LWT - Food Science and Technology 149 (2021) 112040

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

The impact of pirodextrin addition to improve physicochemical parameters of sorghum beer

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ARTICLE INFO

Keywords:
Pyrodextrins
Viscosity
Molecular weight

ABSTRACT

The traditional sorghum beer brewed by African communities differs from the traditional beer obtained from barley, mainly in the absence of hops and in a higher acidity level. People diagnosed with celiac disease find it as an alternative option when enjoying a good beer; however, some of the technological differences could be improved with the use of pyrodextrins obtained from the same cereal. In this work the use of pyrodextrins obtained by a treatment at 120 °C with acid during 360 min is proposed. A composite central design was made varying the concentration of pyrodextrins between 5 and 15 g/L and hop between 30 and 60 mg/L. The results indicate that the use of pyrodextrins up to a concentration of 10 g/L improves α-acid utilisation, bitterness and viscosity of the drink, achieving values similar to a typical lager beer obtained from barley. For 10 g/L pyrodextrin addition the increase in the wort and beer viscosity was 4.4% and 4.9%, respectively.

1. Introduction

Sorghum contains several polyphenol compounds that offer health benefits. The antioxidant action of polyphenols is their most important contribution. The incorporation of antioxidants in the diet helps to balance oxidative damage in cells and tissues (Butnariu & Caunii, 2013). The physiological role of antioxidants is to prevent the destruction of cell components that occur as a result of chemical reactions involving free radicals. Antioxidant activity depends on that part of molecule with important electron donor properties (Butnariu, Raba, Grozea, Vîrteiu, & Stef, 2013).

Tannins, which belong to the polyphenol family, reduce pre- and post-harvest losses, thus reaching high yields (Queiroz et al., 2018). The antioxidant capacity of sorghum is of scientific interest, even though some of its components, such as proteins and starch, are less available because of their interaction with polyphenols (Girard & Awika, 2018; Taylor, Belton, Beta, & Duodu, 2014). In the brewing of sorghum beer, in order to obtain a beer similar to a lager one, the tannin reduction in grains is necessary to allow the action of the alfa-amylase enzyme, and to reduce astringency and unpleasant bitter taste caused by the interaction of the polyphenols with proteins (Belhadi, Djabali, Souilah, & Yousfi, 2013 ; Links, Taylor, Kruger, & Taylor, 2015). Enzymes are necessary to break down both protein and starch in order to be used by yeasts during subsequent fermentation (Aguiar Moraes et al., 2015). In previous studies, it has been possible to use a hydrothermal treatment to obtain a cereal reduced in tannins and suitable to be used in food production. (Acquisgrana, Gomez Pamies, & Benítez, 2019; Acquisgrana et al., 2020).

Sorghum beer is typical of African countries and differs in the production method and, therefore, in its taste compared to the typical beers made with barley. Particularly, the elaboration at pH close to 4, which involves fermentations by lactic bacteria and the absence of hops, is one of the main differences found. Cloudy beers characterized by high starch content are similarly obtained. (Taylor & Duodu, 2019). Nowadays, beer made with sorghum has received greater attention by celiac consumers, since it is gluten-free. Although several studies have been carried out to obtain a drink similar to traditional barley beer, there are still issues to be resolved, such as the low viscosity obtained once the starch is separated by decantation (Igyor, Ogbonna, & Palmer, 2001), low mouthfeel, probably associated with low viscosity (Langenaeken, De Schutter, & Courtin, 2020) and low flavor retention. These are some of the problems detected and which can be improved through the incorporation of pyrodextrins. One of the possibilities is to use maltodextrins, known to be used for this purpose, to improve the viscosity of beverages and to

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https://doi.org/10.1016/j.lwt.2021.112040
Received 6 April 2021; Received in revised form 1 June 2021; Accepted 26 June 2021

provide better stability (Kapusniak & Nebesny, 2017). However, the food code of Argentina does not allow the use of additives from cereals that are not suitable for celiacs. An alternative is the use of dextrins from the same sorghum, which could also be used for other purposes in food. Especially the use of pyrodextrins, considered as dietary fibers, could be beneficial (Kapusniak & Nebesny, 2017) since they would provide the drink with a higher nutritional content. On the other hand, it could also contribute to α-acid utilisation and bitterness, which are issues to be tested in this work. Resistant dextrin, generally known as pyrodextrin, is a starch-derived product that partially resists the enzymatic hydrolysis in human gastrointestinal tract (Han, Kang, Bai, Xue, & Shi, 2018; Nunes et al., 2016). Resistant dextrin is produced via pyroconversion or dextrinization, using the combination of dry heat ($\leq$5% moisture, $\geq$100 °C) and acid ($\geq$0.1% dry starch basis (db)) (Jochym, Kapusniak, Barczynska, & Slizewska, 2012; Laurentin, Cárdenas, Ruales, Pérez, & y Tovar, 2003).

The beer suitable for celiacs produced entirely from sorghum can be made in different ways: from malted or unmalted sorghum, varying the form of production in each case. When starting from unmalted sorghum, the sorghum grain should be gelatinized at a temperature higher than 75 °C, which is the gelatinization temperature of the sorghum starch (Acquisgrana et al., 2020). Once this process has been carried out, it must be cooled to a suitable temperature for the addition of exogenous enzymes, mainly amylases and protease, to obtain free sugars and amino acids for fermentation by yeasts (Holmes, Casey, & Cook, 2016). Insufficient gelation or ineffective enzyme action leads to the production of a nutrient-poor wort for the yeasts.

This work presents the results of pyrodextrins addition to improve the physical-chemical properties of the drink, such as aroma and bitterness, produced by hops, and also to provide more body to the drink.

2. Materials and methods

2.1. Sorghum beer preparation

Sorghum bicolor (L.) Moench, variety TOB 60 T, from the Experimental Agricultural Station - National Institute of Agricultural Technology (INTA) from Argentina, with high content of tannin (Acquisgrana et al., 2020), was selected for this study. Sorghum grains (5 Kg) were steeped in 10 L of 0.5% (v/v) sodium hypochlorite (NaOCl) solution. The procedure was done at 25 °C during 18 h. Subsequently, the preparation was incorporated to a heat bath at 75 °C for 240 min (Acquisgrana et al., 2016, 2018, 2019). Then, the sorghum sample was boiled for 1 h with water in a 1: 4 ratio (sorghum: water) to achieve the gelatinization of the starch. The sample was cooled to 50 °C. The mashing process was carried out following the curve described by Ogbonna (2011) with some modifications in working temperatures. A curve consisting of 50 min at 50 °C, 30 min at 60 °C and 20 min at 70 °C was used. At the beginning of the mashing, the 2% α-amylase enzymes (Alphamalt VC, 5000 SKB, Germany) and 0.5% papain (Biopack, 30000U/mg, Argentina) were added. The non-hydrolyzable starch was decanted and separated for pyrodextrin obtention. After mashing, the samples were boiled for 1 h with the addition of hops, for bitterness and flavor. The different samples were inoculated with 6.3 g/L yeast lager type (Saflager S-23; Fermentis, Marcq-en-Baroeul, France). Fermentation was carried out at 12 °C for a period of 15 days, followed by a maturation of 7 days at 12 °C and a cold rest at 3 °C for 2 other days (Martinez Amezaga, Benítez, Sosa, Peruchena, & Lozano, 2016; Benítez, Acquisgrana, Peruchena, Sosa, & Lozano, 2016).

2.2. Pyrodextrin obtention

The sample for the starch isolation was obtained from the decanted starch after mashing. The 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, Polyclar 10, TUDELA, Argentina) (Benítez, Acquisgrana, Peruchena, Sosa, & Lozano, 2016). The negative reaction resulting from the Bradford method (Bradford, 1976) for proteins and the Foulin-Ciocalteu method for total polyphenols (Singleton, Orthofer, & Lamuela-Raventos, 1999) was used to verify whether those components were removed (Lataza Rovaletti et al., 2014). Non hydrolyzed starch was extracted with ethanol (80%) by precipitation and drying at 40 °C, as described by Segarra, Lao, López-Tamames, and De la Torre-Boronat (1995). As described in the literature, pyrodextrins were obtained using hydrochloric acid. 500 ± 0.5 g of isolated starch were mixed with 0.182% HCl, dry basis (db) (Anedra, Argentina) in a blender to disperse HCl evenly (Kwon, Chung, Shin, & Moon, 2005). However, the conditions for obtaining sorghum pyrodextrins were modified in their time and temperature, since the bibliography indicates that they must be more extreme to achieve a higher degree of conversion, using heating at 120 °C for 360 min (Laurentin et al., 2003).

2.3. Determination of average molecular weight, degree of polymerization and water solubility of pyrodextrins

Viscometry method was used for determining pyrodextrins molecular weight; a capillary viscometer, which is one of the most commonly used tools for determining the molecular weight of dilute solutions of water-soluble polysaccharides, was used (Wan et al., 2018). η (pyrodextrin viscosity), η_s (solvent viscosity, for pyrodextrin dispersion was water), η_w (wort viscosity) and η_b (beer viscosity) were measured at 25.0 ± 0.1 °C in a glass capillary viscometer (Cannon–Fenske) according with the AOAC official method (AOAC, 2000), calibrated with distilled water. Samples of beer after fermentation were degassed for 2 h at 60 °C to remove ethanol and other volatile components. The viscosities are expressed in mPa.s.

A stock solution of pyrodextrin was prepared and diluted to five desired concentrations (which must be below the critical concentration, C*).

The molecular weight (MW) of a polymer in dilute solution can be estimated with the intrinsic viscosity through the Mark-Houwink-Sakurada equation (MHS) (Wan et al., 2018; McClements, 2005):

$$[\eta] = K \times M_w^{\alpha} \tag{1}$$

where α and K are constants for the given solvent, polymer and temperature (Wan et al., 2018).

The specific viscosity (η_{sp}) of the solution can be described by the Huggins equation (1942):

$$\frac{\eta_{sp}}{C} = \frac{(\eta - \eta_s)}{\eta_s c} = [\eta] + K_H[\eta]^2 c \tag{2}$$

where c is the concentration of the polymer in the solution (g/mL) and the K_H is the Huggins coefficient.

Reduced viscosity (η_{red}) is equal to $\frac{(\eta - \eta^s)}{\eta^s c}$ (Linemann et al., 1998). Therefore, the intrinsic viscosity $[\eta]$ is obtained from the extrapolation of η_{sp}/c to zero concentration. It is possible to estimate the molecular weight of the dextrins from the intrinsic viscosity data, with reliable α and K values. Due to lack of information for pyrodextrins from sorghum, maltodextrin data obtained from the bibliography were used: $\alpha = 0.337$ y $K = 2.43 \times 10^{-3}$. The molecular weight (Mw) corresponding to a given degree of polymerization (D_P) for maltodextrin is obtained using $M_W = 162D_P + 18$ (Avaltroni, Bouquerand & Normand, 2004).

Water solubility of pyrodextrins was measured by the Schoch method (1964) with a slight modification. Pyrodextrin solution (10%, w/v) was heated in an 85 °C water bath with stirring for 30 min and immediately cooled in an ice water bath. The solution was centrifuged at 5000×g for 10 min, and the amount of dried matter in the supernatant was weighed after drying in a 60 °C oven overnight, and subsequently in a 130 °C oven for 1 h. Solubility (%) was the weight of dried matter in supernatant × 100/sample weight as dry matter (Kwon et al., 2005).

2.4. Physicochemical determination

The international standards MEBAK (Mitteleuropäische Brautechnische Analysenkommission, 2013) were taken as a reference, according to the technique used. Color was determined spectrophotometrically at 430 nm (MEBAK, 2.12.2) and the value was expressed on the SRM scale (Standard Reference Method) taken from the American Society of Brewing Chemists (ASBC). Free amino nitrogen determination (FAN) was determined spectrophotometrically at 570 nm with the ninhydrin method (MEBAK, 2.6.4.1).

The most important bitter substances in wort and beer are the iso-α-acid from hops (MEBAK, 2013). Bitterness in beer was expressed through IBU (International Bitterness Units). 1 IBU is equivalent to 1 mg/L of iso-α-acids.

The conversion of α-acids (AA) in hops into iso- α-acids depends on several factors such as hop variety, quantity and form, extraction time and temperature, hop dispersion methods (static/dynamic), beer and yeast type (Machado, Faria, Melo, Martins, & Ferreira, 2018). The AA utilisation efficiency is defined as the ratio of the concentration of iso-α-acids present in the final beer to the concentration of AA initially added to the wort (Verzele & De Keukeleire, 1991).

The iso-α acids concentration (IBUS) as well as α-acids were determined spectrophotometrically by carrying out isooctane extractions in alkaline methanol at different wavelengths (255 nm and 360 nm, respectively) and expressed in mg/L (MEBAK, 2.17.2).

Tannins (T) were estimated using the HCl-vainillin midific method (Price, Van Scoyoc, & Butler, 1978). Tannin extraction from the whole sorghum flour samples was performed with HCl in methanol (1% v/v) for 20 min at 30 °C, in a centrifuge. A volume of 1 mL of the extract (supernatant) was then mixed with 5 mL of vanillin reagent and absorbance was read at a wavelength of λ: 500 nm. It was used as a standard reagent catechin (Sigma – Aldrich Inc., St Louis, MO). Tannin content was expressed as mg catechin/Kg of solid matter. Total polyphenols content (TPP) was determined with the Folin-Ciocalteu method (Singleton et al., 1999). All the determinations were done in triplicate.

2.5. Experimental design

Hop concentrations at the beginning of the boiling were used for bitterness (Hop 1) and a second addition was made (Hop 2) for aroma, 2 min before the end of boiling which corresponded with half of the hops added for bitterness. Moreover, hops are usually classified according to AA concentration and categorized as high or low alpha specimens (Machado et al., 2018). In this study, Cascade hops with 7% AA (Hops from Patagonia, Argentina) were used, and the same variety was used for bitterness and flavor. In the experimental design, the concentration of AA of the added hops was expressed as the amount of initial AA reported in each sample multiplied by the mass of hops added, and it was expressed as AA-Hop 1 (H1) for the first addition and as AA-Hop 2 (H2) for the second addition. In all cases, half the amount of hops was added in each experience at the end of boiling, hoping that this contribution will be reflected in the aroma; however, it is known that part of this second addition of hops is also isomerized. For the experimental design, a range of AA-Hop1 between 20 and 40 mg/L was used and added in proportions as indicated in Table 2. For a better comparison, three samples were prepared before and after fermentation with AA-Hop 1 and AA-Hop 2 concentrations without added pyrodextrins, and their physicochemical properties are presented in Table 1.

Response surface methodology (RSM) was used to study the simul-

Table 1
α-acid Hop concentration for bitterness (AA-Hop$_1$) and flavor (AA-Hop$_2$), Experimental data of IBU, alfa-acid, color, tannins, total polyphenols (TPP), free amino nitrogen (FAN) and viscosity for wort and beer of sorghum, with different hop concentration without pyrodextrin addition.

AA-Hop 1 (mg/L)	AA-Hop 2 (mg/L)	Wort							Beer						
		IBU (mg/L)	α-acid (mg/L)	Color (SRM)	Tannin (mg/L)	TPP (mg/L)	FAN (mg/L)	η_w (mPa.s)	IBU (mg/L)	α-acid (mg/L)	Color (SRM)	Tannin (mg/L)	TPP (mg/L)	FAN (mg/L)	η_b (mPa.s)
20	10	14.7 ± 0.4	4.4 ± 0.1	10.5 ± 0.1	102.8 ± 0.2	276.7 ± 0.3	245.5 ± 0.5	1.46 ± 0.03	14.1 ± 0.5	4.3 ± 0.1	19.7 ± 0.1	65.6 ± 0.1	184.8 ± 0.2	76.6 ± 0.3	1.39 ± 0.01
30	15	21.5 ± 0.1	5.8 ± 0.2	10.9 ± 0.1	115.9 ± 0.3	299.1 ± 0.4	250.5 ± 0.4	1.48 ± 0.03	19.8 ± 0.7	5.1 ± 0.3	20.4 ± 0.1	72.1 ± 0.4	218.7 ± 0.3	66.9 ± 0.3	1.37 ± 0.03
40	20	26.7 ± 0.1	6.5 ± 0.2	10.8 ± 0.1	129.6 ± 0.2	293.5 ± 0.1	258.9 ± 0.3	1.46 ± 0.05	25.9 ± 0.5	5.9 ± 0.2	22.6 ± 0.1	79.4 ± 0.3	242.6 ± 0.5	81.5 ± 0.4	1.39 ± 0.04

Data are mean values ± standard deviation.

Table 2
CCD matrix of α-ácid Hop concentration for bitterness (AA-Hop$_1$) and flavor (AA-Hop$_2$), and pyrodextrin concentrations along with experimental IBU, alfa-acid, color, tannins, total polyphenols (TPP), FAN (Free amino nitrogen) and viscosity for wort and beer of sorghum.

						Wort							Beer						
Std order	Run order	Design	AA-Hop 1 (mg/L)	AA-Hop 2 (mg/L)	Pyrodextrin (g/L)	IBU (mg/L)	α-acid (mg/L)	Color (SRM)	Tannin (mg/L)	TPP (mg/L)	FAN (mg/L)	η_w (mPa.s)	IBU (mg/L)	α-acid (mg/L)	Color (SRM)	Tannin (mg/L)	TPP (mg/L)	FAN (mg/L)	η_b (mPa.s)
1	10	Factorial	22.93	11.47	6.46	21.10	9.64	9.63	88.41	296.81	283.83	1.53	17.72	5.40	11.70	80.24	286.61	92.62	1.43
2	9	Factorial	37.07	18.54	6.46	34.15	10.40	9.28	90.38	303.90	290.15	1.50	27.52	5.44	10.93	79.90	254.01	98.42	1.43
3	5	Factorial	22.93	11.47	13.50	17.25	5.00	13.63	67.71	216.74	257.15	1.57	14.04	2.16	13.15	93.55	193.52	58.00	1.46
4	4	Factorial	37.07	18.54	13.50	23.91	5.08	14.18	72.80	193.20	255.81	1.56	21.89	3.16	13.90	91.59	220.03	58.37	1.47
5	1	Axial	20	10	10	18.60	7.40	10.73	81.67	255.68	274.93	1.54	13.29	6.16	15.18	83.85	247.47	66.62	1.45
6	3	Axial	40	20	10	33.77	8.88	11.38	82.06	261.74	276.33	1.53	32.55	6.32	14.98	88.24	253.47	66.87	1.45
7	13	Axial	30	15	5	26.78	10.72	8.75	95.17	307.60	288.45	1.49	21.80	4.32	8.90	75.82	244.12	95.69	1.41
8	2	Axial	30	15	15	17.89	4.84	14.85	63.35	187.58	251.41	1.59	15.27	1.04	11.83	94.25	179.55	45.59	1.48
9	11	central	30	15	10	27.57	8.76	11.50	83.03	249.00	278.52	1.53	18.11	6.00	14.50	86.85	259.05	83.34	1.45
10	12	central	30	15	10	28.17	8.32	11.25	82.89	258.47	266.27	1.54	23.24	5.88	15.05	86.85	253.61	67.21	1.44
11	6	central	30	15	10	25.33	8.32	11.05	80.45	276.25	272.30	1.53	20.57	5.68	13.98	87.07	262.97	72.43	1.45

taneous AA-Hop 1 and Pyrodextrin influence on IBU, α-acid, color, Tannins, TPP and FAN before and after fermentation, since it allowed finding the optimal variation and identifying the influence of those factors. The response surfaces and equation fits were obtained from the first hop aggregate, but they can easily be transformed to consider the sum of both hop aggregates. As a simple reference this was only made to the first hop addition. The 3D response surface was used to determine the individual and cumulative effect of the factors and the mutual interaction between the factors and the dependent variable. Each factor was set at two different levels (k). The total number of experimental runs (N) was calculated as:

$$N = 2^k + 2 \cdot k + x_0 \tag{3}$$

where $x_0 = 3$ is the number of central points. Least squares regression methodology was used to obtain estimates for the parameters (Lataza Rovaletti et al., 2014), for example for IBU:

$$IBU = \beta_0 + \sum \beta_i \cdot X_i + \sum \beta_{ii} \cdot X_i^2 + \sum \beta_{ij} \cdot X_i \cdot X_j + \varepsilon \tag{4}$$

where β_0 is the constant, β_i is the slope or linear effect of the input factor X_i, β_{ii} is the quadratic effect of the input factor X_i, β_{ij} is the interaction effect between the input factors X_i, and ϵ is the residual error (Montgomery, 2003). Assays were carried out randomly using the rotatable central composite design (CCD) methodology, with axial values at a distance a = 1.414 from the central point. The experimental data are presented in Table 2 and the resulting responses for each combination of variables are shown in Table 3.

2.6. *Statistical analysis*

Data points were presented as the mean of the measured values. Variance was analyzed, and the Tukey test was performed at 0.05 level of significance. The statistical software MINITAB® Release 17 Statistical Software for Windows (Minitab Inc., USA) was used for the regression analysis and for the estimation of coefficients of the regression equations for the RSM, and Infostat (2002) was used for the analysis of the steeping procedures and for the starch isolation data.

3. Results and discussion

3.1. *Determination of average molecular weight, degree of polymerization and water solubility of pyrodextrins*

The evaluation of beer mouthfeel is complex and is dependent on various factors, being the taster one of its major attributes for which the terminology, like “viscosity” and “density”, is used. Carbohydrates play an important role in beer viscosity and the addition of adjuncts is a way to improve the mouthfeel (Langenaeken et al., 2020). Dextrins are one of the major contributions to beer viscosity; however, they are degraded during brewing resulting in decreased perception of mouthfeel (Rübsam, Gastl, & Becker, 2013).

In the case of sorghum beer, it is observed that low viscosity values could be improved by adding dextrins from the same cereal, and which, at the same time, could contribute to retain aroma. As regards the use of dextrins to increase viscosity, the current trend is to use pyrodextrins resistant to stomach enzymatic degradation, since they are dietary fibers (Wan et al., 2018). The pyrodextrins addition was carried out as described in the methodology section. From 500 ± 0.5 g of the isolated starch, 295 ± 4 g of pyrodextrins were obtained and they were used to determine the intrinsic viscosity and the experimental design. In this study $[\eta] = 0.0433 \pm 0.0002$ was obtained. The molecular weight (Mw) was 5168 ± 75 Da, which corresponds to a given degree of polymerization (D_P) of: 32 ± 1. Laurentin et al. (2003) found, for the pyroconversion of sorghum dextrins, intermediate molecular weight values between 8 and 105 kDa with an acid treatment at 140 °C and

Table 3

CCD matrix of α-ácid Hop concentration for bitterness (AA-Hop_1) and pyrodextrin concentrations along with experimental IBU, α-acid, color, tannins, total polyphenols (TPP), free amino nitrogen (FAN) and viscosity for wort and beer of sorghum. .

Regression coefficients	Wort							Beer						
	IBU (mg/L)	α-acid (mg/L)	Color (SRM)	Tannin (mg/L)	TPP (mg/L)	FAN (mg/L)	η_w (mPa. s)	IBU (mg/L)	α-acid (mg/L)	Color (SRM)	Tannin (mg/L)	TPP (mg/L)	FAN (mg/L)	η_b (mPa. s)
β_0	27.02	7.94	11.23	80.70	255.09	272.26	1.519	20.54	5.93	14.70	86.21	256.39	73.16	1.387
(AA-Hop 1)βi	7.27	NI	0.20	NI	NI	NI	NI	7.94	NI	NI	NI	NI	NI	NI
(Pyrodextrin)βj	−4.74	−3.24	3.11	−14.75	−63.85	−20.02	0.01	−3.28	−1.81	1.50	9.05	−38.85	−25.79	0.006
(AA-Hop1 x AA-Hop 1)β_{ii}	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
(Pyrodextrin x Pyrodextrin)β_{jj}	−4.78	NI	0.69	NI	NI	NI	NI	−2.33	−3.44	−4.42	NI	−41.80	NI	NI
(AA-Hop 1 x Pyrodextrin)β_{ij}	−3.21	NI	0.45	NI	NI	NI	NI	NI	NI	NI	NI	29.71	NI	NI
Lack of fit	0.276	0.4887	0.0573	5.249	0.944	4.57	0.123	5.351	0.1773	0.1736	2.308	2.004	0.2424	0.026
(Predicho) R^2	0.9542	0.8889	0.9679	0.9275	0.9093	0.8875	0.8147	0.7359	0.9238	0.9370	0.9311	0.7755	0.8601	0.8934
Pure error	2.241	0.0645	0.0508	2.105	1.914	3.752	0.33	6.583	0.0261	0.2863	0.016	0.221	0.677	0.033

NI: No interaction.

6 h, using twice the treatment time to obtain pyrodextrins from other starches, such as corn (maize) or cassava. The explanation of sorghum resistance to pyroconversion emerged from the fact that the dark sorghum used in that study required prior bleaching. However, in this work as pretreated sorghum was used to reduce the tannins present (Acquisgrana et al., 2020), pyroconversion was performed more easily and, therefore, the treatment time was shorter. The molecular weight is likely to be lower since the solubilization properties of the pyrodextrins obtained are improved. Other more recent studies used lower temperatures reaching 130 °C which indicate that they could improve the solubilization properties of the pyrodextrins obtained (Weil et al., 2020). For this reason, in this work, starting from a starch that has undergone a previous thermal and enzymatic treatment, it was decided to test with lower temperatures and periods of 4 h .

Water-solubility is an important factor for the use of pyrodextrins as food ingredients. With increases in the pyroconversion temperature, the amount of added HCl for pyroconversion, and the prethinning period for waxy sorghum starch before pyroconversion, the solubility decreased. The Pyrodextrin prepared in this study had a solubility of 90.7%, similar to the values reported in the bibliography. For example, Kwon et al. (2005) reported values ranging from 98.3% in mild conversion conditions to values of 62.7% for prolonged 24-h treatments. Therefore, it can be concluded that the pyrodextrins obtained were highly soluble and could be used to be incorporated into sorghum beer. These pyrodextrins were incorporated in the concentrations established in the experimental design. Likewise, by the intrinsic viscosity method, the critical concentration 137 ± 0.1 g/L could be determined, indicating that they could be used up to high concentrations. However, as it will be seen in the analysis of the design of experiments, only small doses of them are necessary to achieve the desired flavor retention effect.

For a better comparison regarding the effect of adding pyrodextrins, Table 1 is presented with the values obtained from three samples of model beers, without adding pyrodextrins but with three different levels of hops. In all cases, prior to fermentation, there was a similar viscosity on an average of 1.47 ± 0.01 mPa s, and after fermentation it presented an average value of 1.38 ± 0.01 mPa s. Similar values were obtained from the bibliographic data consulted, thus, for sorghum wort 1.44 ± 0.02 mPa s, this value was obtained from the average of six tests with unmalted sorghum with the addition of different mixtures of exogenous enzymes. However, beers obtained from these experiments presented a value of 1.37 mPa s, with no reported deviation value (Dale, Young, & Omole, 1990). Likewise, others authors reported a viscosity of sorghum wort of 1.30 mPa s at 65 °C mashing temperature and they compared it to a barley wort, under the same brewing conditions, which gave a value of 1.58 mPa s (Igyor et al., 2001). From the analysis of the experimental design in Table 3 it was observed that there was no interaction with the amount of hops added nor with AA-Hop 1 x Pyrodextrin or, Pyrodextrin x Pyrodextrin. A linear interaction was observed with the concentration of pyrodextrins, with the coefficient adjustment of Table 3. In Fig. 1 it can be seen the adjustments of both curves that show an increase with increasing concentration, probably due to the high solubility previously mentioned. When the results without and with pyrodextrin addition were compared it could be highlinghed that sorghum wort and beer viscosity were improved with 10 g/L pyrodextrins addition in 4.4% and 4.8%, respectively.

Likewise, in this analysis, it is important to highlight the effect of increasing viscosity, both wort and beer, reaching to a pyrodextrin value of 15 g/L (maximum value used in the design) and values of 1.59 mPa s and 1.48 mPa s, for wort and beer, respectively. For the 15 g/L pyrodextrin addition the percentage increase to 8.2% and 7.2% respectively. Similar values were obtained from a typical barley lager beer reported in the bibliography and also studied by the working group. One polysaccharides major influences on fermented beverages is the viscosity increase, through intermolecular forces modification that keep colloidal constituents in suspension (Martinez Amezaga et al., 2016). Therefore, an increase in polysaccharides concentration, in this case

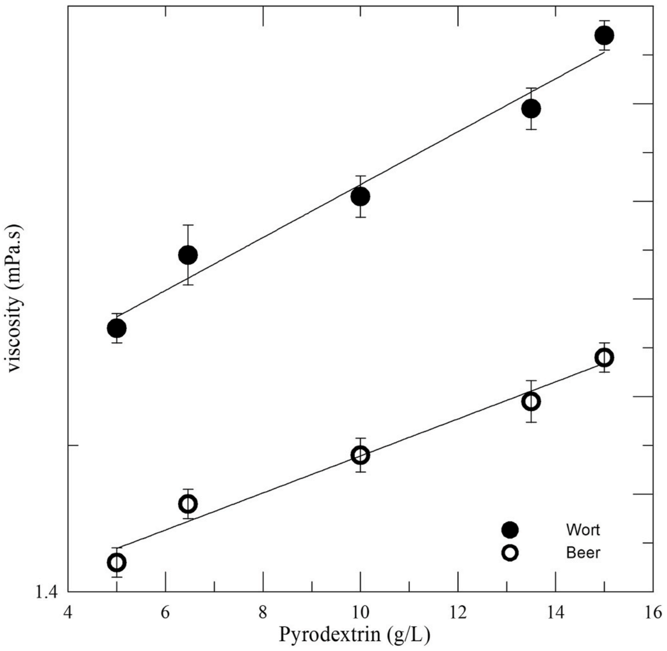

Fig. 1. Effect of pyrodextrin additions on viscosity of wort and beer of sorghum.

pyrodextrins, would increase the suspension viscosity, by increasing colloidal stability. Based on what is was stated above, it can be said that the use of pyrodextrins obtained from sorghum contributes to improving the technological aspect such as viscosity, and this increase could contribute to improving the low mouthfeel obtained in this type of beer.

3.2. *Bitterness and α-acid utilisation*

In the analysis of IBU, before fermentation, the quadratic regression coefficients for pyrodextrin (−4.78) and the regression coefficient for the interaction between AA-Hop1 and pyrodextrin (−3.21) were significant, but not for the quadratic regression coefficients for AA-Hop1. It can be observed that a positive constant (27.02), a negative linear regression coefficient for pyrodextrin (−4.74) and a positive linear regression coefficient for AA-Hop 1 (7.27) were obtained, suggesting an antagonistic effect on IBU. The negative coefficients of pyrodextrin x pyrodextrin and pyrodextrin x AA Hop 1 indicated an antagonistic effect on IBU in the studied zone, resulting in a decrease of IBU after the maximum peak, of near 10 g/L. The high values of R^2 (95.42%) and the low value of the lack of fit indicated a high dependency and a correlation between the observed values and the predicted response ones. Before fermentation, the interaction of pyrodextrins at concentrations greater than 10 g/L was unfavorable for bitter retention, causing a reduction in the values of IBUs obtained. The same behavior of the adjustment coefficients found before fermentation was observed after fermentation. However, from Fig. 2, it can be seen that at low concentrations of hops, the addition of pyrodextrins is favored, while at high concentrations, a change in the shape of the curve is observed, indicating a reduction in bitter retention with concentrations of pyrodextrins greater than 10 g/L.

The AA utilisation efficiency is limited to 40% in the traditional brewing process with barley (Briggs, Boulton, Brookes, & Stevens, 2000, pp. 543–587; Steenackers, De Cooman, & De Vos, 2015). From Table 1

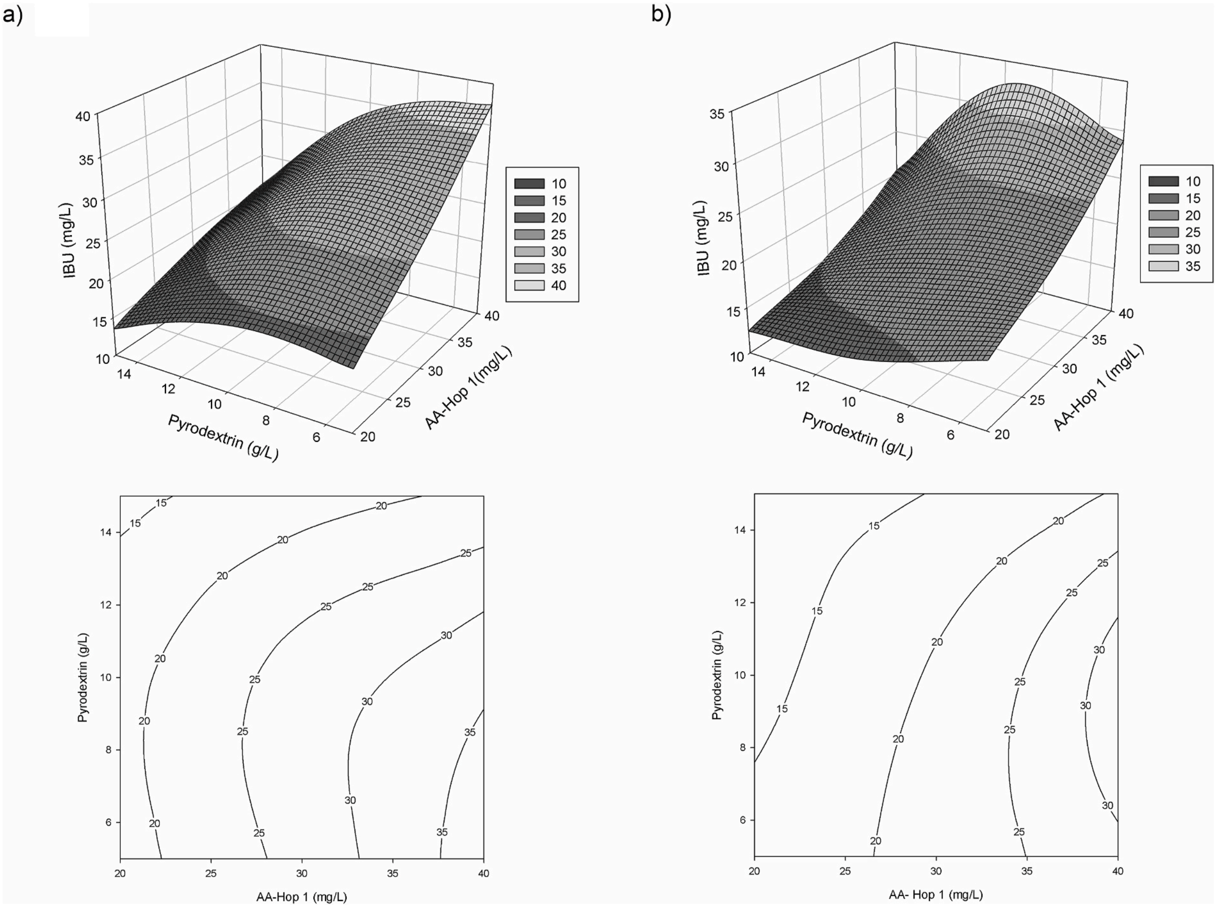

Fig. 2. Response and contour surfaces of IBU versus Hop and pyrodextrin concentrations: a) before fermentation (wort) and b) after fermentation.

the AA utilisation efficiency of the hops could be obtained before fermentation, being 49%, 48% and 44.5%, for 30, 45 and 60 mg/l of total hops added (sum of both additions), respectively. After fermentation, values of 47, 44 and 43% were obtained for the same hop concentrations. In both cases, it was observed that as the hop concentration increases, the AA utilisation efficiency decreases (Fig. 3); these results are in accordance with those found in the bibliography (Machado et al., 2018). Taking into account the values of the experiment design, evaluating the axial points for 30 and 60 mg/l of hops and the average of the central points for the concentration of 45 mg/l, if the optimal addition of dextrin of 10 g/l is considered, 56%, 57% and 59% α-acid utilisation efficiency was obtained before fermentation and 54%, 48 and 47% after fermentation. These results indicated a better utilisation efficiency of hops with pyrodextrin addition.

3.3. Total polyphenols and tannins analysis

The experimental design for TPP followed a behavior similar to those previously obtained for bitterness retention. The values from 187.6 to 307.6 mg/L before fermentation and 179.6–286.6 mg/L after fermentation were obtained. Sorghum is known to provide both condensed tannins and simple polyphenols (Acquisgrana et al., 2019; Taylor et al., 2014). From previous studies it is known that the determination of polyphenols by the foulin method is not specific for tannins and it only reflects the presence of simple polyphenols (Acquisgrana et al., 2019). Considering bibliographic data in highly hopped beers, TPP values of 288, 214 and 209 mg/L are obtained for the use of Hersbrucker, East Kent Goldings (EKG) and Zeus hops respectively (Oladokun et al., 2017), similar to the values obtained in the samples analyzed in this work. The analysis of the experimental design has shown that before fermentation, only the linear adjustment with respect to pyrodextrins was significant and with a negative coefficient, indicating a reduction in polyphenols with increasing additions of pyrodextrins. According to previous studies on beer obtained from barley and carried out by the same working group (Lataza Rovaletti et al., 2014) it is known that polysaccharides notably influence the formation of colloidal particles formed by proteins and polyphenols which increase in size and which finally precipitate both before and after fermentation. Therefore, the observed behavior coincides with the phenomenon previously explained. After fermentation, however, it was observed that in addition to the linear adjustment, the quadratic interaction of pyrodextrins and AA-hop 1 x pyrodextrins was significant. The quadratic interaction coefficient of pyrodextrins was negative (−41.80), indicating a maximum, and then, a decrease in the concentration of polyphenols with the addition of pyrodextrins, and the positive quadratic coefficient of the AA-Hop 1 x pyrodextrin interaction (29.71) indicates an increase enhanced by the use of both additions. It is likely that the greater decrease in polyphenols with the addition of hops is due to the increase in the size of the colloidal particles previously explained, due to the interaction of polysaccharides-proteins-polyphenols.

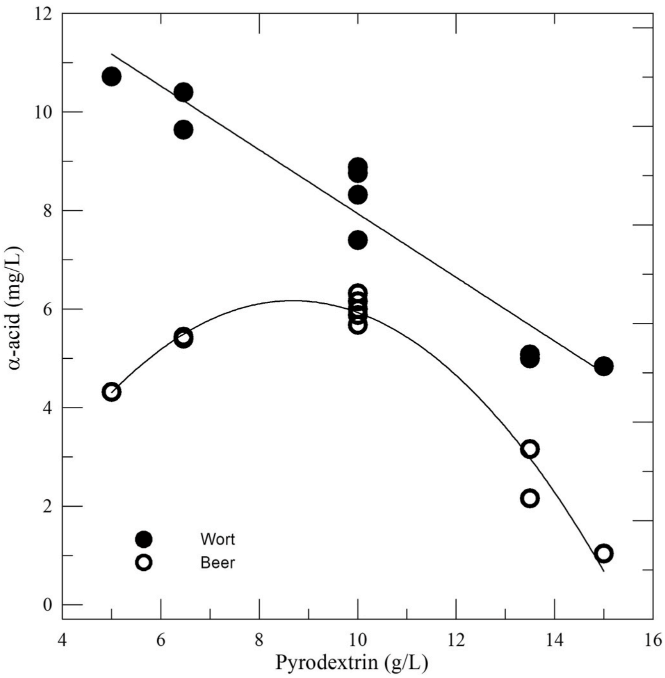

Fig. 3. Effect of pyrodextrin addition on α-acid generated before and after fermentation of sorghum beer.

The sorghum used in this work had a high tannin content, which is significantly reduced after the previous hydrothermal treatment carried out (Acquisgrana et al., 2020). In addition, malt and hops also provide tannins (Martinez-Gomez, Caballero, & Blanco, 2020). In this work it was important to previously reduce the tannins so that the fermentation could take place to acceptable levels and so that they can then contribute to the antioxidant and astringent properties which are sought and should be balanced in beers. The behavior of the experimental design was similar to that of the polyphenols, varying in the quadratic interactions AA-Hop1 x pyrodextrins and pyrodextrins x pyrodextrins resulting not significant after fermentation. The linear adjustment indicated an increase in tannins with the addition of dextrins, probably due to the greater capacity of dextrins to keep both polyphenols and tannins in suspension; therefore, it could be stated that the addition of pyrodextrins caused an additional benefit in the greater availability of precursors of antioxidant compounds such as polyphenols and tannins. In TPP and tannins, an optimal value close to 10 g/L was observed for pyrodextrins.

3.4. Color and free amino nitrogen analysis

Color in beers is a factor mainly influenced by malt, and is a distinctive aspect of each style of beer. Each style is mainly known for its physicochemical parameters, being the style guide published by the *Beer Judge Certification Program* (Beer Judge Certification Program, 2015), a clear example of this (Chan, Chua, Toh, & Liu, 2019). In international amber lager beers, according to the aforementioned guide, the color values expressed in the SMR scale are between 7 and 14. For a dark international lager it presents values of 14–22, while for a pale international lager it is between 2 and 4. In this study it was able to compare the style elaborated with the international amber style. Tannins by themselves give a remarkable dark coloration; however, after a previous treatment to reduce tannins, beers, with an intermediate coloration between the styles mentioned, could be obtained. To brew certain styles of beer, this range of colors must be respected to be accepted by consumers (Moura-Nunes et al., 2016). The analysis of the experimental design indicated that prior to fermentation only the AA-Hop1 x AA-Hop1 interaction was not significant, while the other interactions presented positive adjustment coefficients with statistical values suitable for a good fit. This indicated that the concentration of pyrodextrins increased as well as the hops increased the color in the wort (Fig. 4). This is expected since the pyrodextrins present a reddish hue due to the source of sorghum used. However, after fermentation, the linear and quadratic adjustments with respect to pyrodextrin were significant, having a negative quadratic coefficient and obtaining the maximum color for approximately 10 g/L of pyrodextrin. This allowed the pyrodextrins obtained from sorghum to be used to graduate the color in sorghum beer, which is an added benefit of using them.

Finally, one of the reasons why it is necessary to reduce tannins in this type of beer is that it does not allow the action of proteolytic and amylolytic enzymes during mashing, which causes unmalted sorghum beers to present low FAN values due to the low digestibility of kafirin, the sorghum protein (Dlamini, Buys, & Taylor, 2015). For the fermentation to be efficient, it is known that an optimal FAN level of 150 mg/L

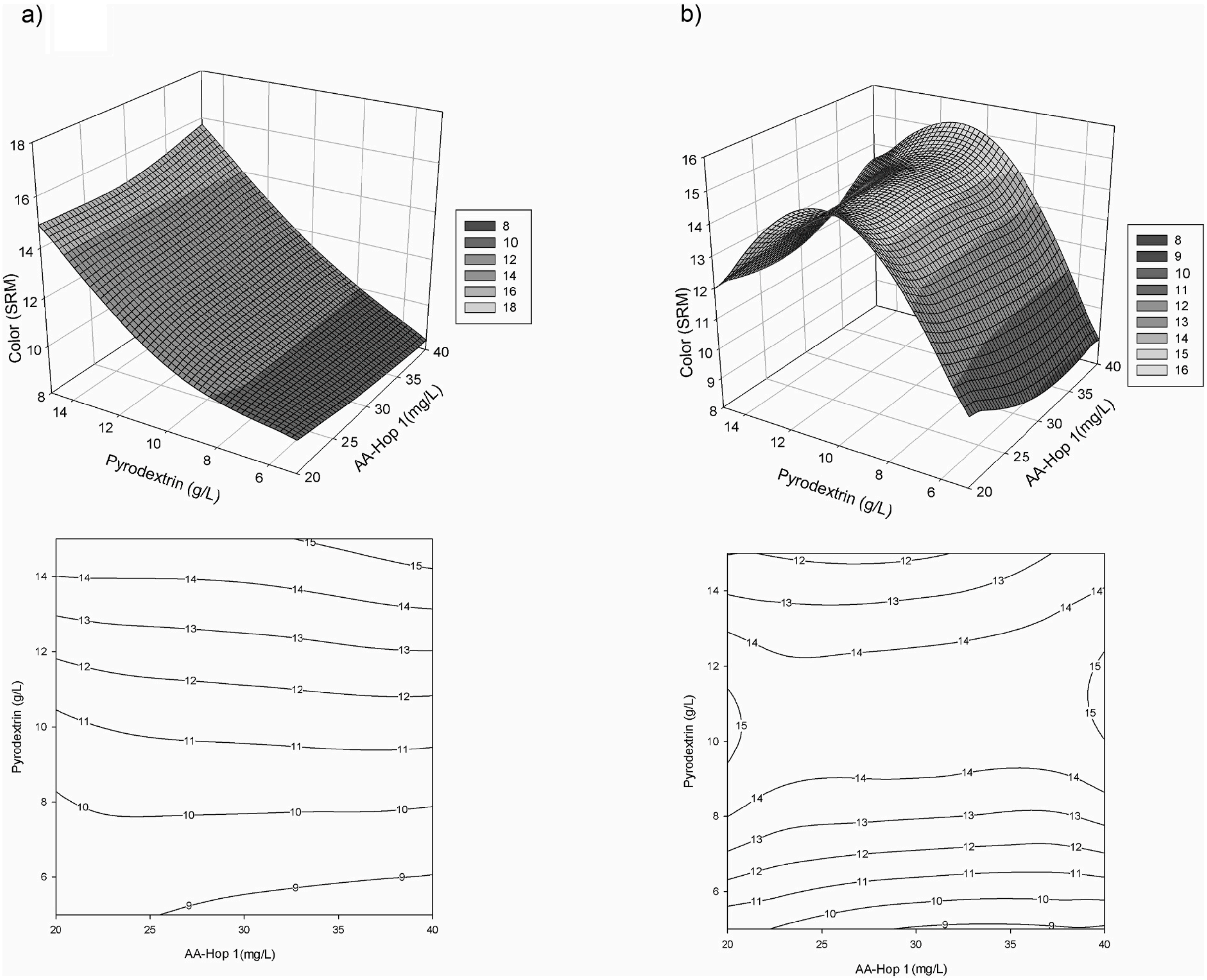

Fig. 4. Effect of pyrodextrin addition on Color a) before and b) after fermentation of sorghum beer.

must be attained (Fontana & Buiatti, 2009; Dlamini et al., 2015). The presence of FAN influences the growth of the yeasts, and in the final product, the flavors profile, the foam and the formation of haze (Fontana & Buiatti, 2009). Therefore, a FAN value far below optimal leads to slow and incomplete fermentations and to the appearance of unwanted compounds such as sulfides (Dlamini et al., 2015). The optimal value is controversial, since, like the color, there are numerous styles with higher values than the optimal one. This may lead to beers with a greater body, since it implies a higher proportion of nitrogen compounds. In the study presented, values between 251.41 and 290.15 mg/L prior to fermentation and 45.49–98.42 mg/L after fermentation were observed. The values found were in agreement with those reported in the bibliography, for example, for red and white sorghum wort beers FAN content was 195.14 ± 5.62 and 206.02 ± 2.04 mg/L, respectively (Tokpohozin, Fischer, & Becker, 2019).

Considering the experimental design analysis, it was observed that only the linear adjustments of the pyrodextrins before and after fermentation were significant and with negative coefficients, indicating a decrease as the concentration of pyrodextrins increased, mainly due to the interaction polyphenols-proteins-polysaccharides mentioned previously. It is important to take into account this last fact to avoid a high reduction of FAN with the increase of pyrodextrins; therefore, according to the global analysis of this experience, it can be stated that the use of dextrins from sorghum up to a concentration of 10 g/L would be beneficial to improve some technological aspects of sorghum beer without compromising desirable FAN levels.

4. Conclusions

The use of pyrodextrins isolated from the sorghum brewing up to a concentration of 10 g/L resulted in remarkable benefits to improve the technological properties of the beer, mainly viscosity, α-acid utilisation and bitterness retention. It also helps to optimize the proportions of polyphenols and tannins that could improve the functional properties of the beer, without compromising desirable FAN levels needed for a good growth of the yeasts during fermentation.

CRediT authorship contribution statement

Laura Cecilia Gómez Pamies: Conceptualization, Methodology, Formal analysis, Data curation, Visualization. **María Mercedes Lataza Rovaletti:** Methodology, Formal analysis, Data curation. **Nancy María Jimena Martinez Amezaga:** Methodology, Conceptualization, Writing – review & editing, Zhen-Ming Lu: Methodology, Conceptualization, Writing – review & editing. **Elisa Inés Benítez:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration, Supervision.

Acknowledgements

The authors acknowledge Universidad Tecnológica Nacional – Facultad Regional Resistencia, for helping them publish the research topics that are being developed by the research team of Experimental Theoretical Chemistry (QuiTEx). They also thank UTN and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) for financial support.

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