

*Full Length Research Paper*

## **Hydrocolloids as beers foam stabilizer**

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Received 19 February, 2020; Accepted 17 April, 2020

**Beer foam is one of the most important parameters for consumers, affecting their purchase decision and satisfaction. Studies indicate that foam stability is positively influenced by its viscosity, and based on this fact the brewing industry uses propylene glycol alginate (PGA) as a stabilizer. However, PGA has its use restricted by Brazilian legislation to 0.07 g/L of beer. The objective of this research was to present alternatives to PGA, improving beer foam stability by adding other hydrocolloids, which does not have a maximum amount established by Brazilian legislation, and determining those with the greatest influence on the foam stability without significantly changing the colloidal stability and pH of the beverage. Colloidal stability, viscosity and foam shaking tests showed that the higher the hydrocolloid concentration in the beer, the greater the foam stability. PGA exhibited the best performance among the hydrocolloids tested, followed by Genu® Pectin type 106 HV and Genu® GUM type RL 200-Z, which had a significantly better foaming capacity than the control. Differently from the initial hypothesis, the foam stability was found to be more influenced by the chemical structure of the hydrocolloids, mainly their degree of esterification, than by foam viscosity.**

**Key words:** Beverages, technology, viscosity.

### **INTRODUCTION**

Beer is defined by Brazilian legislation as the beverage resulting from alcoholic fermentation, using brewer's yeast, malted barley wort or malt extract, previously submitted to a cooking process, added with hops or hop extract. A portion of the malted barley or the malt extract may be replaced in up to 45% by beer adjuncts, comprising unmalted barley and other cereals suitable for human consumption, malted or not, as well as starches and vegetable origins sugars (Brasil, 2009, 2011, 2019).

The most consumed beers in Brazil are of Pilsen type,

also called mainstream beers, which belongs to the Lager family (Mintel, 2013; AAFC, 2019). However, compared with the original Pilsen beers from the Czech Republic, these beers are lighter and more refreshing, less bitter and less full-bodied, what can be attributed to the addition of beer's adjuncts, such as corn, rice and syrups (BJCP, 2015; Justdrinks, 2018; Mega et al., 2011; Mintel, 2013). These beers are more correctly classified as American Light Lagers or American Standard Lagers and generally have original gravity content between 1.028

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and 1.050, final gravity between 0.998 and 1.010 g/cm<sup>3</sup>, the color varying from 2-4 SRM, bitterness from 8-18 IBU and alcohol by volume (ABV) from 2,8 to 5,3% (BJCP, 2015).

World beer production in 2018 was estimated in 1.90 billion hl (BarthHaas, 2019) and the market revenue in approximately USD 570 billion (Statista, 2020), in which lager beers accounted for USD 366.94 billion. In volume, Madson (2017) and Arthur (2018) estimate that lagers represent around 90% of all produced beers, a market shared mainly by standard and premium lagers (Marston's, 2018).

Although they still represent a large market, mainstream standard lagers are losing space for premium and super-premium beers, in a trend known as "drink less, but drink better". Looking for new experiences, consumers are moving to different styles, flavored beer and mixed drinks (Arthur, 2018). Also, considering the growing population with gluten-related-diseases, breweries are seeking alternatives for offering high-quality gluten-free beers, as using gluten-free cereals or enzymes treatments (Hager et al., 2014; Rubio-Flores and Serna-Saldivar, 2016). These alternatives can interfere in quality parameters appreciated by consumers, as beer foam (Hughes and Baxter, 2001; Viejo et al., 2019; Deotale et al., 2020).

Beer foam is one of the first aspects perceived by beer consumers, along with color and turbidity, after the beer is poured into a glass. It is a very appreciated attribute, affecting consumer purchase decision and satisfaction (Hughes and Baxter, 2001). The foam layer is also important to protect beer from direct oxidation, to continuously release volatile compounds into the air as bubbles pop, contributing to the perception of beer aroma, and to the beer texture (Farber and Barth, 2019).

Barley proteins, especially protein Z (Niu et al., 2018), hop acids and non-starch polysaccharides are the main factors responsible for the formation and maintenance of beer foam. However, other factors such as the pH of the beverage, solubility and diffusivity of the gas in the liquid and the viscosity of the liquid also influence its stability (Bamforth et al., 2009, Jarpa-Parra et al., 2016). Inversely, the most damaging substances for foam are lipids because they affect surface tension (Gordon et al., 2018).

Therefore, the overall composition of the raw materials has a great impact on beer foam formation and stability, and it varies with the choice of malts and grains; the use of specialty malts or adjuncts; the proportion of ingredients; the addition of unusual ingredients to achieve different tastes and flavors; the alternatives used to produce gluten-free beers; the amount of hops; and the addition of foam stabilizers.

Foam is a colloidal system formed by a continuous solid or liquid phase and a discontinuous gaseous phase. Foam stability is correlated to the presence of

foam-positive substances and absence of foam-negative negative ones (Bamforth, 2017). The amount of liquid present in the foam is also time-dependent; it leaves the foam under the influence of gravity and "plateau border suction" in a process called drainage (Evans and Sheehan, 2002). For most beer foams, drainage precedes coalescence, which is the combination of two or more small bubbles to form larger bubbles. Occurring concomitantly, the dismutation or maturation of Ostwald consists in the fact that large bubbles increase in volume through the migration of the gas from the small bubbles, generating the weakening of the film around the gas bubble, with consequent rupture of the bubble. These latter two processes are more noticeable to consumers than the drainage itself, but of equal importance in terms of overall foam stability (Hughes and Baxter, 2001; Ronteltap et al., 1991).

Ronteltap et al. (1991) concluded that the forces that counteract drainage are the viscosity of the beer and the capillary effects of the foam surface. The influence of viscosity on foam stability is consistent with observations made, which show increased foam stability at low temperatures.

Increased beer viscosity can be achieved by the addition of thickening agents, such as gums, pectin, and alginate, which may indirectly contribute to an increased film thickness between the gas bubbles, decreasing drainage rate. Furthermore, the hydrocolloids structure is composed by chemical groups that can interact with other components of the film of the gas bubbles, contributing to the maintenance of its integrity (Hughes and Baxter, 2001; Azizpour et al., 2017).

Studies have shown that foam stability is positively influenced by the increase in the beer viscosity. Therefore, it can be achieved by the addition of stabilizing agents as gums, pectins and alginates, allowed for use in Brazil in accordance with Resolution RDC 65 of November 29, 2011, which provides for the approval of the use of food additives for brewing (Brasil, 2011).

Hydrocolloids are high molecular weight polysaccharides extracted from plants, algae or produced by microbial synthesis. They are mostly water-soluble and have thickening and/or gelling properties under specific conditions. They are currently used in all areas of the food industry, with increasing application in pharmaceutical and cosmetics (Cargill, 2018; Li and Nie, 2016).

The alginates are the hydrocolloids currently used by the brewing industry; however, the propylene glycol alginate (PGA) has its use restricted by Brazilian legislation to 0.07 g per 1000 ml of beer, while other gums and pectins do not have a maximum established amount. Among the commonly used hydrocolloids in the food industry, either as gelling or as thickening agents, are xanthan gum; sodium carboxymethyl cellulose or CMC; the alginates; gellan gum; pectin; and locust bean

**Table 1.** Summary of the characteristics of the hydrocolloids used in the research.

Code	Commercial name	Supplier	Family
PGA	KIMILOID® BF	Kimica Vogler Ing.	Alginate
GG	Gelan Kelcogel® HF-B	CP Kelco	Gellan
CG	CMC Cekol® 30.000	CP Kelco	Carboxymethylcellulose
CGH	CMC Cekol® HVD	CP Kelco	Carboxymethylcellulose
L200	Genu® GUM RL 200-Z	CP Kelco	Locust
P106	Genu® Pectin 106-HV	CP Kelco	Pectin
P121	Genu® Pectin 121 Slow Set	CP Kelco	Pectin
P102	GENU® Pectin LM 102-AS	CP Kelco	Pectin
KG	Carragen GENUVESCO® CSM-2	CP Kelco	Carrageenan
KGK	Carragen GENULACTA® K-100	CP Kelco	Carrageenan
XRD	Xantan Keltrol® RD	CP Kelco	Xanthan
X521	Xantan Keltrol® 521	CP Kelco	Xanthan

gum or LBG (Mahmood et al., 2017), which were tested in this study.

The objective of this study was to evaluate the low cost and widely available hydrocolloids on the market as an alternative to propylene glycol alginate (PGA) as a stabilizer for Pilsen type mainstream beer and others in which substitution of barley malt by other grains or adjuncts is relevant. The influence of hydrocolloids on foam stability was studied, as well as side effects on colloidal stability and beer pH.

## MATERIALS AND METHODS

Twelve hydrocolloids, from 7 different families, were chosen, based on their functionality, application, and availability in the Brazilian market (Table 1). They were obtained from CP Kelco (Limeira - SP, Brazil) and Kimica Vogler Ing (São Bernardo do Campo - SP, Brazil).

For the colloidal stability, viscosity and foam stability preliminary tests, a commercial beer without any additive or adjunct was used. For the subsequent tests, 40 L of an American lager beer (Pilsen type beer) was produced in the pilot plant of the Department of Food Technology of the School of Food Engineering, with 20% of adjuncts (rice), a similar amount to those beers found in the market, using the following ingredients: Pilsen malt (Cooperativa Agrária Agroindustrial, Guarapava - PR, Brazil); rice (Pileco® Nobre Alimentos Ltda, Alegrete - RS, Brazil); drinking water; cluster hops pellets with 5.7% alfa-acids (Lamas Brew Shop, Campinas - SP, Brazil); and dehydrated lager yeast Saflager w-34/70 (Fermentis Lesaffre, Marcq-en-Barœul, France).

Palmitic, oleic and linoleic acids, all  $\geq 99\%$  purity, obtained from Sigma Aldrich (São Paulo - SP, Brazil), were used to evaluate foam stability.

### Preparation of hydrocolloid base solutions

The hydrocolloids were dispersed in water at 1 g/L using a homogenizer (IKA Labortechnik® T-25 Basic, with IKA Labortechnik® S 25 N-25F rod) at 8000 rpm for one minute. The samples were then placed in a water bath (Buchi® b-480) with

boiling water, coupled to a piece of variable-speed mixing equipment (Technical® TE039/1, 3.5 cm diameter naval impeller, 800 rpm), and warmed up to 85°C. For the preliminary tests, in water or commercial beer, stock solutions (1 g/L) were made in triplicate. For the final tests, in the produced beer, only one stock solution of each hydrocolloid was made, which was dissolved in the degassed beer at the concentrations to be tested, in triplicate.

### Beer degasification by helium injection

The procedure was carried out by injection of 50 kPa helium as a degasser (Shimadzu® DGU-2a) into a 2 L glass beaker containing about 700 ml of beer at 8°C. To avoid excessive foaming during helium degasification, a second 5-mm diameter aperture diffuser was connected in the helium outlet channels of the apparatus to form large bubbles, to dismantle the foam by causing its rapid collapse. The beer was then transferred to a 4-L beaker partially immersed in an ultrasonic bath (Sharp® UT-204) for 10 min to remove residual gases. The degassed beer was stored in amber screw-capped glass bottles at 4°C and used as quickly as possible to avoid degradation.

### Dilution of hydrocolloids in commercial beer

Samples were prepared with 90% degassed beer and 10% hydrocolloid solutions (aliquots from the base solution of hydrocolloids plus the necessary water) to obtain beers with 0.01; 0.03; 0.05; 0.07; 0.09 and 0.1 g/L of each hydrocolloid, as exemplified in Figure 1. A control was prepared to contain 10% of water. By adding the already dissolved hydrocolloid in the degassed beer, the process to be used in the industry was simulated, where the hydrocolloid solution is added to the filtered beer before the gasification and beer filling step.

### Hydrocolloids pre-selection

A qualitative colloidal stability test was carried out based on Chapon's methodology (1968) using the degassed commercial beer (without adjuncts). Aliquots 10 ml of beer samples containing the different hydrocolloids at concentrations of 0.01, 0.05, and 0.1 g/L were incubated in test tubes at 8°C for 24 h to evidence precipitation

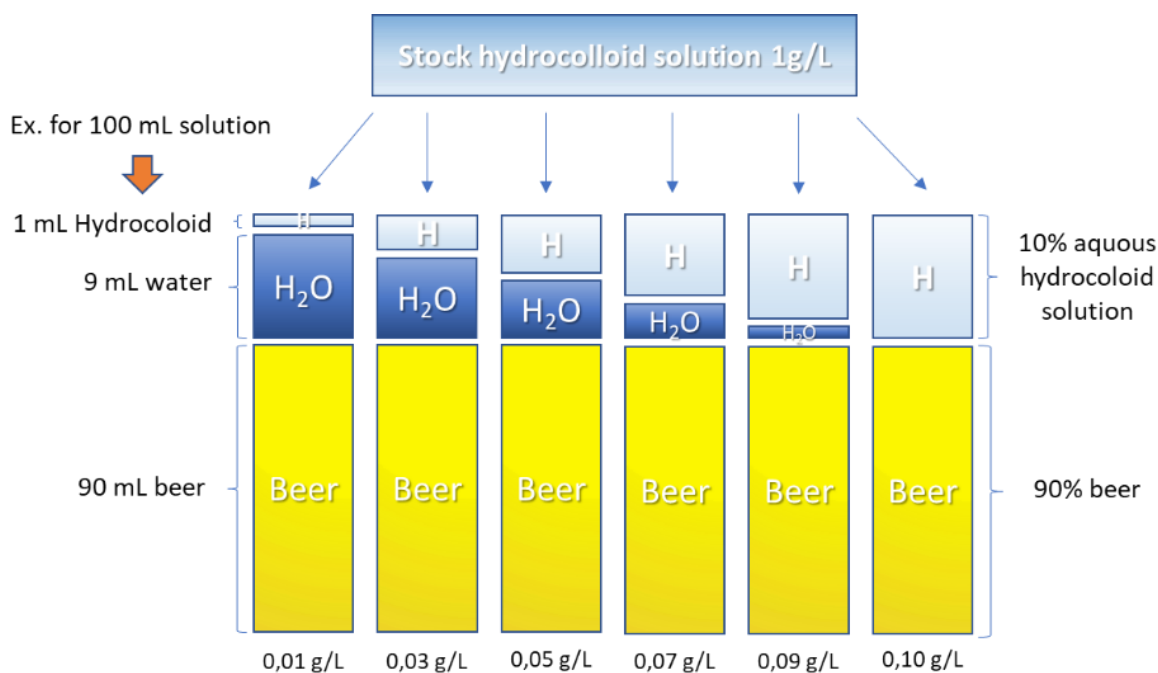


Figure 1. Hydrocolloid dilution scheme in beer.

or turbidity of the beer. After 24 h, the tubes were visually analyzed and compared to the control (beer plus distilled water). Then, 1 ml of anhydrous ethanol was added on the samples, which were incubated for another 24 h, to force the precipitation of the hydrocolloids in those samples which, in the first moment, had no turbidity neither precipitate. Images were recorded with a Canon® 6D camera.

### pH

The pH of the hydrocolloid solutions in beer was measured using a pHmeter (Digimed DM 20) according to AOAC (2010).

### Viscosity test

The assays were carried out at 20°C in a viscometer (Brookfield® LVDV-IIT with spindle and Brookfield ULA cup camera) coupled to a bath (Brookfield® TC-550). The tests were performed using 16 ml samples, at 60 rpm, according to the manufacturer's specifications. The solutions' viscosity was evaluated in triplicate, in solutions of distilled water and degassed beer in the concentrations 0.01; 0.03; 0.05; 0.07; 0.09 and 0.10 g/L.

### Shaking test

The shaking test was based on the method developed by Knapp and Bamforth (2002). The test tubes were placed in a rack and images were recorded at times 0 and 30 min, respectively with a camera (Canon® SX150IS) attached to a tripod, then, analyzed with the help of the software program Charten software® Bitruler. Assays were carried out with samples of degassed beer containing hydrocolloids at concentrations of 0.01, 0.05 and 0.10 g/L, in

triplicate. The percentage of residual foam was calculated by difference with the initial and final foam data. Only the best performance hydrocolloids were chosen to be evaluated in the following foam stability tests using a produced beer with 20% of adjunct.

### Beer preparation

For the final tests, an American Lager beer was prepared. The ground rice (Quadrimat Senior Brabender® roller mill) was mixed with water and boiled in a jacketed kettle until complete gelatinization of the starch, resulting in 2 h of processing. The gelatinization was verified by polarized light microscopy (Olympus BX51) with a magnification of 10x and 100x in slides, with an aqueous dispersion of starch (0.1 g of starch with 5 ml of water) (Zambrano et al., 2001).

The previously milled malt (Guzzo mill cod. 2508) was mixed with the adjunct and mashing was conducted at 63-64°C for 1 h. The end of the process was verified through the 0.01 N Iodine test, in which the absence of purple color in the wort indicates the complete hydrolysis of the starch into smaller sugars.

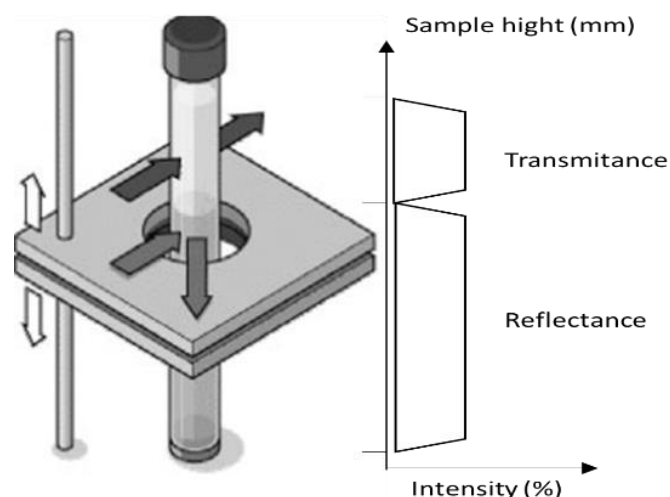
The wort was separated from the malt husks and other insoluble materials by centrifugation. The clarified wort was boiled for 1 h and hopping was conducted in two steps: 70% at the beginning of the boil and 30% at the last 15 min.

The cooling was carried out in a plate heat exchanger (0.7 m<sup>2</sup> of exchange surface), in counterflow, until a temperature < 27°C was reached. The soluble solids concentration of the wort was 11 °Brix. It was placed in 15 L fermenters and inoculated with 11.5 g of the dehydrated yeast.

The fermentation was carried out at 8°C for 18 days. On the 19<sup>th</sup> day, temperature was decreased to 4°C, initiating maturation. On the 28<sup>th</sup> day, maturation was finished, and beer was stored at 2°C. For the analysis, the beer was filtered at 4°C on filter paper

**Table 2.** Production parameters of Pilsen clear lager with 20 % adjunct.

Adjunct	20% w/w
Apparent fermentation	92.7%
Original extract	11 °Brix
Alcohol by volume	6.45%v/v
Bitterness	8.2 IBU
pH	4.32

**Figure 2.** Turbiscan measurement principle.  
Source: Buron et al. (2004).

(Whatman 1 Cat No 1001 110) with the aid of a vacuum pump, then degassed as described opportunely. Table 2 presents the process parameters.

### Foam stability test by light scattering

The foam stability was also evaluated through light scattering using a vertical scanner (Turbiscan™ LAB expert), which produces a series of backscattering (BS %) and transmittance (T %) profiles as a function of time and tube length (Pasin et al., 2014). For this research, only the transmittance value was used. To measure foam stability, 10 ml of the sample at 20°C was added in the equipment's tube and manually shaken in the same way as in the shaking test described above. Immediately after that, the tube was disposed of in the apparatus for the starting of the measurements, as illustrated in Figure 2. This procedure was performed 3 times for each sample and read at every 30 s over 30 min, as suggested by Knapp and Bamforth (2002).

For the result analysis, the peak thickness measurement performed by the device software, TurbSoft 2.0.0.28 (Formulation SAS, 2013), was used. The two peaks identified as correspondent to the beginning and the end of the foam in the tube length were selected in the Delta Transmission ( $\Delta T\%$ ) chart, as described in Figure 3, which uses as the reference curve the scan transmittance

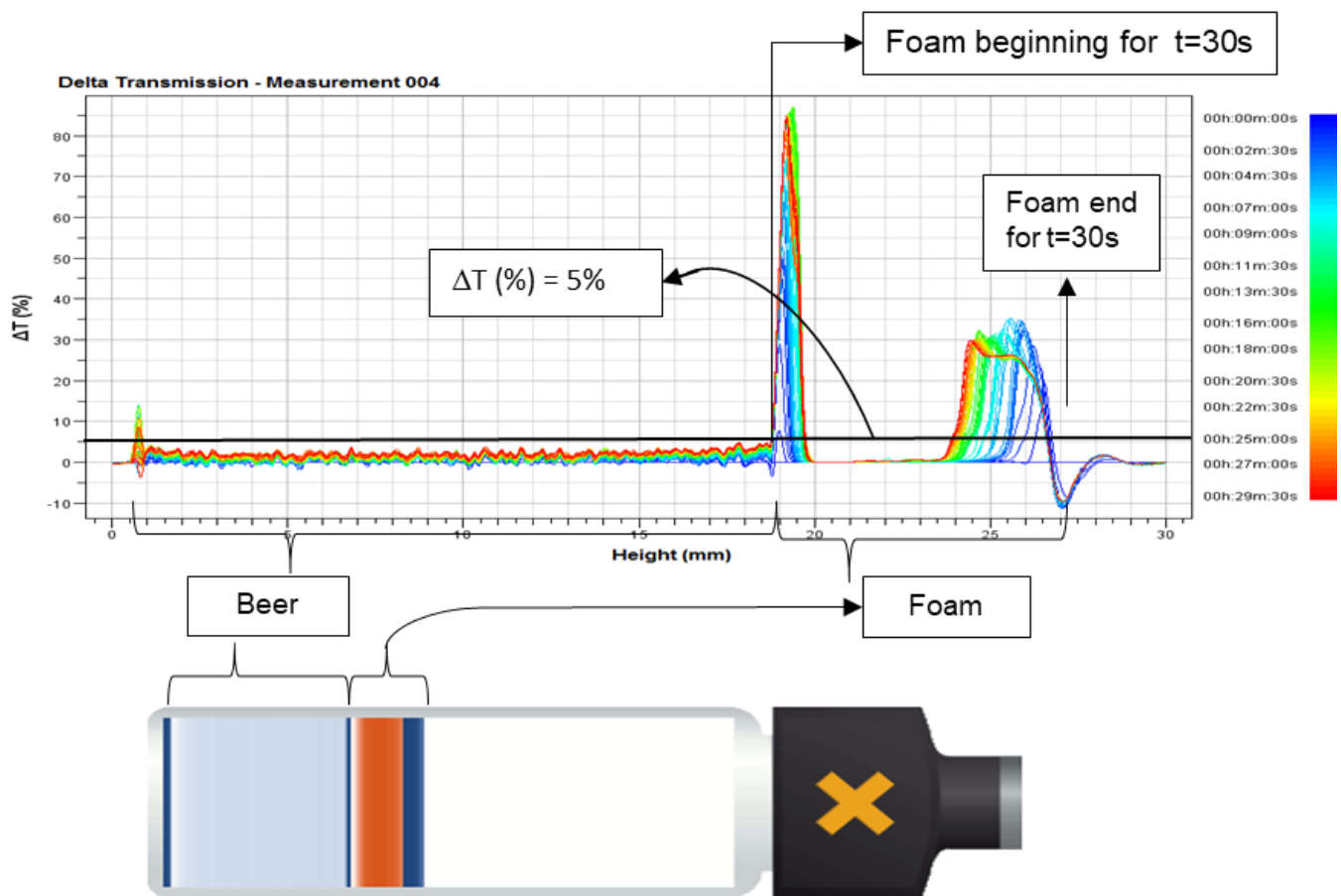
reading performed in 30 s. The software measured the width of each peak at  $\Delta T = 5\%$ , the value chosen to avoid noise readings. The peak width represents how much each reading is different from the reference reading ( $t = 30$  s), that is, the width of the set of the two peaks represents the collapse of the foam over time. Thereafter, the smaller the peak width, the greater the hydrocolloids stabilizing power.

In addition to the analysis with every single hydrocolloid, to investigate synergetic effects between PGA and the other products, light scattering analysis was also performed for samples containing 0.05 g/L of PGA and 0.05 g/L of the other hydrocolloids.

From these data, a graph of foam collapse by time was built, which represents what happened to the foam height in the Turbiscan tube for 30 min, and it was possible to evaluate which hydrocolloid was able to improve the foam stability compared to the control.

### Shaking test with free fatty acids (FFA) using the produced beer

Based on the work of Knapp and Bamforth (2002), a shaking test, similar as described previously, was carried out, using an intermediate hydrocolloid concentration of 0.05 g/L and an aliquot of 10  $\mu$ L of free fatty acids (FFA) dissolved in ethanol PA to reach a



**Figure 3.** Transmittance (%) by height (mm) of the White sample. The darker blue line represents the first reading at  $t = 30$  s (00 h: 00 min: 00 s in the graph). The red line represents the last reading at  $t = 30$  min (00 h: 29 min: 30 s on the graph).

concentration of 1 mg/L of palmitic, oleic or linoleic acids. The agitation was done immediately after the introduction of the FFA, evaluating the damage generated in the beer just after the contact with the lipids, simulating problems related to beer service and consumption (Robert et al., 1977).

The foam height in the test tubes was recorded using a Canon® 6D camera with a Canon® 24-105 f4 L lens, mounted on a tripod, so that the videos were digitally treated (Adobe® Premiere Pro CC) to insert a time counter, and used to measure the foam height as function of the time, for the different hydrocolloids.

#### Statistical analysis

Data was analyzed with SAS statistical software (SAS, 2002), using Tuckey Tests ( $p \leq 0.05$ ) for mean comparison.

## RESULTS AND DISCUSSION

### Hydrocolloids pre-selection

Concerning the preliminary tests, the hydrocolloids KG,

CG, CGH, XRD, and X521 were withdrawn from the study after 24 h incubation, because they caused haze, while KGK caused precipitation in the commercial beer, making their use impossible at the conditions tested. In addition, during the hydrocolloid dissolution and dilution stage, GG formed a visible gel or high viscosity solutions, even at low concentrations, making it difficult to handle, so it was also disqualified. After the addition of anhydrous alcohol, P102 formed a suspension gel and it was also removed.

Through the preliminary visual analysis, the hydrocolloids PGA, L200, P106, and P121 were chosen to remaining assays, at concentrations 0.01; 0.03; 0.05; 0.07; 0.09 and 0.10 g/L.

### pH

The pH value of beer solutions, independent of the hydrocolloid used and its concentration, remained between 4.57 (P106 – 0.01 g/L) and 4.65 (control), with

**Table 3.** Viscosity (mPa.s) at 60 rpm of solutions of distilled water or beer with hydrocolloid at different concentrations.

g/L	PGA	L200	P106	P121
<b>In distilled water</b>				
0.01	1.13±0.01 <sup>i</sup>	1.14±0.01 <sup>hi</sup>	1.13±0.01 <sup>i</sup>	1.14±0.01 <sup>hi</sup>
0.03	1.15±0.01 <sup>ghi</sup>	1.16±0.01 <sup>fghi</sup>	1.16±0.01 <sup>fghi</sup>	1.18±0.02 <sup>fghi</sup>
0.05	1.20±0.01 <sup>defgh</sup>	1.19±0.02 <sup>efghi</sup>	1.20±0.02 <sup>defgh</sup>	1.24±0.05 <sup>bcde</sup>
0.07	1.22±0.02 <sup>bcdef</sup>	1.21±0.05 <sup>cdefg</sup>	1.26±0.01 <sup>abcd</sup>	1.25±0.03 <sup>abcde</sup>
0.09	1.27±0.03 <sup>abc</sup>	1.28±0.03 <sup>abc</sup>	1.29±0.02 <sup>abc</sup>	1.27±0.00 <sup>abc</sup>
0.10	1.26±0.01 <sup>abcd</sup>	1.28±0.02 <sup>ab</sup>	1.29±0.02 <sup>a</sup>	1.29±0.01 <sup>a</sup>
<b>In beer</b>				
0.01	1.64±0.01 <sup>d</sup>	1.68±0.03 <sup>d</sup>	1.65±0.03 <sup>d</sup>	1.64±0.01 <sup>d</sup>
0.03	1.66±0.02 <sup>d</sup>	1.71±0.01 <sup>cd</sup>	1.65±0.02 <sup>d</sup>	1.66±0.02 <sup>d</sup>
0.05	1.67±0.02 <sup>d</sup>	1.75±0.01 <sup>bc</sup>	1.68±0.03 <sup>cd</sup>	1.66±0.02 <sup>d</sup>
0.07	1.67±0.02 <sup>d</sup>	1.79±0.02 <sup>ab</sup>	1.68±0.02 <sup>cd</sup>	1.68±0.01 <sup>cd</sup>
0.09	1.71±0.05 <sup>cd</sup>	1.82±0.03 <sup>a</sup>	1.67±0.02 <sup>d</sup>	1.69±0.01 <sup>cd</sup>
0.10	1.68±0.03 <sup>cd</sup>	1.83±0.02 <sup>a</sup>	1.69±0.01 <sup>cd</sup>	1.69±0.01 <sup>cd</sup>
Control	1.64±0.01 <sup>d</sup>	1.64±0.01 <sup>d</sup>	1.64±0.01 <sup>d</sup>	1.64±0.01 <sup>d</sup>

Means with different letters differ significantly by the Tukey test ( $p < 0.05$ ).

no significant difference between the samples ( $p < 0.05$ ). Despite this small difference, this is an important result, since pH influences hydrocolloid performance and solubility (CP Kelco, 2001; 2009; Ngouémazong et al., 2015). Jarpa-Parra et al. (2016) investigated the stability mechanisms of lentil legumin-like protein and polysaccharide foams at different environmental pH conditions (3.0 to 7.0), and the best foam stabilization occurred at pH 5.0, followed by pH 3.0, while pH 7.0 led to phase separation.

### Viscosity tests

As expected (Li and Nie, 2016), the viscosity of the hydrocolloid solutions in water increased with the concentration (Table 3); however, there was no significant difference between the samples of the different hydrocolloids at the same concentration. Regarding the formulations with beer, viscosities did not differ statistically from the control either, except for the formulation with hydrocolloid L200, where from the concentration 0.05 g/L, the viscosity increased significantly. However, it was noted that hydrocolloids have a slight tendency to increase viscosity as their concentration increases. In addition, it is important to note that the hydrocolloid used in industry, PGA, at the maximum concentration allowed by legislation, was not able to provide a significant increase ( $p < 0.05$ ) in beer viscosity.

The difference in the viscosity profile of the

hydrocolloids when in distilled water or beer solutions probably occurs due to the small differences in pH and the complexity in the beer matrix, whose components can interact with hydrocolloid molecules, altering their performance. In addition, beer contains mono and divalent metal ions that interfere with the viscosity of solutions containing hydrocolloids.

When beers were produced using mixtures of hydrocolloids PGA/P106, or PGA/L200, at 0.05 g/L (total 0.10 g/L), there was no significant difference ( $p < 0.05$ ) in their respective viscosities, when compared to beers formulated alone with 0.10 g/L of P106 or PGA, respectively. As the effect on beer viscosity by the addition of hydrocolloids (Table 3) was statistically similar ( $p < 0.05$ ) at all the concentrations tested, except for L200, we chose to continue with only three concentrations, two extremes, 0.01 and 0.10 g/L, and an intermediate, 0.05 g/L.

### Foam stabilization

In the shaking tests (Table 4), the hydrocolloids showed a tendency to increase foam stability with increasing concentration, the higher the percentage of residual foam, the greater the foam stability provided by the hydrocolloids. Azizpour et al. (2017) showed a similar effect on the foaming properties of shrimp puree using different hydrocolloids (xanthan gum, tragacanth methylcellulose, and Arabic gum). Jarpa-Parra et al. (2016) revealed that guar, xanthan, and pectin improved

**Table 4.** Percentage of residual foam in hydrocolloid beer solutions after shaking test.

Solution (g/L)	PGA	L200	P106	P121
0.01	63.15±4.63 <sup>bA</sup>	58.98±3.44 <sup>bcA</sup>	63.96±6.19 <sup>abA</sup>	60.52±2.97 <sup>abA</sup>
0.05	76.32±5.45 <sup>aA</sup>	69.00±2.38 <sup>abAB</sup>	75.65±5.35 <sup>aAB</sup>	61.09±8.05 <sup>abB</sup>
0.10	76.27±0.24 <sup>aA</sup>	72.36±3.87 <sup>aAB</sup>	72.17±3.10 <sup>aAB</sup>	67.09±0.82 <sup>abB</sup>
Control	52.92±5.38 <sup>b</sup>	52.92±5.38 <sup>c</sup>	52.92±5.38 <sup>b</sup>	52.92±5.38 <sup>b</sup>

Means followed by the same letters (uppercase in the row and lowercase in the column) do not differ significantly by the Tukey test ( $p < 0.05$ ).

**Table 5.** Variation in the foam height (%) of beers when fatty acids are added, in relation to the control with each FFA.

Solution (g/L)	Palmitic		Oleic		Linoleic	
	0	30 min	0	30 min	0	30 min
Control*	-39.33	-90.05	-36.95	-56.12	-56.93	-74.95
PGA 0.05	25.42	25	58.45	57.89	99.75	100
L200 0.05	-38.90	-39.28	-2.29	-2.02	73.55	73.75
P106 0.05	-8.87	-8.92	12.40	12.55	57.21	57.44
P121 0.05	-9.21	-8.90	64.69	65.18	103.36	103.54

\*Variation in relation to the control without FFA.

the stability of lentil legumin-like protein foams at mildly acidic pH, by the formation of aggregates and a dense network, which helped mitigate drainage and avoid coarsening.

There was no significant difference ( $p < 0.05$ ) between 0.05 and 0.10 g/L for all hydrocolloids, and, as the concentration of 0.01 g/L was insufficient for foam stabilization, we concluded that the concentration of 0.05 g/L was optimal.

According to Table 4, P121 showed the lowest residual foam averages by concentration, while PGA, L200, and P106 were similar at concentrations of 0.05 and 0.10 g/L. Thus, only the latter hydrocolloids were evaluated in the light scattering assays.

When comparing Tables 3 and 4, it was noted that the increase in viscosity is not necessarily related to the increase in the foam stabilizing property conferred by the hydrocolloids, as initially assumed. The hydrocolloid stabilizing power can then be attributed to their chemical nature and interactions with the different foam-forming chemical compounds (Hughes and Baxter, 2001), as proteins (Wijaya et al., 2015).

This hypothesis is reinforced when comparing the two hydrocolloids of the same family, but different in degrees of esterification: P106 has a better performance as a foam stabilizer than P121 and presents a 10% higher degree of esterification (69.2 and 59.4%, respectively) (CP Kelco, 2009). Freitas et al. (2017) also demonstrated high-methoxyl pectin shows a higher solubility in a greater pH range, increasing the stabilization of protein-pectin

complexes. It is known that the protein content is significantly correlated to parameters representative of foam stability (Condé et al., 2017), especially protein Z (Niu et al., 2018). Thus, it is confirmed that the higher the degree of esterification, the greater its foam stabilizing capacity, which has already been described in the literature for alginates (Hughes and Baxter, 2001).

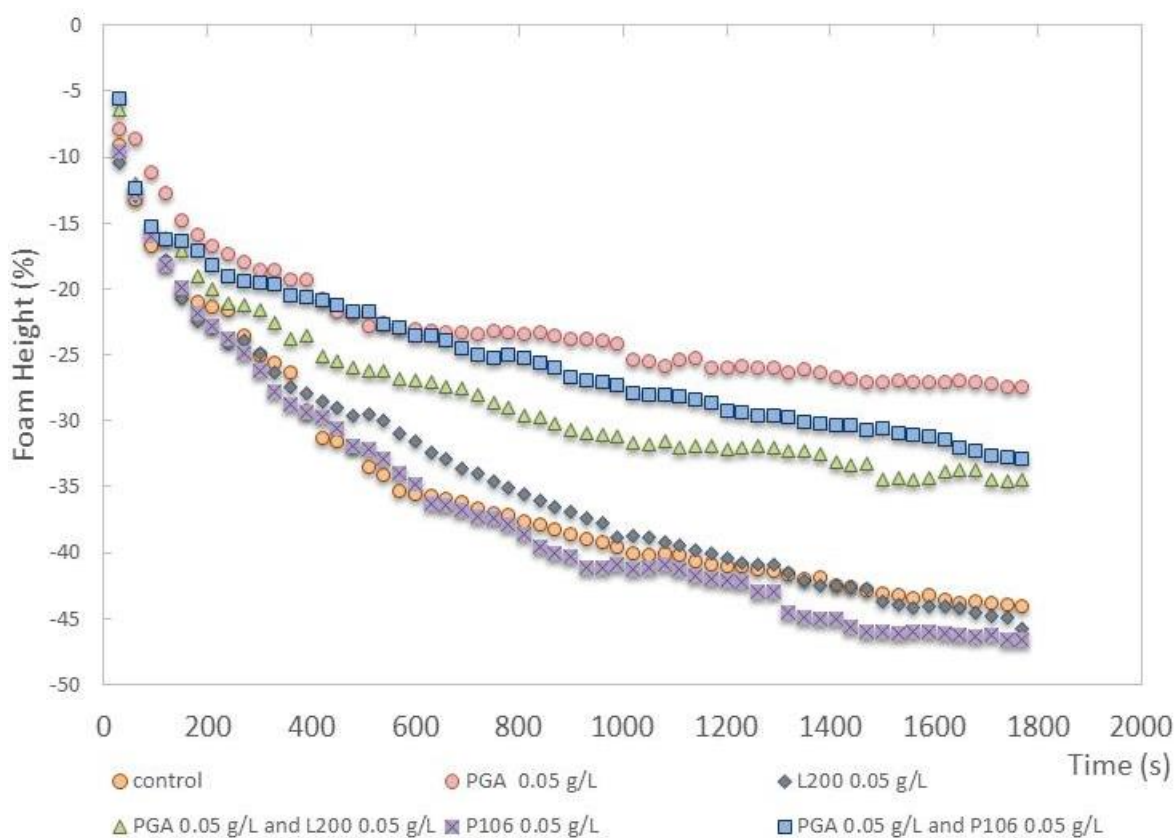
### Shaking test with free fatty acids

Lipids and high ethanol concentrations are the main foam-negative substances (Bamforth, 2017). Kosin et al. (2018) considered foam stability dependent on both foam-stabilizing and foam-damaging compounds and Kosin et al. (2017) stated the importance of the presence of foam-negative compounds in the beer foam study since they can be present in many recipes. When studying model beer-foam solutions, Kosin et al. (2017) demonstrated the importance of the presence of linoleic acid in the model foam due to its interactions with specific components and its impact on the foam structure and behavior.

In this study, the influence of palmitic, oleic and linoleic acids on the beer-hydrocolloids solutions was determined. Table 5 shows the reduction (- signal) or increase (+ signal) in the foam height (%) when fatty acids are added in the beer with or without the addition of hydrocolloids.

Only PGA at 0.05 g/L showed a good performance





**Figure 4.** Foam collapse (%) by time (s). The mean height of the foam of the replicates at each time, relative to the initial height, is plotted.

when fatty acids were added, increasing the foam height in the presence of all interfering agents. Hydrocolloids P106 and especially P121, both at 0.05 g/L, also showed good results for oleic and linoleic acids. According to Qin et al. (2018), PGA shows emulsification-stabilizing characteristics which make it highly efficient in acid-protein beverages.

### Foam stability test by light scattering

For their higher performance, L200 and P106 were chosen to be compared to PGA and the control, when applied to the beer produced in our Pilot Plant, at the concentration of 0.05 g/L. Figure 4 shows the foam collapse of the samples containing the three hydrocolloids evaluated at this stage (PGA, L200, and P106) and the mixtures of PGA with each of the other two in equal concentrations by the light scattering test. The 0% value of foam height at time zero represents the initial sample foam, which, as the analysis proceeds, collapses, acquiring a negative height value, as a percentage of the initial foam.

PGA was the only hydrocolloid capable of significantly decreasing foam collapse. It was verified that, from 660 s onward, PGA presents a significantly ( $p < 0.05$ ) higher foam retention than the other samples. However, it should be noted that foam stabilization for 5 min (300 s) already represents sufficient time for a suitable consumption of the product. In this aspect, again PGA and its mixtures showed good results.

### Conclusion

Although the viscosity of the hydrocolloid solutions in water increased significantly with the increase of its concentrations, the same did not occur when the hydrocolloids were added to beer, which can be attributed to the complexity of the beer matrix and possible interactions of the hydrocolloids with its components.

It was noted that the increase in the beer viscosity is not necessarily related to the increase in the foam stabilization conferred by the hydrocolloids at the concentrations tested, as originally supposed, but

probably to their chemical nature, mainly to their degree of esterification, and interactions with the different foam-forming chemical compounds.

The shaking test indicated that the ideal concentration of hydrocolloid to be used is 0.05 g/L since there was no significant difference ( $p < 0.05$ ) between 0.05 and 0.10 g/L, and neither between 0.01 and control. In addition, in this test, the PGA presented the best foam stability indexes, followed by hydrocolloids L200 and P106.

PGA at 0.05 g/L showed the best performance when fatty acids were added, but P106 and especially P121, both at 0.05 g/L, also showed good results for oleic and linoleic acids.

When assessing the height of the foam for 30 min, it was noted that the PGA hydrocolloid was the only one able to significantly decrease ( $p < 0.05$ ) its collapse. When foam stability was tested for PGA blends with L200 and P106, there was observed no synergy in terms of viscosity increase in the tested formulations; also, no positive synergistic effect when evaluating the foam height over the 30 min in the formulations tested.

Therefore, it is concluded that to improve the stability of industrialized beer foam, the addition of P121 and P106 are good alternatives, although PGA still shows the best results.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for the Scientific and Technological Development (CNPq), for the Master Fellowship of the second author, and the Undergraduate Fellowship of the third author, respectively; in addition to Research Support Foundation of the State of São Paulo (FAPESP) for funding part of the project.

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