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Evaluation of Treatments to Reduce Hardness of *Agave americana* Core

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Summary

Agave americana contains inulin as storage carbohydrate. Therefore, agave is interesting to be used for the extraction of inulin by pressing. The yield of the process is low due to the high hardness of the core. The objective of this work was to evaluate pretreatments to reduce the hardness in the process of obtaining inulin by pressing. Treatments with water, sulphuric acid 1 % (by mass) or sodium hydroxide 1 % (by mass) were tested and optimized. The pretreatment of the core of *A. americana* with sulphuric acid 1 % allowed the reduction of hardness from 30 000 *g* to 2000 *g* of breaking force. The mathematical model obtained predicts an optimum processing at 84 °C during 75 min. The treatment with sulphuric acid 1 % also allows white core of *A. americana* to be obtained, while the other treatments provide yellow core. These results open a good alternative to obtain value added products from this resource.

Key words: *Agave americana*, inulin, hardness, colour

Introduction

The genus *Agave* belongs to the *Agavaceae* family with more than 300 species and occurs natively in arid and tropical regions, particularly in Mexico and Central America. *Agave americana* L. is a very large plant with rosettes up to 3.5 m wide. The agave can be used to produce two distinct kinds of beverages. The first is dropped as sap from the living plant and consumed fresh, known as aguamiel or fermented, known as pulque. The second kind is the distilled liquors known as mezcal or tequila. The market for tequila is growing due to the recently recognized origin denomination for it by the European Union, which means more tequila will be produced with a substantial improvement of the process and tequila quality. The main *Agave* used for the production of tequila is *Agave tequilana*, but analogous products can be obtained with other species of *Agave*. For instance, *A. americana* is used to obtain high-fructose fermentable liquor for making mezcal, a distilled alcoholic beverage similar to tequila, by using natural or controlled fermentations.

Although agave plants are associated with Mexico, several agave species were introduced in Spain in the 1940s. Actually, in the southeast Spain, a problem exists due to the invasive characteristics of these plants, with high growth rate in sandy soils (*1*). Finding applications for the agave could promote a reasonable control of their population.

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A new application for this plant is the production of inulin. This is an indigestible oligosaccharide (fructan) that is found in many plants as a storage carbohydrate. Inulin has been defined as a polydisperse carbohydrate material consisting mainly, if not exclusively, of $\beta(2\rightarrow1)$ fructosyl-fructose links. It is present in many regularly consumed vegetables, fruits and cereals such as leek, onion, garlic, wheat, chicory, artichoke and banana. Inulin is a dietary fiber that can be used as food additive or ingredient for food products (*2*). Inulin has good fat mimic properties when combined with water. It can stabilize low-fat mousses and spreadable products, and it shows synergetic gelling effects when combined with gelling agents. Inulin is a prebiotic. Recent nutrition research has shown interesting results with regard to calcium absorption and colon cancer prevention (*3*). Nowadays, the inulin commercially available on the food ingredient market is either synthesized from sucrose or extracted from chicory roots (*Cichorium intybus*).

A. americana and *A. tequilana* contain inulin as storage carbohydrate. Therefore, an interesting use of agave is the extraction of inulin by pressing, but the yield of the process is low due to the high hardness of the agave core.

After the extraction by pressing, the agave bagasse can be used to obtain agave fiberboards that have moisture and mechanical properties comparable to medium specific gravity fiberboards made by using aspen fiber (*4*). For that reason, the waste from inulin production process would be eliminated.

The objective of this work was to reduce the hardness of the core of *Agave americana* as a pretreatment in the process of obtaining inulin by pressing. Treatments with water, sulphuric acid or sodium hydroxide were tested and optimized.

Materials and Methods

Raw material

The *A. americana* used in the experiments was collected in San Carlos county (Tamaulipas, Mexico). The stem was cut to separate the core and the leaves. The core was saved in a single lot in plastic bags, frozen and stored until needed.

Treatments of samples

The pretreatment using water to reduce hardness of the core for the extraction of inulin from *A. americana* was selected because it is the usual treatment in the production of tequila. Alternative treatments with acid or alkali solutions were selected on the basis of studies of hydrolysis of other lignocellulose material, such as sorghum straw or wood (*5,6*).

The influence of temperature and processing time during pretreatments on hardness was assessed using the response surface methodology, which has been successfully used to optimize biochemistry and biotechnology processes related to food systems (*7*). The set of experiments followed a second-order factorial structure (*8*).

Treatments were performed at 80, 100 or 122 °C in media containing 1 g of H_2SO_4 per 100 g of water, 1 g of NaOH per 100 g of water or only water. The values of acid or alkali concentrations were selected according to the literature for hydrolysis in similar materials (*5–9*). All experiments were performed using a water/solid ratio (WSR) of 5 g of water per 1 g of agave on dry basis. Samples were collected at treatment times 60, 100 and 120 min and filtrated to separate the core and the liquor. The treated cores were washed with cold sterile water and the hardness was tested as it is shown below.

The liquor was used to determine the concentration of carbohydrates released (fructose, glucose and xylose) by HPLC (Agilent Technologies, Palo Alto, CA, USA) and furfural by spectrophotometry (Shimadzu, Kyoto, Japan). The analyses were carried out as shown in the literature (*10*).

Mechanical properties

Samples of products of 5 cm × 5 cm × 2 cm were obtained and equilibrated to room temperature for 30 min in a plastic bag to avoid dehydration before the mechanical properties were measured. The mechanical properties were determined using a TA-XT2i Stable Micro Systems Texturometer (Viena Court, England).

The hardness test (Warner Bratzler test) was performed by compressing the samples of the core to 25 % of the initial height using a compression speed of 60 mm/min and an AACC probe of 36 mm. The breaking force expressed in gram-force (*g*) for each treatment was measured (1 gram-force is 0.0098 N). Samples were placed on the base of the texturometer, taking care that the probe reached the sample at the centre (*11*). Six samples were analysed for each treatment.

Colour attributes

Spectral reflectance of *A. americana* core after the treatments was determined using a HunterLab colorimeter (Model Miniscan, Reston Virginia, USA) calibrated against white tile. CIE L*, a*, and b* values, chroma ([a*2+b*2]1/2), and hue angle (arc tan b*/a*) were calculated based on illuminant C and the 2° standard observer, as referenced in the literature (*12*). Six samples were analysed for each treatment.

Statistical analysis

The interrelationship between operational and dependent variables was established through the following equation including linear, interaction and second-order terms: $y_i=b_{0i} + b_{1i}x_1+b_{2i}x_2+b_{3i}x_1{\cdot}x_2+b_{4i}\ x_{11}+b_{5i}\ x_{22}$, where y_i (i=1–3) are the dependent, normalized variables and $b_{0i}...b_{5i}$ are regression coefficients calculated from the experimental data by multiple linear regressions. A second-order multiple regression analysis using the least squares regression methodology was performed using Microsoft Excel 7.0 (Microsoft Corporation, Redmond, WA, USA) software. Microsoft PowerPoint 7.0 (Microsoft Corporation, Redmond, WA, USA) was used to plot the experimental data and models. The treatments were performed in triplicate. All texture and colour data presented are mean values of six determinations.

Results and Discussion

Hardness analysis

The temperature and time were considered as operational variables (denoted θ and t, respectively) and their effects on the selected dependent variable hardness after the treatment (y_i) were calculated for each treatment: water y_1, acid y_2 and alkali y_3. For computation purposes, the normalized, dimensionless variables x_1 and x_2 were defined as: $x_1=(\theta-100)/20$ and $x_2=(t-120)/60$.

Table 1 summarizes the variables involved in the optimization of the pretreatment for the core of *A. americana.* The operational conditions assayed (in terms of dimensional and dimensionless operational variables) as well as the experimental results determined for y_i (i=1 to 3) are shown in Table 2. Empirical mathematical models were obtained by processing these data. Table 3 shows the values of the coefficients from the models and their statistical significance.

The range of study for the variable temperature was selected between 80 and 120 °C because 100 °C is the usual temperature commonly recommended for extraction of sugar in the production of tequila or mezcal. The range for the variable time was selected at 60–180 min because the objective is only to decrease the hardness and longer time periods can release sugars to the medium.

The experimental values for hardness after the treatment with water (y_1) varied over the range 9981–28443 *g*. The analysis of the main experimental trends and the values of coefficients listed in Table 3 suggest that the effect of temperature and time in the treatment using

Table 3. Regression coefficients and statistical significance of the empirical models that predict the relationship between temperature and time of the pretreatment and the hardness obtained in the core

Coefficients	y_1 (j=1)	y_2 (j=2)	y_3 (j=3)
b_{0j}	12480[a]	6046[b]	21425[c]
b_{1j}	963	7335[c]	1466
b_{2j}	2998	–73	–4201[a]
b_{12j}	1201	–1652	–1022
b_{11j}	6897	5372[b]	–12066[b]
b_{22j}	5842	843	1134

[a]coefficients significant at 90 % confidence level
[b]coefficients significant at 95 % confidence level
[c]coefficients significant at 99 % confidence level
y_1 is the hardness after the treatment with water, y_2 is the hardness after the treatment with sulphuric acid and y_3 is the hardness after the treatment with sodium hydroxide

Table 1. Variables used in the study and their nomenclature, units and ranges. The ratio liquid/solid was 5 g of water per 1 g of agave on dry basis

(a) Dimensional independent variables	Nomenclature	Units	Variation range
Temperature	θ	°C	(80–120)
Processing time	t	min	(60–180)
(b) Dimensionless, normalized independent variables	**Nomenclature**	**Definition**	**Variation range**
Dimensionless temperature	x_1	$(\theta-100)/20$	(–1–1)
Dimensionless time	x_2	$(t-120)/60$	(–1–1)
(c) Dependent variables	**Nomenclature**	**Units**	
Hardness after the treatment with water	y_1	*g* (gram-force)	
Hardness after the treatment with sulphuric acid	y_2	*g* (gram-force)	
Hardness after the treatment with sodium hydroxide	y_3	*g* (gram-force)	

Table 2. Operational conditions assayed and experimental results achieved for the pretreatments with water, sulphuric acid or sodium hydroxide

Experiment	Independent variables				Dependent variables		
	Dimensional		Dimensionless				
	θ/°C	t/min	x_1	x_2	y_1/g	y_2/g	y_2/g
1	80	60	–1	–1	20254	4085	10114
2	80	120	–1	0	18701	2800	8325
3	80	180	–1	1	22976	7050	7567
4	100	60	0	–1	10703	6958	31096
5	100	120	0	0	9981	5672	20376
6	100	180	0	1	28443	7196	15074
7	120	60	1	–1	23813	20591	14941
8	120	120	1	0	22556	20412	11443
9	120	180	1	1	26340	16947	8303

θ is temperature and t is time, x_1 and x_2 are the related coded variables, y_1 is the hardness after the treatment with water, y_2 is the hardness after the treatment with sulphuric acid and y_3 is the hardness after the treatment with sodium hydroxide water do not have a clear effect on the hardness of the core of *A. americana*. The resulting variation pattern is shown in Fig. 1. Although it appears that a minimum value of hardness exists, the coefficient of determination r^2 was not significant (0.65). The effect of the treatment with water did not decrease hardness of the core enough to be a good pretreatment for the pressing of the core.

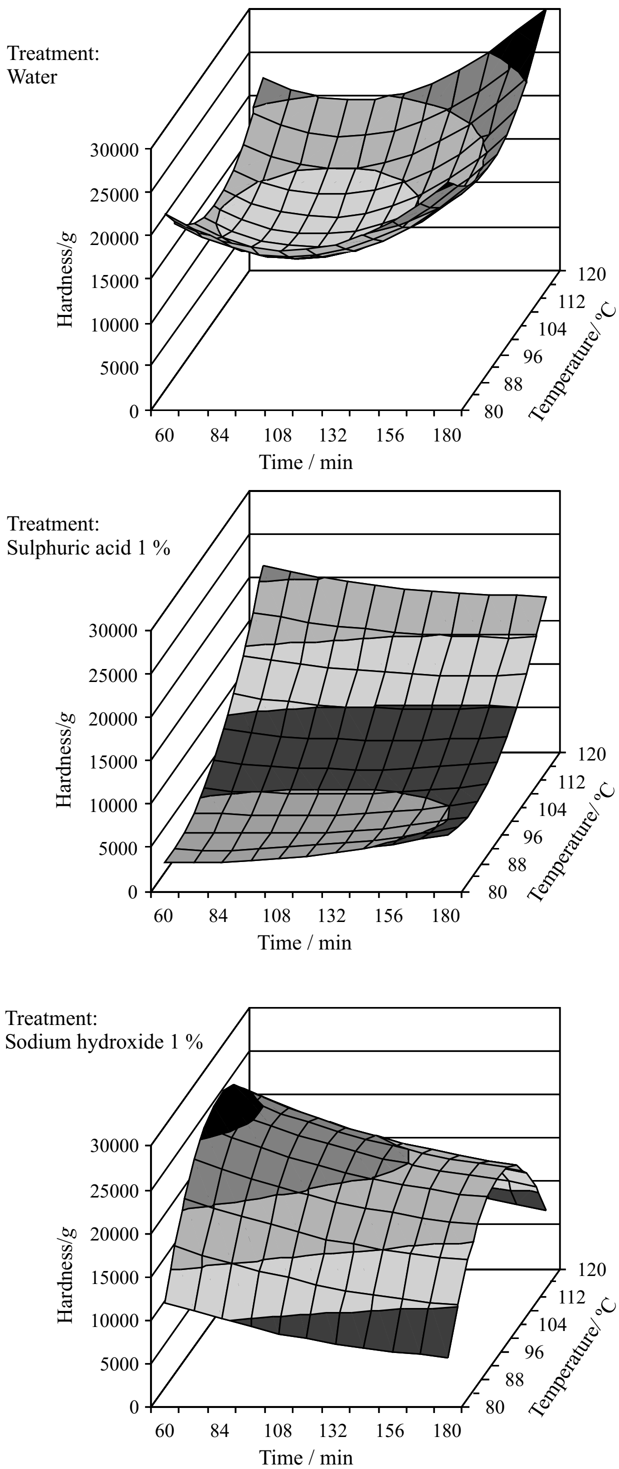

Fig 1. Effect of temperature and time on the hardness parameter for the three pretreatments assayed to reduce hardness in the core of *Agave americana*

The treatment with sulphuric acid 1 % (y_2) showed a behaviour different from the treatment with water (y_1). After the treatment, the hardness varied within the wide range of 2800–20 591 *g*. The most favourable conditions were defined by low value of temperature and intermediate value of time (experiment 2). The significance analysis of the regression coefficients for y_2 showed that the hardness was significantly affected by the variable temperature and the second-order term of this variable but was not significantly affected by the variable time. In this case the coefficient of determination r^2 was significant (0.98). The predictions of the empirical model for the dependence of hardness after the treatment with sulphuric acid 1 % on temperature and time are shown in Fig. 1. The optimization of the treatment using the model obtained shows that the best conditions are 84 °C and 75 min, given a core with a predicted hardness of 3154 *g*. Visual analysis of Fig. 1 allows to conclude that low values of temperature and time produce a core with low hardness (<5000 *g*). These values of hardness are adequate for the pressing used in the extraction of inulin or the grinding for the hydrolysis of sugars that can be used for fermentation in the production of mezcal or tequila.

The last pretreatment, with sodium hydroxide 1 %, allowed hardness in the range of 8303–31 096 *g* to be obtained. The predictions of the empirical model for the dependence of hardness after this treatment on temperature and time are also shown in Fig. 1. Time and the second-order term of the variable temperature were the terms most influential in the model obtained shown in Table 3. The coefficient of determination r^2 was significant (0.90), but the values of hardness were not low enough for the posterior processing by pressing.

Sugar released

It was clear that the best results to reduce the hardness were obtained using sulphuric acid 1 % at low temperature and time. However, in addition to low hardness, it is also interesting to maintain the sugars in the core for a posterior extraction by pressing or lixiviation (*2*). Fig. 2 shows the main sugars released to the medium in the pretreatment with water. Fructose, glucose and xylose were always under 3 g/L. The concentration of sugars in the medium increased with temperature and time.

The effect of the treatment with sulphuric acid 1 % on the sugars released is shown in Fig. 3. Fructose concentration increased with temperature and time and it was released up to 9.6 g/L. The fructose was a product of the hydrolysis of the inulin from the core. Therefore, a high concentration of fructose in the medium after the treatment is not favourable for reducing hardness if a high yield in the posterior extraction of inulin by pressing is desired. In the conditions for the lowest hardness (around 80 °C and 60 min), the fructose concentration was only 2.6 g/L. The other sugars (glucose and xylose) also showed low concentrations (1.2 and 0.4 g/L).

Fig. 4 shows the results for the sugars obtained in the medium after the treatment with sodium hydroxide. The values of fructose concentration were under 1.8 g/L for temperature of 80 or 100 °C. The concentration increased up to 3.2 g/L when 120 °C was selected. Glucose and xylose concentrations also gave values under 1.4 g/L. Low concentrations obtained for all sugars confirm that the alkali hydrolysis in the conditions of temperature and time studied was moderate and it does not allow a modification of the structure of the system to reduce hardness.

Therefore, the acid hydrolysis in these conditions of temperature and time was moderate enough to release sugar, but strong enough to change the structure of the lignocellulose matrix of the core, with the consequent decrease of hardness. These results confirm that the treatment of the core of *Agave americana* with sulphuric acid 1 % is an adequate pretreatment to reduce hardness.

The analysis of oligomers released from inulin confirmed that the conditions assayed for the three treatments were moderate because the oligomer concentration was always under 1 g/L. The results obtained suggest that a small release of inulin sugars promotes a restructuring of the hemicellulosic matrix causing low hardness of the core. This was observed at low tempera-

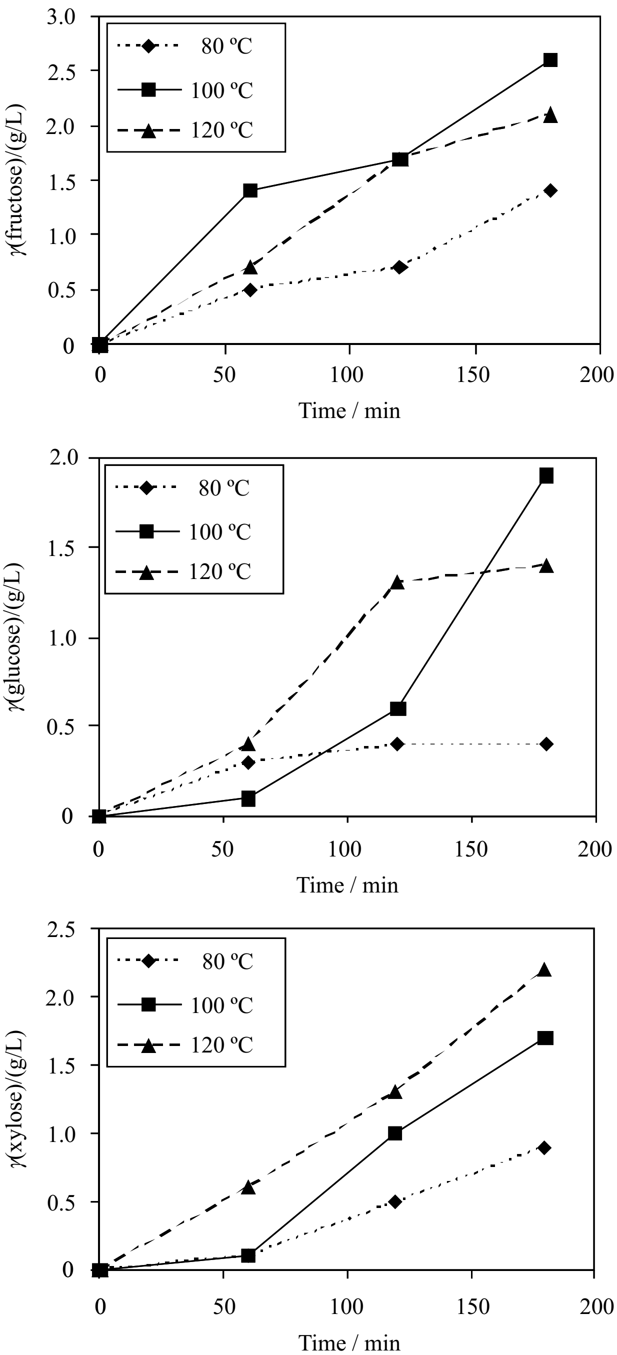

Fig. 2. Effect of the treatment with water on the released sugar concentrations from the core of *Agave americana*

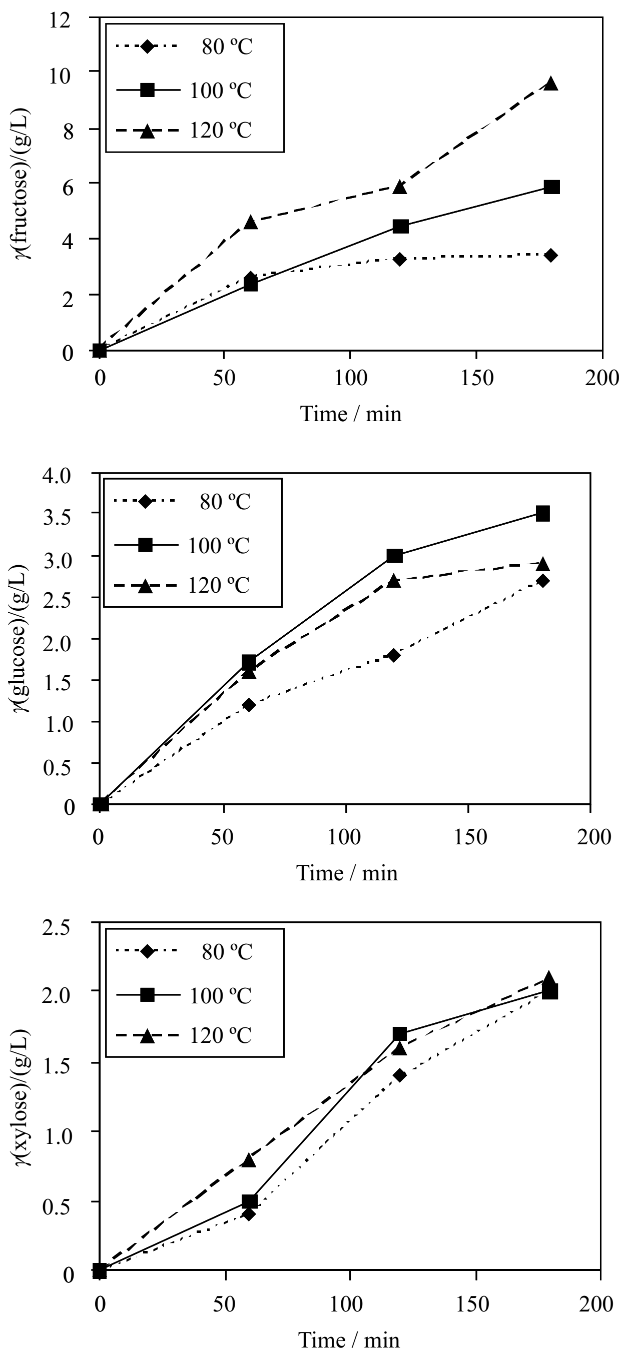

Fig. 3. Effect of the treatment with sulphuric acid 1 % on the released sugar concentrations from the core of *Agave americana*

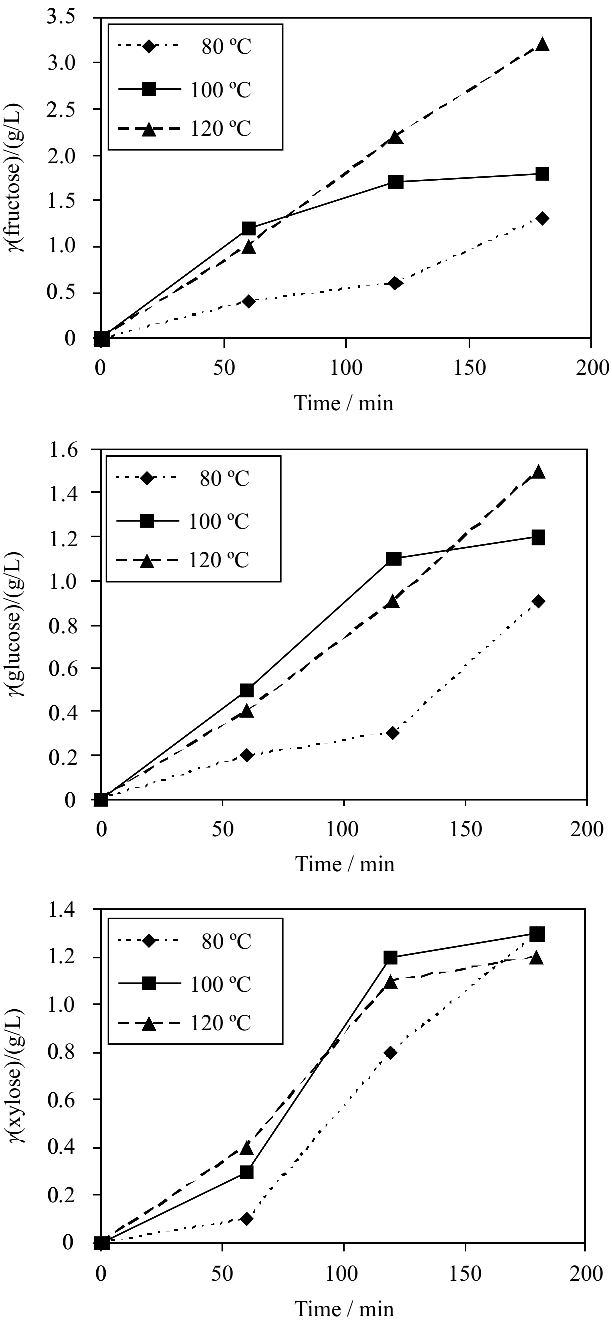

Fig. 4. Effect of the treatment with sodium hydroxide 1 % on the released sugar concentration from the core of *Agave americana*

tures. Using high temperatures, the initial sugars released can react with other compounds of the core forming new polymers, causing higher hardness and making further release of inulin sugars difficult.

Colour parameters

Other effects of the treatments of the core of agave could be the changes in the surface colour due to Maillard reactions and caramelization. These reactions should have low intensity because their rates are increased with temperature over 170 °C (*13*).

Table 4 compares the colour attributes for the samples after the treatment with water. The L* values de-

Table 4. Colour attributes (L*, a* and b*) of cores of *Agave americana* after the treatments

θ /°C	t/min	L*	a*	b*
Treatment with water				
	60	68.2	3.7	28.7
80	120	63.1	3.8	21.5
	180	60.1	2.2	12.4
	60	65.9	2.2	22.3
100	120	58.5	3.8	22.6
	180	57.8	3.9	14.4
	60	65.9	5.4	27.4
120	120	62.5	3.5	23.5
	180	62.1	4.5	18.1
Treatment with sulphuric acid 1 %				
	60	72.9	–0.2	10.5
80	120	72.3	–0.7	11.1
	180	68.0	3.6	21.9
	60	66.1	0.6	18.0
100	120	62.2	8.5	31.3
	180	58.3	3.3	14.6
	60	71.0	1.0	13.9
120	120	66.6	8.5	32.1
	180	61.0	3.9	12.4
Treatment with sodium hydroxide 1 %				
	60	68.2	0.5	19.2
80	120	63.8	3.3	19.5
	180	63.5	5.0	26.3
	60	63.5	3.9	23.2
100	120	53.6	5.3	18.7
	180	51.1	3.6	18.8
	60	65.1	2.6	23.1
120	120	59.9	3.1	18.5
	180	61.0	3.9	12.4

θ is temperature and t is time

creased with time from 68.2 to 57.8 at 80 °C, from 65.9 to 57.8 at 100 °C and from 65.8 to 62.1 at 120 °C. This decrease indicates a decrease in the whiteness but the values of L* remain in the range in which the samples can be considered as white.

The a* parameter was in the range of 5.4–2.2. There were not significant differences between the samples for this parameter. In the treatment at 80 °C, the b* parameter also decreased from 28.7 to 12.41 with time. This means that the samples lost some yellowness but still showed a colour between white and yellow.

Table 4 also shows the colour attributes after the treatment with sulphuric acid 1 %. A high value of L* (72.9) was observed in samples treated at 80 °C for 60 min. The a* parameter was in the range –0.7 and 8.5. This parameter indicates the tendency between green (negative values) and red (positive values). The samples were close to zero, therefore there is not a clear appreciation of these colours in them. The values of b* show that with this treatment a core that was whiter than in the treatment with water was obtained.

The colour attributes after the treatment with sodium hydroxide 1 % (Table 4) showed a similar behaviour to the treatment with water. The L* values decreased with time from 68.2 to 63.5 at 80 °C, from 63.5 to 51.0 at 100 °C and from 65.0 to 59.8 at 120 °C. This is indicative of a decrease in whiteness. The a* parameter remains very low and the b* parameter shows a trend to yellowness.

After the analysis of the colour attributes, it can be concluded that in the treatment with sulphuric acid 1 % white core of *A. americana* was obtained, while the other treatments provided yellow core.

Conclusions

The pretreatment of the core of *A. americana* with sulphuric acid 1 % allows the reduction of the hardness by a factor of 15, with low release of sugars and remaining white colour of the core. The core after this treatment is suitable to be processed to extract inulin by pressing. This opens a good alternative to obtain value added products from this resource.

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