IJFR 5 (2018) 13-19 ISSN 2056-9734

International Journal of Food Research
www.bluepenjournals.org/ijfr

# Particle size distribution of polysaccharides in beer before the filtration process

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Article History
Received 06 December, 2017
Received in revisedform 21 December, 2017
Accepted 27 December, 2017

**Keywords:**
**Polysaccharides, Fermentation, Viscosity, Hydrocolloids.**

**Article Type:**
***Full Length Research Article***

**ABSTRACT**

**Conventional beer filtration with diatomaceous earth as a filter aid causes a remarkable flow reduction due to the presence of yeast and colloidal particles formed principally by polysaccharides. The main polysaccharides of beer are dextrins, arabinoxylans (AX) and β-glucans (BG). Dextrins affect beer viscosity but not filterability, therefore the aim of this study was to quantify and characterize the two most influential polysaccharides in flow filtration: AX and BG. Scanning electron microscopy (SEM) and particle size distribution for each of the polysaccharides involved was performed. It was determined through SEM images a more compact and bigger formation of AX colloidal aggregates, even though both polysaccharides have the same diffusion limited method of aggregation. The greater size of AX compared to BG, might indicate that the concentration and the average size of AX could have a greater influence on filtration, resulting in a further reduction in the filtration flow.**



## INTRODUCTION

Prior to filtration, beer shows significant turbidity due to the presence of colloidal particles resulting from wort processing (Martinez Amezaga et al., 2016; Benítez et al., 2013). The polysaccharides remaining after fermentation are the main reason for a reduced filtration rate, interacting to a greater or lesser extent with the solvent depending on the type and molecular weight of the polysaccharide (Fox, 2009).

Arabinoxylans (AX) and β–glucans (BG) are the main components of cell walls in barley endosperm, causing further problems such as reduction in filterability, mainly attributed to incomplete degradation during barley germination (Li et al., 2015). Dextrins are the residual products of the enzyme action of α– and β–amylases on starch. Beer viscosity is affected by the presence of AX, BG and dextrins, the latter being responsible for causing the most significant effect on this property (Muñoz-Insa et al., 2013), but not for decreasing filtration rate. It has been reported that AX and BG molecular weights (MW) determined viscosity and influenced flow reduction in beer. Although initially the decrease in filterability was mainly attributed to BG, it was found that the concentration and MW of the AX are the most influential factors as to increasing viscosity and reducing filterability (Sadosky et al., 2002).

Wort models used to predict filterability added AX and BG with high MW which overestimated their effect on filtration (Sadosky et al., 2002; Jin et al., 2004). Since AX and BG degrade during malting due to the enzymes produced during germination and the mashing, boiling and fermentation stages carried out along the brewing process, it is important to use polysaccharides that have already gone through these phases, in order to use models with ranges of MW similar to those existing in beer before filtration.

Based on literature regarding MW, viscosities and mechanisms of aggregation of these polysaccharides, it

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has been postulated that they quickly aggregate even in very dilute aqueous solutions (Li et al., 2011; Shelat et al., 2010). For BG at temperatures below 100°C, it has been postulated that the aggregates have a bimodal distribution, and are very stable and cannot be removed by heat treatment (Li et al., 2011). This bimodal distribution is due to the presence of individual particles of 5 µm and aggregates of 50 µm in diameter. In that same study, a dominant cluster-cluster aggregation was postulated, which would correspond to low fractal dimensions ($D_f$). However, the said study obtained $D_f$ values in agreement with the fact that aggregates may not be large enough to form fractal structures (Li et al., 2011). Little is known about the mechanism of AX aggregation; the high diffusion coefficients might explain their aggregation and an average size of 100 µm might indicate a significant aggregation in water. Also, it has been postulated that there are varying size aggregates and branched structures (Shelat et al., 2010).

While previous studies explore the aggregation mechanism, they do not show images of the aggregates that could help characterize them morphologically. The methodology based on the determination of beer $D_f$ (Benítez et al., 2013) from images of particles obtained by SEM, has been very useful to explain the behavior of colloidal aggregates and can be applied in this case.

Since AX and BG have a greater influence on flow reduction, they were characterized and quantified. The characterization consists in the determination of size, shape and aggregation mechanism to understand their influence on the filtration flow.

## MATERIAL AND METHODS

### Beer preparation

Mashing was carried out with 7.5 kg of milled malted barley (Cargill Malt Division, Argentina) with water at 62 ± 2°C for 90 min. The water/malt ratio was 4:1 during mashing. Lautering was performed in order to separate liquid (wort) from solid (spent grains) and more water was added to complete 40 L. Afterwards, wort was boiled for 60 min with the addition of hops for bitterness and aroma. Subsequently, wort was cooled to 12 ± 2°C and inoculated with 0.63 g $L^{-1}$ of Lager yeast (Saflager Fermentis S-23, France). Fermentation was accomplished in 15 days at a temperature of 12 ± 2°C, followed by a maturation period of7 days and a cold rest at 3 ± 2°C for another 2 days (Martinez Amezaga et al., 2016;Benítez et al., 2016). For the preparation of the conventional filtered (CF) beer, filtration was performed in a Büchner funnel (Ø = $5.0\times10^{-2}$ m) with a filter bed of diatomaceus earth (1 g Standard Super-Cel, mean porosity = 3.5 µm, permeability = $2.8\times10^{-13}$ $m^2$, Refil, Argentina) over a filter paper Whatman N° 3 under vacuum (-50 kPa) was used (Martinez Amezaga et al., 2016; Lataza Rovaletti et al., 2014; Benítez et al., 2013). The cross section area was $1.96\times10^{-3}m^2$.

### Total polysaccharides isolation

For the isolation of total polysaccharides (TPS) a beer sample was treated according to the method proposed by Lataza Rovaletti et al. (2014). Proteins and polyphenols were removed by precipitation and filtration. Proteins were extracted with bentonite (0.5% weight/volume commercial sodium bentonite type I) (La Elcha; Mendoza, Argentina) and polyphenols were extracted with polyvinyl polypyrrolidone (15 g $L^{-1}$, Polyclar 10, TUDELA, Argentina) (Benítez et al., 2016).The negative reaction resulting from the Bradford method (Bradford, 1976) for proteins and the Foulin-Ciocalteu method for total polyphenols (Singleton et al. 1999) was used to verify whether those components were removed (Lataza Rovaletti et al., 2014). Polysaccharides were extracted with ethanol (80%) by precipitation and drying at 40°C, as described by Segarra et al. (1995).

### Arabinoxylans and β-glucans isolation

The separation of AX and BG from the TPS solution was carried out according to the technique described by Sadosky et al. (2002). The sample was then treated enzymatically with α–amylase (UI:191) (Saporiti, Argentina) to degrade dextrins for 48 h at 20°C with constant stirring. After that period, the sample was boiled for 30 min to inactivate enzymes, and subsequently a 5% bentonite solution was added to precipitate the remaining proteins. Ammonium sulfate was added until saturation was reached and afterwards the sample was refrigerated at 4°C for 2 days. This process caused the precipitation of AX that were separated by centrifugation and re-dissolved in distilled water. After AX precipitation, the supernatant of the TPS solution contained the BG, which was recovered.

### Determination of polysaccharide concentration

The polysaccharide solutions obtained were dissolved in water to achieve the concentrations stated in Table 1. These solutions were prepared in order to work with a concentration equal to the starting beer. Polysaccharide concentration was determined after the enzymatic treatment. The difference with the initial TPS showed the concentration of dextrins. After AX and BG isolation, polysaccharide concentration in the precipitate (AX) and in the supernatant solution (BG) was determined, and a volume correction was made to express both

**Table 1.** Type and concentration of beer polysaccharides before filtration process.

| Beer polysaccharides | *c* (g/L) |
|---|---|
| Total polysaccharides (TPS) | 43 ± 2 |
| Dextrins | 32 ± 1 |
| β-glucans (BG) | 11 ± 1 |
| Arabinoxylans (AX) | 0.36 ± 0.04 |

Polysaccharides concentration data are shown as mean values ± standard deviation.

**Table 2.** Molecular weights (MW), fractal dimensions ($\overline{D}_f$) and particle ($\overline{D}_p$), and aggregates ($\overline{D}_A$) diameters of AX and BG after filtration.

| Parameters | AX | BG |
|---|---|---|
| MW | 19.99 | 2.12 |
| $\overline{D}_f$ | 2.5 ± 0.1 | 2.31 ± 0.03 |
| $\overline{D}_p$ (µm) | 9±1 | - |
| $\overline{D}_A$ (µm) | 147 ± 9 | 131± 18 |
| **Volume (%)** | | |
| $V_{A1}$ | 5.8 | 100 |
| $V_{A2}$ | 94.2 | - |

The data $\overline{D}_f$, $\overline{D}_p$ and $\overline{D}_A$ are the mean values ± standard deviation.

polysaccharides according to the initial concentration in the beer sample.

TPS, AX and BG concentration were determined with the Phenol-Sulfuric method (Segarra et al., 1995). Each sample was measured in triplicate.

### Analysis of particles and aggregates

Dilute samples of AX and BG were studied through SEM analysis (LEO, EVO 40, Cambridge, Ing.). Further details of the methodology are given in Lataza Rovaletti et al. (2014). Twenty different SEM images of the aggregates were subjected to the FERImage program which calculates $D_f$ by means of a variogram and a Fourier power spectrum (Bianchi and Bonetto, 2001). The methodology described was previously used with aggregates of beer (Benítez et al., 2013).

Particle diameter and size distribution of the AX and BG samples were measured using a laser-scattering particle size distribution analyzer (LA-950, Horiba Ltd., France) at 25°C. Particle size calculation was based on the Mie-Scattering theory. Results obtained are the average of five determinations per sample. The methodology was applied to beer (Benítez et al., 2013).

In order to calculate the volume percentages of the two aggregates of BG and AX, Origin 6.0 software (Origin Lab Corporation Northampton, MA, USA) was used. This program calculates the total area under the curve and of each peak. Particle size distribution and the values obtained are reported in Table 2. As size distribution was bimodal for AX, two values of area under the curve were obtained ($VA_1$ and $VA_2$), being their sum equal to 100%.

### Statistical analysis

Collected data were subjected to statistical analysis of variance and the Tukey test at the 0.05 level of significance, using Infostat (2002). Results reported in this study are the averages of three repetitions, unless otherwise stated.

## RESULTS AND DISCUSSION

### Polysaccharides analysis before beer filtration

Table 1 shows type and concentration of beer polysaccharides before filtration. Dextrin was the major constituent of the TPS of beer (74.1%), followed by BG (25.1%) and AX (0.8%). It could be noted that the

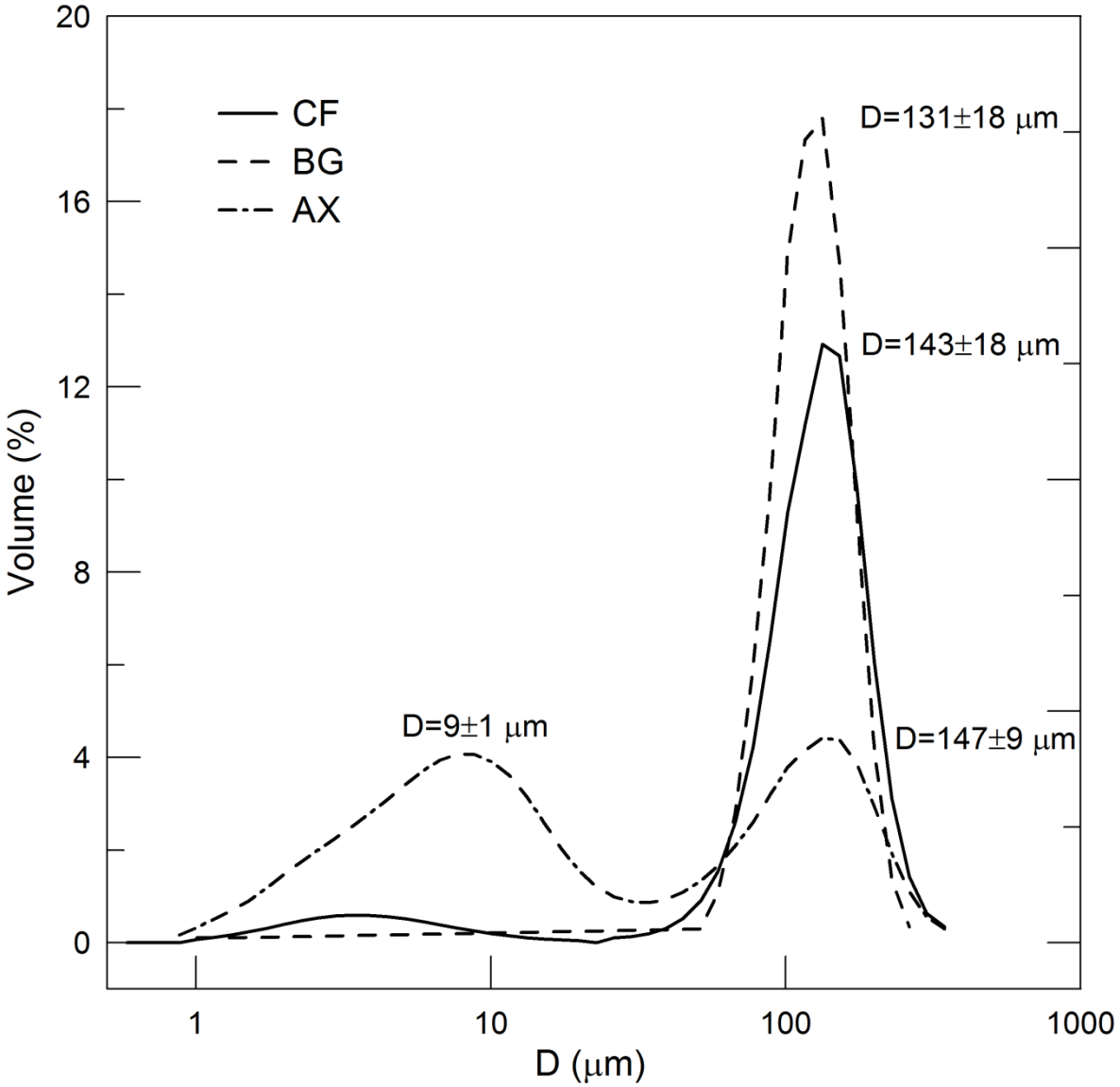


**Figure 1.**Particle size distribution for colloidal suspensions of AX, BG and filtered beer.

concentration of BG was 30 times higher than AX.

In some brewing industries, beer is treated with enzymes to degrade the polysaccharides, thus reducing filtration problems (Buttrick, 2010; Sensidoni et al., 2011).However, this practice also reduces viscosity, an important characteristic in liquid food products. Even though viscosity affects pumping, filtration, clarification and certain other processes, it has a positive effect on beer, contributing to foam stability (Bamforth, 2009). Furthermore, enzyme treatment would increase filterability by reducing the size of polysaccharides (Buttrick, 2010), allowing them to pass through the filter and may subsequently form colloidal aggregates, increasing turbidity and decreasing colloidal stability (Sensidoni et al., 2011). Therefore, enzymes were not used in the present study, which explains the high BG concentration.

**Particle size distribution**

Particle size distributions of the two main polysaccharides isolated from beer are compared with the particles isolated from CF beer, as shown in Figure 1.Previous studies have shown a bimodal distribution of beer particles before filtration, with small individual particles of a mean size of 0.06 μm and colloidal aggregates of 17 μm (Benítez et al., 2013). Therefore, it has been stated

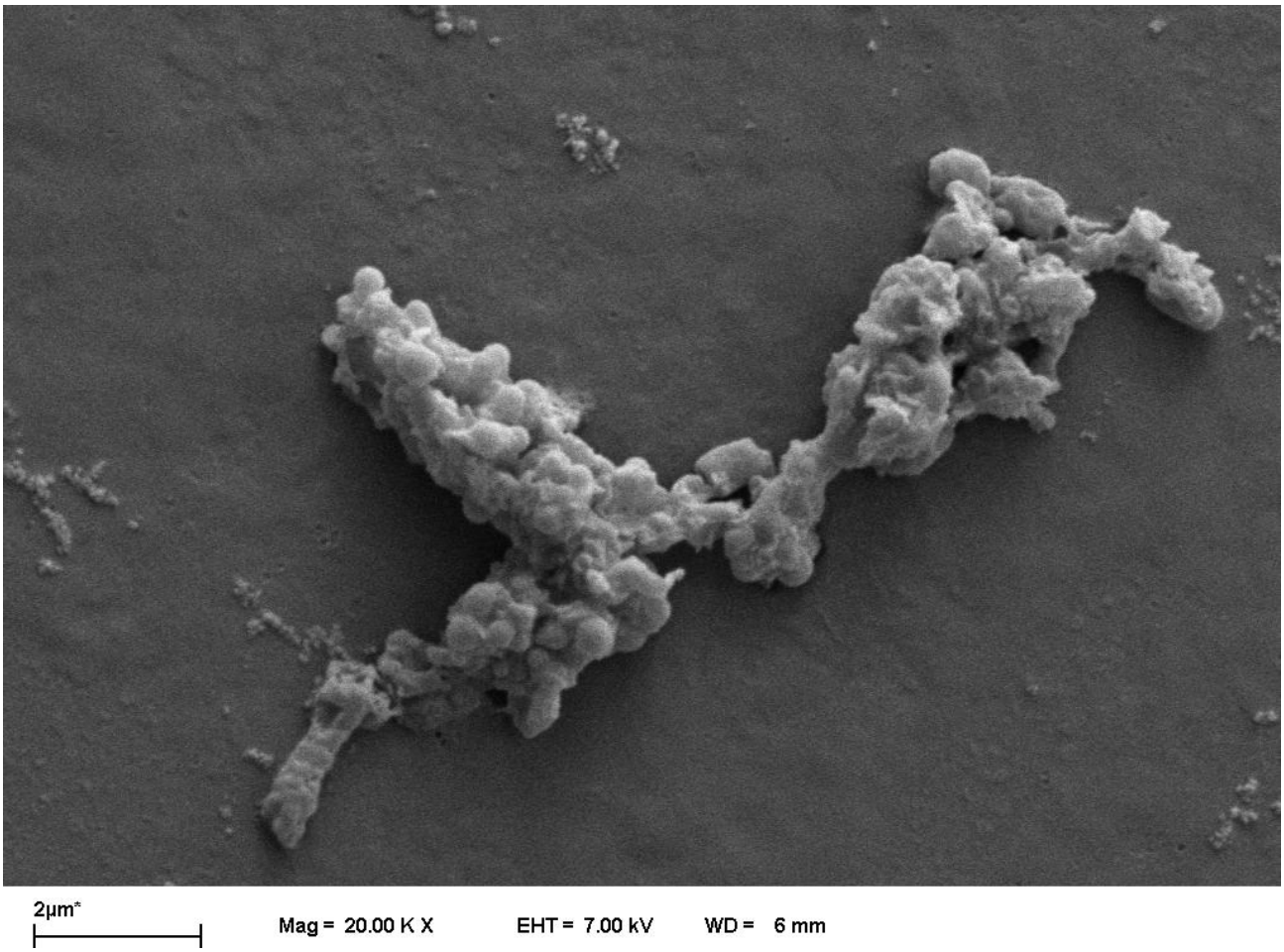


**Figure 2.** SEM image of a colloidal aggregate of AX (Magnification: 20000 X).

that due to the small size of the individual particles, a large amount of them may pass through the filter. This study suggests that these small particles that passed through the filter have aggregated afterwards to form colloidal particles of an average diameter of 143 ± 18 µm, similar to the BG and AX that were isolated from beer samples. Aggregates of similar size were found in both isolated samples of AX and BG, even with the CF. The values are shown in Table2. BG shows a monomodal behavior; where individual particles were not found, this may have occurred given the low MW of these polysaccharides. However, BG aggregates have a similar size (131 ± 18 µm) to AX and those found in CF, without significant differences. On the other hand, AX show a bimodal histogram, with small aggregates of 9 ± 1 µm (5.8% of the total volume of AX) and bigger size aggregates of 147 ± 9 µm (94.2%). Therefore, AX are more likely to show greater retention during filtration than BG, because of the formation of aggregates of AX, having an intermediate size of aggregates unlike BG.

**Analysis of scanning electron microscopy images**

The $D_f$ obtained from each polysaccharide shown in Table 2 reveals that a fast aggregation occurred due to a diffusion limited aggregation (DLA) for both aggregates. The slightly higher $D_f$ value for AX suggests a tighter aggregation than for BG (Bragaet al. 2015). The value found for BG indicates that particles adhere to the colloidal aggregates and that there would not be a subsequent re-accommodation−at least this was not observed over the study period. However, for AX, the value was found within the expected limits for re-accommodation existence, so this is likely to occur; therefore the colloidal aggregate is more compact than for BG.

Figures 2 and 3 show two SEM images of the aggregates obtained. The size of the BG aggregates is smaller (9.1 µm) and less compact than AX (11 µm), which is in agreement with the $D_f$ found. In BG, each of the particles in the aggregate can be clearly

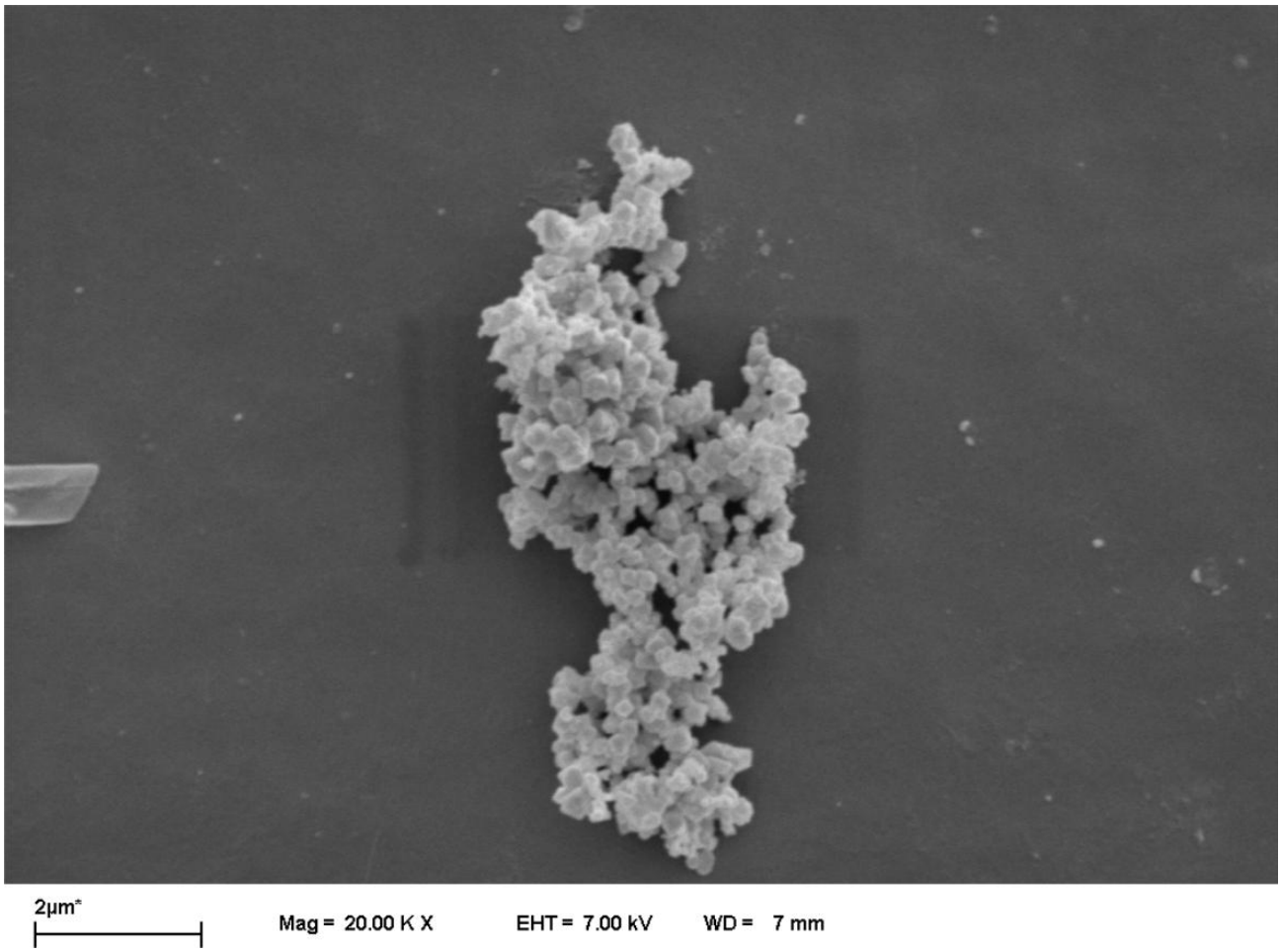


**Figure 3.**SEM image of a colloidal aggregate of BG (Magnification: 20000 X).

distinguished.

However, in the size distribution shown in Figure 1, smaller BG aggregates was not detected (9.1 µm), perhaps attributed to its low concentration, thus no corroborating the viscosity studies for these particles.

## Conclusion

These studies detected the presence of three polysaccharides: AX, BG and dextrins. In addition, both colloidal aggregate-forming polysaccharides AX and BG could be characterized morphologically.

In this study, it was determined that BG content is 30 times greater than that of AX, which contributes to understanding the reasons for previous studies to attribute so much influence to the loss of filterability caused by BG. Furthermore, it was determined through SEM images a more compact and bigger formation of AX colloidal aggregates, even though both polysaccharides have the same DLA method of aggregation. However, it could not be corroborated through particle size distribution, the existence of smaller colloidal aggregates of BG.

## ACKNOWLEDGEMENT

The authors thank the Facultad Regional Resistencia-UTN and CONICET for financial support.

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