

## Original article

**The role of polysaccharides on the stability of colloidal particles of beer**Nancy Maria J. Martínez Amezaga,<sup>1</sup> Elisa I. Benítez,<sup>1\*</sup> Gladis L. Sosa,<sup>1</sup> Nélide M. Peruchena<sup>2</sup> & Jorge E. Lozano<sup>3</sup>

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**Summary** This study aimed at understanding the influence of beer colloidal composition and size on physical stability of the colloidal suspensions in beer before filtration. The percentages of retention during filtration indicate that colloidal particles consist principally of polysaccharides (97.04%) and smaller amounts of proteins (2.87%) and polyphenols (0.22). The viscosity of a colloidal dispersion can be modelled as the sum of a hard-sphere contribution and a colloidal force contribution. The colloidal force contribution is determined from the volume fraction of particles, the viscosity colloidal dispersion and the beer viscosity without particles. The volume fraction of particles could be estimated from the particle density and the total solid present in the beer. The fitting parameters of the correlation between the particles volume fraction and the colloidal force viscosity could explain the low stability of the colloidal particles of beer. This last determination is more specific than the simple viscosity of beer and could help to improve the prediction of the filtration flow.

**Keywords** Hydrocolloid, polysaccharides, viscosity.

**Introduction**

Beer before filtration shows a significant turbidity due to the presence of colloidal particles resulting from mashing and fermentation (Benítez *et al.*, 2013; Van der Sman *et al.*, 2015). The colloidal particles in beer modify not only the turbidity but also the viscosity of a dispersion (Gassara *et al.*, 2015). The viscosity is an important property of liquid food products, because it affects pumping, filtration, clarification and some other processes (Yanniotis *et al.*, 2007). However, in beer, it has a positive effect contributing to foam stability (Gassara *et al.*, 2015).

The viscosity of a colloidal dispersion ( $\eta$ ) can be modelled as the sum of a hard-sphere contribution ( $\eta^{\text{hs}}$ ) and a colloidal force contribution ( $\eta^{\text{cf}}$ ) (Benítez *et al.*, 2007, 2009):

$$\eta = \eta^{\text{hs}} + \eta^{\text{cf}} \quad (1)$$

The term  $\eta^{\text{hs}}$  is considered to be the viscosity of an ideal suspension of rigid, noninteracting (inert) spherical particles. Einstein's equation [eqn (2)] predicts  $\eta^{\text{hs}}$  in terms of the volume fraction of particles ( $\phi$ ) and solvent viscosity ( $\eta^{\text{s}}$ ):

$$\eta^{\text{hs}} = \eta^{\text{s}}(1 + 2.5\phi) \quad (2)$$

This equation is valid in the dilute regimen (Tan & Kerr, 2015). By combining eqns (1) and (2), the following expression is derived to estimate the contribution of colloidal forces to the relative viscosity, using viscosity data at different volume fractions:

$$\eta_{\text{r}}^{\text{cf}} = \frac{\eta}{\eta^{\text{s}}} - (1 + 2.5\phi) \quad (3)$$

The term  $\eta/\eta^{\text{s}}$  is the relative viscosity ( $\eta_{\text{r}}$ ).

Therefore, based on the data of the colloidal system viscosity to different volume fractions, it is possible to determine the contribution of colloidal forces. For diluted systems, the following correlation of  $\eta_{\text{r}}^{\text{cf}}$  in terms of the energy barrier and the volume fraction has been proposed (Genovese & Lozano, 2006):

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$$\eta_r^{cf} = \alpha \left( \frac{U_{Max}}{k_B T} \right) \varphi \quad (4)$$

where  $\alpha$  is a numerical constant.

The energy barrier ( $U_{Max}$ ) is the point of maximum repulsive energy between two interacting particles. To agglomerate two particles, the particles must have enough kinetic energy to overcome that barrier. The energy depends on their speed and mass (this because of the Brownian movement of the particles, expressed by the product of  $k_B$  with  $T$ , where  $k_B$  is the Boltzmann constant and  $T$  the temperature in °K). Further details about the theory that involve the energy barrier with the colloidal force viscosity are given in Benítez *et al.* (2009, 2007).

For the study of colloidal particles of apple juice (Genovese & Lozano, 2006), the experimental data were adjusted with a power law:

$$\eta_r^{cf} = \varepsilon \cdot \varphi^\sigma \quad (5)$$

where  $\varepsilon = 391$  and  $\sigma = 1.22$ . When comparing eqn (5) with eqn (4), it can be observed that  $\varepsilon$  is comparable to the product of  $\alpha$  and the factor in brackets that involves the energy barrier. The difference in the exponent  $\sigma$  from the eqn (4) with respect to the experimental data adjusted by eqn (5) was attributed to attractive van der Waals and repulsive hydration forces not considered in eqn (4).

Subsequent work (Benítez *et al.*, 2009) found values for  $\varepsilon$  ranging from 174 to 48 for pH from 4.5 to 2.1, respectively. In the colloidal system of apple juice, these values correspond to a strong hydration that makes the particles inherently stable. In this system, the aggregate formation occurs, but practically no precipitation is observed (Benítez *et al.*, 2007, 2009). In the present work, all these concepts were used to evaluate the values of  $\varepsilon$  and  $\sigma$  that could explain the tendency of the beer colloidal system to form aggregates and precipitate.

Bubbles of gas could modify the 'hard-sphere' contribution, and the volatile components could modify the solvent viscosity. While these modifications were not considered in previous studies (Jin *et al.*, 2004; Lu & Li, 2006), the beer samples analysed in our study were pretreated before viscosity determination to avoid such modifications. The methodology used to pretreat samples was also used in previous studies (Benítez *et al.*, 2007, 2009) and resulted to be very sensitive to the composition of the liquid medium surrounding the colloidal particles.

To provide more accurate predictions of the influence of colloidal particles on the filtration flux, the concept of colloidal force viscosity applied to beer was presented. We also provide percentages of retention ( $\%R_X$ ) of proteins (P), total polysaccharides (TPS) and total polyphenols (TPP) during filtration.

## Material and methods

### Preparation of samples for the global fermentation of wort in relation to the formation of colloidal particles

Initial mashing was carried out in a 40-L stainless steel container, by mixing 7.5 kg of barley malt from Argentina (Cargill Malt Division) with water at 62 °C for 90 min. The resulting wort was boiled for 1 h with the addition of hop for bitter and flavour. This bitter wort was then settled down for 30 min before it was cooled down to 12 °C. A part of the wort was separated for sample preparation as described below. The remaining wort was kept in a fermentation container to evaluate the global physicochemical properties of the manufactured beer and was inoculated with yeast Lager type (Saflager S-23; Fermentis, Marcq-en-Baroeul, France). Fermentation was carried out at 12 °C for a period of 15 days (Taylor *et al.*, 2015), followed by a maturation of 7 days at 12 °C and a cold rest at 3 °C for another 2 days. Filtration was performed in a Büchner funnel ( $\varnothing = 5.0 \times 10^{-2}$  m) with a filter bed of diatomaceous earth (1 g Standard Super-Cel, mean porosity = 3.5  $\mu$ m, permeability =  $2.8 \times 10^{-13}$  m<sup>2</sup>, Refil, Argentina) over a filter paper Whatman No 3 under vacuum (−50 kPa) was used (Benítez *et al.*, 2013; Lataza Rovaletti *et al.*, 2014). The cross-section area was  $1.96 \times 10^{-3}$  m<sup>2</sup>.

### Determination of particle densities

A volume of 250 mL of the 100% wort sample were filtered over a microfiltration (MF) membrane to retain all the colloidal particles and then retrieve the particles after filtration. The MF was made with an all-glass filter holder assembly (Glassco, UK, <http://glasscolabs.com/index.php>) using a 0.45- $\mu$ m cellulosic membrane (E04WP04700, MSI, Westboro, MA, USA) under a pressure difference of 50 kPa with a effective diameter of  $3.5 \times 10^{-2}$  m, cross-section area of  $9.62 \times 10^{-4}$  m<sup>2</sup>. In this methodology, the pore diameter of membrane is the typical used for beer (0.45–0.65  $\mu$ m) (Cimini *et al.*, 2013; Sensidoni *et al.*, 2011). The particles were washed with deionised water over the membrane and then vacuum-dried during 24 h. The mass of the particles was obtained by the difference in the weight of the dry membrane before and after filtration. The particles were dispersed in water. The volume of particles was calculated from the difference in volume of water and of particles in water with a pycnometer (10 mL capacity). The density of the particles was calculated using the relationship of mass/volume. Density was determined in triplicates at 25 °C.

### Determination of the colloidal particles composition

Five fermenters of 500 mL were prepared with varying concentration of the wort: 20, 40, 60, 80, up to 100%

(v/v). The most concentrated fermenter consisted exclusively of 500 mL of wort. The 80% (v/v) concentration consisted of 400 mL of wort and 100 mL of deionised water, the 60% consisted of 300 and 200 mL of deionised water, and so on for the other concentrations. Then, lager yeast (Saflager S-23, Fermentis, France) was pitched in the fermenters. Yeast concentration in each fermenter was  $0.63 \text{ g L}^{-1}$ , giving a initial concentration cell of  $N_y = 1.44 \times 10^6 \text{ (CFU mL}^{-1}\text{)}$  (Benítez *et al.*, 2013). The samples were used for the correlation of TPS and the total solid (TS).

The percentage of retention of the various components during filtration is calculated as follows:

$$\%R_X = \frac{X_{\text{Before}} - X_{\text{After}}}{X_{\text{Before}}} \times 100 \quad (6)$$

where  $X$  represents any of proteins (P), TPS or TPP concentrations. The resulting  $\%R_X$  and concentrations of P, TPS and TPP before filtration were used to calculate the amount of colloidal particles retained on the filter. Then, particle weight was converted to volume using the particle density ( $\rho_p$ ), hence determining the particle volume fractions of each sample (Benítez *et al.*, 2007, 2009).

### Measurements

$\eta$  and  $\eta^s$  were measured at  $25 \text{ }^\circ\text{C}$  in a glass capillary viscometer (Cannon–Fenske) according with the AOAC official method (AOAC, 2000), calibrated with distilled water. Samples were degassed for 2 h at  $60 \text{ }^\circ\text{C}$  to remove ethanol and other volatile components.  $\eta^s$  was determined after filtration.

P concentration was estimated using the Bradford method (Bradford, 1976) and TPP concentration using the Folin–Ciocalteu method (Singleton *et al.*, 1999). Both methods were mentioned by Bamforth (2009). TPS concentration was estimated using the Phenol–Sulphuric method (Segarra *et al.*, 1995). All three methods have already been used with beer samples (Benítez *et al.*, 2013; Lataza Rovalletti *et al.*, 2014).

The TS, before and after filtration, was determined following AOAC's official method (AOAC 2000). TS is a measure of the amount of the solids present in a sample. Measurements were done in triplicate. All results were expressed as  $\text{g L}^{-1}$ , to allow the comparison among them.

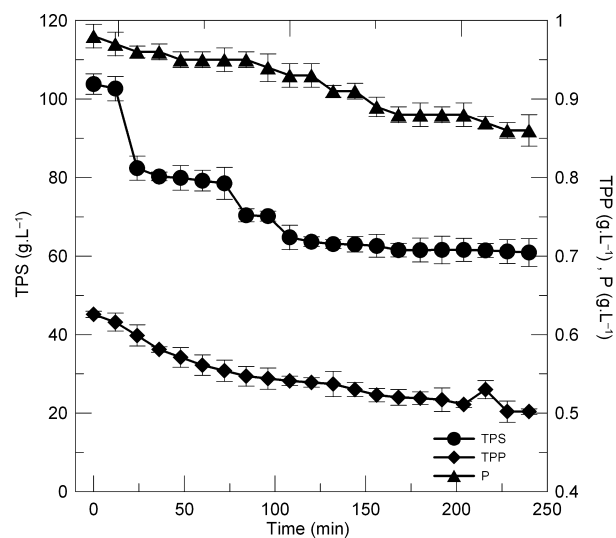
### Statistical analysis

The mean of measured values was calculated, and the software Infostat (2002) was used to analyse the variance and carry out a Tukey test at the 0.05 significance level.

## Results and discussion

### Changes in the composition of beer during the global fermentation of wort in relation to the colloidal particles formation

Several transformations occurred during wort fermentation that modified the composition of the principal components of the beer. Concentrations of TPS, P, TPP decreased from  $104 \pm 5 \text{ g L}^{-1}$  to  $64.1 \pm 0.5 \text{ g L}^{-1}$  (41.3%), from  $0.98 \pm 0.06 \text{ g L}^{-1}$  to  $0.86 \pm 0.05 \text{ g L}^{-1}$  (12.2%), and from  $0.63 \pm 0.01 \text{ g L}^{-1}$  to  $0.50 \pm 0.01 \text{ g L}^{-1}$  (20.6%), respectively (Fig. 1). Concentrations decreased significantly only during the first 10 days of fermentation remaining constant afterwards. Furthermore, the temperature drop during fermentation favours the formation of a complex between P and TPP (Gassara *et al.*, 2015), and the beverage gains colloidal stability. At the same time, the TPP become attached to yeast cell walls and are removed during yeast cropping (Bamforth, 2009). At the end of fermentation, the TPS concentration was seventy-five times higher than P concentrations and 130 times higher than TPP concentrations (Fig. 1). Our results on the composition variation of manufactured beer after fermentation and before and after filtration (Table 1) reveal a high concentration of TPS after fermentation. The filtration reduced 13.3%, 16.5% and 43.8% of the TS, TPS and P, respectively. In our study, the TPP concentration was only slightly reduced, which could be explained by the fact that TPP were able to pass through the filter due to their small size, but were retained when complexed with proteins.



**Figure 1** Variation in the composition of total polysaccharides, proteins and total polyphenols during fermentation.

**Table 1** Global physicochemical properties of manufactured beer, after fermentation and before and after filtration

	TS (g L <sup>-1</sup> )	TPS (g L <sup>-1</sup> )	P (g L <sup>-1</sup> )	TPP (g L <sup>-1</sup> )
Before Fermentation	–*	104 ± 5	0.98 ± 0.03	0.63 ± 0.01
After Fermentation	104 ± 5a	64 ± 1a	0.86 ± 0.05a	0.50 ± 0.01b
Before Filtration	54 ± 1b	41 ± 1b	0.48 ± 0.03b	0.20 ± 0.02a
After filtration	47 ± 1c	34 ± 1c	0.27 ± 0.02c	0.19 ± 0.01a
%R <sub>x</sub>	13.3	16.5	43.8	–

Mean values ± SD ( $N = 3$ ). Means in same column with different lowercase letters are significantly different ( $P < 0.05$ ).

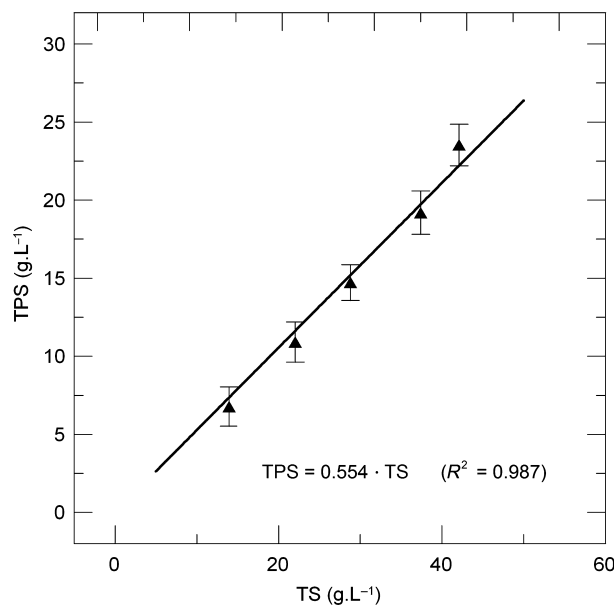
\*Not determined.

In some brewing industries, beer is treated with enzymes to further degrade the polysaccharides that could hamper the filtration process (Buttrick, 2010; Sensidoni *et al.*, 2011). Nevertheless, the use of enzymes before the filtration process reduces the size of colloids, allowing them to cross the filter; but after passing the filter they agglomerate, decreasing the colloidal stability after filtration (Sensidoni *et al.*, 2011). This approach is principally used with MF, where the fouling mechanism is more pronounced. To preserve the nutritional value of beer and maintain colloidal stability after filtration, we did not use enzymes in this study.

The invention of MF was thought to lead to a replacement of traditional filters using powder treatments, like diatomaceous earth (Buttrick, 2010). However, this did not happen: because of high investment costs plus other current uncertainties associated with MF, many brewers still opt for conventional systems (Buttrick, 2010). Regardless of the method used to filter colloidal particles, such particles are mainly formed by TPS and then deepen in the composition of colloidal particles and their interaction with the liquid medium that surrounds the particles will improve and predict the filtered flow.

### Composition of colloidal particles

There is a linear correlation between TPS and the TS values (Fig. 2). TPS were not consumed by yeast and thus remained in suspension, but part of them aggregated (Benítez *et al.*, 2013) and precipitated as consequence of the increased size. With the increase in wort in the samples, the fermentation activity by the yeast increased as well, while the TPS/TS ratio remained constant. An equilibrium was reached when fermentation ended. Independently of the quantity of yeast nutrients, residual material could not be degraded. It is possible to make a linear regression to describe the relationship between the TPS and the TS, as seen in eqn (7):



**Figure 2** Correlation between total polysaccharides and total solid. Full lines represent eqn (7).

$$\text{TPS} = a \cdot \text{TS}, \quad (7)$$

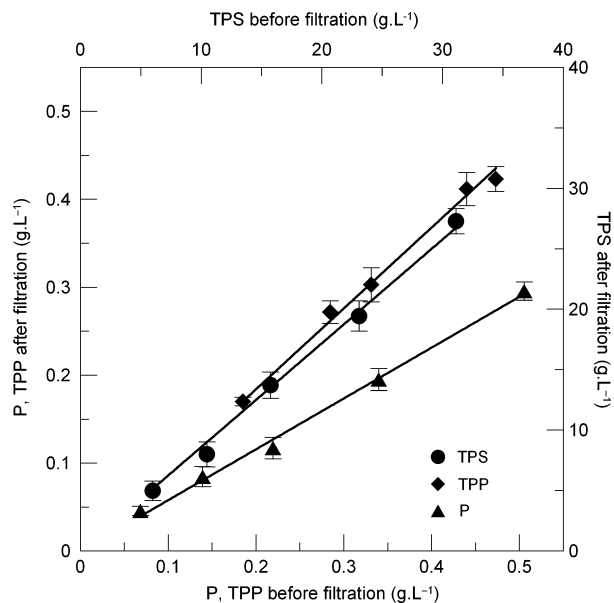
where  $a$  is an adjustable constant. Our results suggest the fitting parameter  $a = 0.554$  ( $R^2 = 0.987$ ). This fitting was valid even when yeast concentration was high and wort concentration was low. The variation in the composition of wort was due to the effect of fermentation, allowing us to detect a high concentration of TPS in the TS (> 50%).

With this analysis, we estimated the concentration of TPS from a simple test, such as the determination of TS, for the type of beer studied. TPS determination is highly time-consuming and is thus not suitable to estimate the amount of filter aid needed for breweries. Estimation of TPS concentration from the TS could be useful in the calculation of the influence of the colloidal particles on the colloidal force viscosity, which is more specific than the simple viscosity of beer and could actually help to improve the prediction of the filtration flow.

After filtration, beer components were not retained in the same proportion. Independently of wort concentration, the proportion in which TPP, TPS and P were retained, they maintained a linear relation among each other (Fig. 3). Consequently, the experimental data were fitted with straight lines-through origin:

$$X_{\text{After}} = b \cdot X_{\text{Before}} \quad (8)$$

where  $b$  is a numerical constant.



**Figure 3** Effect of filtration on the composition of beer samples. Full lines represent eqn (8).

**Table 2** Results for the retention percentages ( $\%R_X$ ) for total polysaccharides (TPS), proteins (P) and total polyphenols (TPP) and fitting parameters,  $b$  and  $R^2$ , for eqn (8)

	$\%R_X$	$b$	$R^2$
TPS	$17 \pm 3$	0.832	0.997
P	$43 \pm 5$	0.569	0.979
TPP	$8 \pm 2$	0.924	0.998

By combining eqns (6) and (8), the following expression was derived to estimate the retention percentages from the slope of each line for TPS, P and TPP (Table 2):

$$\%R_X = (1 - b) \cdot 100, \quad (9)$$

Values of  $\%R_X$  for the samples used in the micro-fermenters were close to the values of  $\%R_X$  of the overall process (Table 1). The main difference in the concentration of TPP between the two analyses could be due to the fact that the change in TPP concentration during filtration was not significant in the overall process. Therefore, the study using the microfermenters is representative of the overall process at a pilot scale.

An incipient interaction between P and TPP after fermentation and before filtration is less likely to occur, because TPP permeate through the filter with minimal retention, independently of their concentration. It was assumed that the interaction between both components increases after filtration. This supports the idea postulated by Siebert (2009) that polyphenols in

drinking beverages have to polymerise before they interact with proteins, producing haze in bottled beer. Consequently, because they are not yet polymers at the beginning of the filtration (or precipitated with proteins during fermentation), interactions with P do not occur.

The fact that filtration retained colloidal particles and the retention was linear for TPS, P and TPP could explain a constant composition of colloidal particles in this study. Filtered material remains retained in about 13.3% of the TS (Table 2). With the composition of the wort before filtration (Table 1) and the retention percentages (Table 2), this last analysis also indicates that colloidal particles have a uniform composition and are formed by polysaccharides (97.04%), P (2.87%) and TPP (0.22%). This composition agrees with previous work (Benítez *et al.*, 2013).

### Contribution of TPS to viscosity

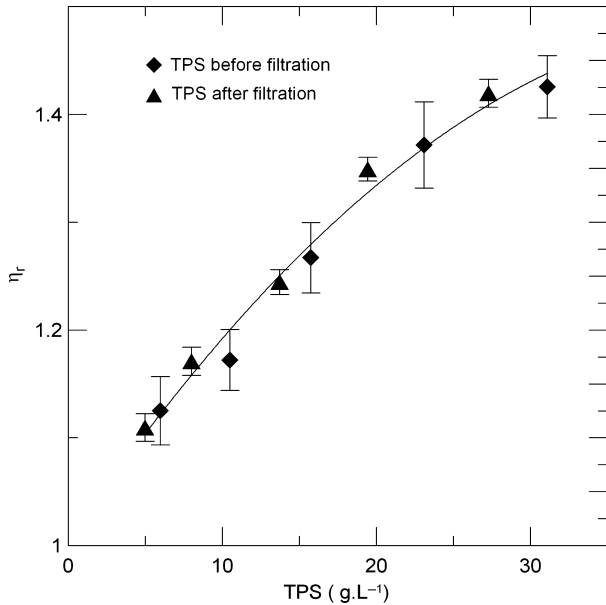
Because TPS concentration was considerably higher than P and TPP, it is reasonable to think that they would significantly contribute to the relative viscosity. An empirical polynomial of second-order equation was obtained that explains the influence of TPS on viscosity ( $R^2 = 0.985$ ):

$$\eta_r = 1 + 2.11 \times 10^{-2} \cdot \text{TPS} - 2.29 \times 10^{-4} \cdot \text{TPS}^2 \quad (10)$$

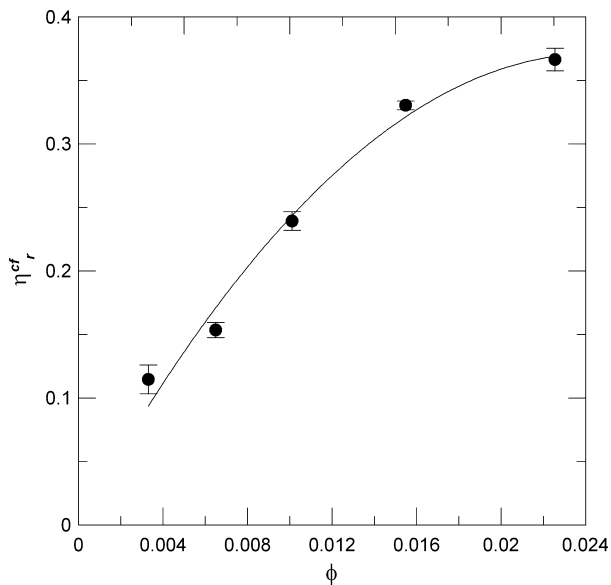
The shape of the curve after filtration, with the negative adjustment parameter for the quadratic term, indicates that the polysaccharides had low molecular weight, agreeing with viscosity values found for beer models obtained with concentrations of beta-glucans smaller than 31 kDa (Jin *et al.*, 2004). Furthermore, over a certain concentration of TPS, the interparticle distance is reduced and allowed the aggregation and the precipitation of them. Nevertheless, more studies with a more concentrated solution, that is not present in this type of beer, are needed.

Removing the colloidal particles, the 73.8% of the TPS remaining in solution had an influence on the viscosity similar to that of the sample before filtration. No difference was observed between the effect of polysaccharides on viscosity, before or after filtration (Fig. 4). The correlation between TPS and sample viscosity shows no variation before and after filtration ( $P < 0.05$ ), and only one empirical correlation for the samples before and after filtration was obtained.

The overall results support the possibility of obtaining a single fitting to residual polysaccharides after fermentation as a function of the relative viscosity (after samples are degassed and boiled). In Fig. 4, if each pair of data, before and after filtration, was analysed, it could be observed that the concentration of TPS



**Figure 4** Effect of total polysaccharides on viscosity, before and after filtration. Full line represents eqn (10).



**Figure 5** Contribution of colloidal forces to the relative viscosity vs. volume fraction.

was reduced after filtration and consequently the relative viscosity, but the correlation between both  $\eta_r$  and TPS concentration remained constant.

#### Colloidal force viscosity

The particle volume fraction could be determined considering the colloidal nature of the particles retained

on the filter, the composition of the particles before filtration, the  $\%R_X$  from Table 2 and the relative particle density ( $\rho_p^p = 1.36 \pm 0.06$ ). The contribution of colloidal forces to the relative viscosity is presented in Fig. 5. The fitting parameters of eqn 5 were ( $R^2 = 0.991$ ):

$$\eta_r^{cf} = 29.4 \cdot \phi^{0.77} \quad (11)$$

Directly related to the energy barrier, the value of  $\varepsilon$  indicates the stability of colloidal particles. A value of  $\varepsilon = 29.4$  in addition to the  $\sigma$  value  $< 1$  could explain the low stability of the colloidal particles of beer before filtration. The colloidal force viscosity excludes the influence, for example, of the dextrin that modifies the viscosity but not the filterability of the sample; therefore, this new methodology to estimate the influence of colloid particles over the colloidal force viscosity could contribute to the estimation of the filtration flow.

The contribution of colloidal forces is more specific than the relative viscosity of a sample, because it mainly represents the interactions among particles, and the viscosity of the surrounding liquid is excluded. The interactions among particles would explain the tendency of these colloids to agglomerate or remain free (Benítez *et al.*, 2007, 2009) and could be important in the reduction of the flux filtration. However, more analyses are needed to confirm this importance.

#### Conclusions

This study contributes to the understanding of the effects of colloidal particles on the colloidal force viscosity of the beer before filtration.

The fitting parameters of the correlation between the particles volume fraction and the colloidal force viscosity could explain the low stability of the colloidal particles of beer.

The influence of colloidal particles on the colloidal force viscosity could be more significant than the relative viscosity of the solution in the influence on the filtration flux. However, more analyses are needed to confirm this significance.

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