumin. *LWT - Food Science and Technology*, **63**, 376–382.
Zhao, H. (2014). Endogenous antioxidants and antioxidant activities of beers. In: *Processing and Impact on Antioxidants in Beverages*. (edited by V.R. Preedy). Pp. 15–24. Burlington, USA: Academic Press.