

Thermal and storage stability of color in juice and fructose syrup from sugar cane

J A Solís-Fuentes^{1*}, R C Ayala-Tirado¹ and M C Durán-de-Bazúa²

¹Instituto de Ciencias Básicas, Universidad Veracruzana, Av. Dos Vistas s/n carretera Xalapa-Las Trancas, 91000 Xalapa, Ver., México.

²Facultad de Química, UNAM, Conjunto "E", Labs E301-E302-E303, Ciudad Universitaria, 04510 México.

Received 8 October 2014; revised 17 June 2015; accepted 10 August 2015

In this paper the thermal stability of the color of purified, *guarapo*^a and clarified, sugar cane juices after treatment with activated carbon and ultrafiltration was studied, and the color stability during the storage of fructose syrup made from them with *S. cerevisiae* invertase treatment, were evaluated. The results showed that the purification treatments removed up to 99% of the color juices. The remnant color of these purified juices increased with heat treatment at 100°C and 1 h duration, this increment being dependent on the type of purification treatment, the invertase treatment of the sucrose contained in them, and the pH of the juice. Syrups prepared from these purified and hydrolyzed juices had soluble solids over 50°Brix with a color of 25.44 IU for *guarapo*'s cane juice syrup and 76.33 IU for the clarified juice syrup. They showed increases in color during storage at room temperature reaching around 500 for the first and 1000 IU for the second one after 8 weeks. In refrigerated conditions the syrups showed more stability in their pH value and color. After 10 weeks of storage at 8°C, the color levels were 63.3 and 116.3 IU for *guarapo* and clarified juice syrups, respectively, with optical densities (OD) lesser than 0.050 ODU, corresponding subjectively to a light straw color.

Keywords: Sugar cane industry; *guarapo*; sugar cane juice; fructose syrup; ICUMSA color.

Introduction

Color is an important feature relating to the quality of both raw and manufactured foods. Natural dyes are unstable, so the color of food may provide an indication of biochemical and chemical changes during processing and storage. The color of foods results from the presence of natural pigments, colorants added, or those generated during processing and/or shelf life. Among the natural, plant pigments are those of the plant body; these are present in unprocessed foods and in some processed ones where their presence is desirable, and sometimes they are found in the presence of other dyes that can be formed as a result of physicochemical and thermal treatments occurring during the processing. In the sugar cane industry, the manufacturing process for sucrose is directed largely to ward obtaining a substance free of chromophores. Therefore, the entire treatment takes place at the factory in order to eliminate different compounds such as natural chromophores and precursors of color: chlorophylls, carotenoids, flavonoids, phenolic compounds, etc.^{1, 2}. This global treatment, however, generates many other compounds such as caramel, melanoidins, alkaline degradation

products, etc., that tend to be composed of a mixture of substances that are highly complex and highly sensitive to the chemical environment of the system, such as the pH value^{3, 4, 5}. The color appears in the different physicochemical treatment processes of sugar cane juice, during defecation or clarification, evaporation, crystallization and even during the storage of the product^{6, 7, 8, 9}. The nonenzymatic formation of color in the sugar manufacturing process (known as the Maillard reaction), for example, is caused by thermal degradation reactions of sugars with or without the participation of amino acids^{10, 11, 12, 13}. In recent years, the Mexican sugar industry (like the ones from other Latin American, Asian, and African countries) has faced a complex problem due to various elements of financial, marketing, and technological origin. Among the latest, the industry still maintains machinery and processes with outstanding levels of obsolescence which reduce its ability to compete in the international market of sweeteners, which is characterized by its great dynamism for generating new products. One possibility that has emerged often refers to obtaining liquid sugars or fructose syrup directly from sugar cane juice^{14, 15, 16}. These possibilities would point directly to solve one of the main problems of the sugar industry: competition in the market for corn

*Author for correspondence
E-mail: jsolis@uv.mx

syrups with negative repercussions for sucrose. For any of these cases, the process for sugarcane syrups production is seen as one that can be integrated into any stage of the traditional manufacturing process of sucrose. Then, a central problem to be solved is related to the purification treatments of the sugar cane juice to obtain stable products free of impurities and color, similar to sweeteners syrups obtained from other sources. ^a*Guarapo* is a Quechua word used for the cane sugar juice extracted by pressure from which sugar is produced (Quechua is the language of the members of a South American people in Peru who were formerly the ruling class of the Inca empire). This paper examines some processes for further purification, mainly those for removing color from cane juices obtained from a sugar mill, as well as the thermal stability of the color remaining in these purified and hydrolyzed juices and its stability during the storage of syrups obtained from these juices.

Materials and Methods

Obtaining sugarcane samples

Guarapo (first extraction sugarcane juice) and clarified sugarcane juice samples were obtained from the sugar mill Mahuixtlán, SA de CV, located in Coatepec, in the state of Veracruz, Mexico. The samples were transported and stored at a temperature of -10 °C until treatment and/or analysis.

Physicochemical and analytical determinations

The samples were subjected to determinations of pH, °Brix, optical density, and ICUMSA color at different times of the investigation in accordance with official procedures of analysis⁴. pH was measured using a potentiometer OAKTON pH mod. 310 (USA). The soluble solids determination was realized with a digital refractometer Sper Scientific model 300035. The reading was adjusted by calculating the value in °Brix at 20°C.

Color determination

Optical density (OD): The OD of the original and processed samples was determined according to the method reported by Pancoast and Junk¹⁷. In this procedure, the samples were analyzed in a spectrophotometer (Spectronic Genesys 5, USA) at 450 and 600 nm, for recording transmittance (T) and the sample OD calculated in optical density units (ODU), according to the expression: $\log \text{ODU} = [T(600 \text{ nm}) - \log T(450 \text{ nm})] / \text{cell thickness}$.

Subjective color equivalent: The subjective color of the samples was related to the estimated samples ODU according to the classification given by Guzmán-Maldonado¹⁸. In this classification optical density values are allocated to various subjective colors of syrups and pure water: water has 0.025 ODU; very light straw color of syrup, 0.035; light straw color, 0.050; straw color, 0.060; very light straw yellow 0.075; light yellow, 0.125; yellow, 0.150 and bright yellow, 0.200 ODU¹⁸.

ICUMSA Color: It was determined based on the measurement of absorbance at 420 nm of a sugar solution. Previously, the measured sample had been through a membrane of 0.45 µm, after which the concentration of soluble solids was determined and the pH was adjusted to 7.0 units. For the measurement of color, where this was required, each juice sample was diluted so that the transmittance of the sample at 420 nm was between 20 and 80%. The absorbance of the sample was measured by using distilled water in a 1cm-cell, as standard¹⁹. The ICUMSA color of the sample was calculated according to the expression:

ICUMSA color = $(As / bc) 1000$

where: As = absorbance; b = cell length (cm); c = total solids concentration (g/cm³) measured by refractometry and calculated from the density. Finally, the increase in color was calculated as the color difference between the initial (color of the sample before the treatment) and final ICUMSA units of color of the sample (after the treatment).

Purification and color removal treatments

Filtration and centrifugation (F&C)

The *guarapo* and the clarified cane juice from the mill were filtered through filter paper by using a vacuum flask and pump with a Buchner funnel to remove suspended solids; in addition, some samples were processed in a centrifuge (Hettich, mod. Universal 32 R, Germany) for 30 minutes at 4000 rpm.

Ultrafiltration (U)

The filtered and centrifuged juice was processed with ultrafiltration equipment (LabScale TFF system, Millipore), equipped with a Biomax 30 polyethersulfone membrane of 50 cm². The permeate fraction was recuperated as ultra-filtered juice.

Activated carbon treatment (AC)

Removing the color of the juice with activated carbon required previous filtering and/or centrifuging. To this end, 500 mL of juice were placed in a beaker of 1 L to which 2 g of powdered activated carbon

(G-60, -100 mesh, SIGMA-ALDRICH, USA) per 100 mL of juice were added, and the resulting mixture was stirred for 45 minutes. Then, it was filtered and centrifuged for 20 minutes at 4000 rpm. The procedure was repeated through a second treatment with AC for the treated juice.

Combined treatment of activated carbon and ultrafiltration (AC&U)

A sample of 200 mL of juice previously treated with activated carbon was then processed by using the ultrafiltration equipment described above.

Sucrose hydrolysis by invertase in raw and purified sugar cane juices

Hydrolysis of the sucrose contained in the juice samples at different levels of purification was realized with the enzyme invertase of *S. cerevisiae* (Enmex, SA de CV, Mexico). The reaction was realized at pH 4, 30°C with continuous agitation at 300 rpm with 150 µL of the enzyme invertase. The hydrolytic reaction was maintained for 2 hours¹⁵.

Elaboration of fructose sugar cane syrups

Samples of raw (*guarapo*) and clarified sugar cane juices were purified with AC&U, hydrolyzed with invertase, and processed in a lyophilizer (Labconco, model Freezone 4.5, USA) to concentrations above 50°Brix, following the methodology for the preparation of fructose syrups from sugar cane juice reported by Solís-Fuentes *et al.*^{14, 15}.

Color stability of purified and hydrolyzed juice and cane syrup

The color stability was estimated by measuring the increase in ICUMSA color between the original samples and after heat treatment or storage period under controlled conditions¹⁹.

Thermal stability of color in raw *guarapo* and clarified juice before and after they were purified

Thermal color stability was studied in raw (*guarapo*, G), clarified sugar cane juice (CJ) and samples subsequently purified in a laboratory by treatment with activated carbon (AC), ultrafiltration (U), and by a combined treatment of activated carbon and ultrafiltration (AC&U). The samples purified according to the above treatments were then hydrolyzed with the enzyme invertase to obtain the respective hydrolyzed juices, and these were analyzed too for their color stability. The color stability was measured by increasing the color caused by the heating treatment; the sample was kept at 100°C

during 1 h at pH 4.0 or 7.0. For this two samples of 6 mL from each of the studied juices were placed in sealed test tubes and submerged in a boiling water bath for 1 hour. The absorbance of the samples was measured at 420 nm, and the initial and final ICUMSA color, were calculated. The increase in color - by the difference between the initial and final color of each sample - was estimated.

Storage color stability of fructose syrups from *guarapo* and clarified sugarcane juice

The stability of the color of syrups made from *guarapo* and clarified juice previously purified by AC&U treatments, hydrolyzed with invertase, and concentrated -through lyophilization- at concentrations above 50°Brix were analyzed for their color stability. The syrups were stored in sealed plastic bottles in both ambient (approx. 22°C) and refrigeration (8°C) conditions. The color stability was estimated on the basis of increased ICUMSA color during storage under ambient temperature and refrigeration conditions. Each week the ICUMSA color of the stored syrup samples was determined during 8 to 10 weeks.

Results and Discussion

Physicochemical characteristics of the *guarapo* (raw sugar cane juice) and clarified juice before and after their purification treatments

The samples of raw juice and clarified juice from the manufacturing process of sugar in the mill had the characteristic dark brown-green color and the suspended solids contents. The simple filtering operation and centrifugation made it possible to obtain juices free of suspended matter. The *guarapo* juice had 16.4°Brix, a pH of 4.93 and a color level over 35000 ICUMSA units (IU). The clarified juice had 15.2°Brix, 6.41 pH units, and over 13000 IU of color. The subsequent purification process led to minor changes in the values of these characteristics. For example the pH values of *guarapo* and clarified juice samples after the purification treatments of ultrafiltration (U), activated carbon (AC), and combined AC&U processes in the laboratory were 4.60, 5.74 and 5.86 respectively. The °Brix value showed a slight decrease from 16.4 for *guarapo* to 15.1 °Brix for the juice purified by AC&U. The clarified juice presents too minor changes with the purification treatments, with a AC&U treatment it had had a pH of 7.20 and a °Brix of 14.1; the hidrolized form of this clarified juice was 15.4. The changes in the color of the juice from

the effect of the physicochemical purification treatments carried out in the laboratory are shown in Table 1. Ultrafiltration treatment by itself caused a decrease of color in the *guarapo* or raw juice from 35663 to 7350 UI. Treatment with AC achieved a decrease in color to only 1846 UI, and the successive treatment of AC and U produced a *guarapo* practically free of chromophore components to a level of 50 IU. In the case of clarified sugarcane juice the reduction in color achieved with the ultrafiltration operation was from an initial value of 13980 IU to 10537 IU at the end of said treatment. The powdered activated carbon made it possible to obtain a clarified and purified juice with only 162 IU, and the combination of these two treatments of purification achieved a clarified juice with 120 IU of color. The differences in the effect of purification treatments in both types of sugar cane juices can be understood from the nature of the chromophore components present in them, and as a result of physicochemical and thermal treatments to obtain the clarified juice in the mill during the sucrose manufacturing process.

Thermal stability of the color of the juices and purified samples

Table 1 also present the results of assays for studying the effect of heat treatment on the color of the samples subjected to purification. The heat treatment at 100°C for 1h was studied at the two pH level, first conditioning the pH of the juice to 7.0 and second, at pH 4.0. Besides, the table also presents the effect of heat treatment on color, before and after the enzymatic hydrolysis of sucrose contained in the juices. The results show that, in general, the level of color generation through the effect of the thermal treatment depended on the degree of purification (initial color level), the greater or lesser presence of color precursors, and the reducing sugars content produced by enzymatic hydrolysis. Thus, the color of the filtered and centrifuged *guarapo* or raw juice increased around 2414 IU whereas this juice treated with AC&U had an increment of only 295 UI in color. In the corresponding hydrolyzed *guarapo* greater effects were achieved by heating the juice: 6 984 and 858 IU, respectively. Such behavior showed smaller increments for the clarified juice because of its minor

Table 1—Effect of purification treatments on the thermal stability (at pH 7.0 and pH 4.0) of color in juice and clarified juice of sugar cane

Juice type	Purification treatments ^a and pH of heat treatment							
	F&C		U		AC		AC&U	
	pH 7.0	pH 4.0	pH 7.0	pH 4.0	pH 7.0	pH 4.0	pH 7.0	pH 4.0
<i>Guarapo (G)</i>								
Initial color ^b	35663.34	35663.34	7350.69	7350.69	1846.10	1846.10	50.36	50.36
Final color	38077.41	37765.30	8794.58	7985.12	2533.54	1990.24	346.20	173.10
Color increment	2414.07	2101.96	1443.89	634.43	687.44	144.14	295.84	122.74
SD	176.55	617.94	92.82	92.81	39.20	62.72	17.79	7.27
<i>Hydrolyzed guarapo(HG)</i>								
Initial color	36585.37	36585.37	7941.37	7941.37	1831.28	1831.28	108.8	108.8
Final color	43569.84	43126.39	10464.63	9579.23	2015.65	1875.28	967.23	560.36
Color increment	6984.47	6541.02	2523.26	1637.86	184.37	43.99	858.43	451.56
SD	1097.50	1254.28	443.97	94.95	21.98	62.21	15.19	23.08
<i>Clarified juice (CJ)</i>								
Initial color	13980.03	13980.03	10537.87	10537.87	162.5	162.50	120.97	120.97
Final color	14764.63	14336.66	11374.87	10908.35	382.56	328.39	396.51	369.62
Color increment	784.60	356.63	837.00	370.48	220.06	165.89	275.54	248.65
SD	100.86	100.86	446.30	58.21	23.94	23.94	9.51	0.00
<i>Hydrolyzed clarified juice (HCJ)</i>								
Initial color	11014.31	11014.31	10519.48	10519.48	57.05	57.05	48.97	48.97
Final color	12177.99	12383.32	12080.87	12156.97	550.0	469.10	652.06	570.55
Color increment	1163.68	1369.01	1561.39	1637.49	492.95	412.05	603.09	521.58
SD	331.20	176.00	662.47	310.47	35.35	8.97	51.22	46.9

^aF&C: Filtration and centrifugation; U: Ultrafiltration; AC: Activated carbon treatment; AC&U: Combined treatment of activated carbon plus ultrafiltration

^bIn ICUMSA units

charge of initial color. At pH 7.0, the F&C clarified juice increased its color to 784 IU through heating. The corresponding juice purified with AC&U did so by 275 IU, and the two hydrolyzed juices showed increments of 1163 IU, for the first, and an average of 603 IU, for the second. The effect of the environmental pH of the juices on the development of more color in them during the heat treatment can be seen by comparing the results described in Table 1. As shown in the results, the raw juice *-guarapo-* and clarified juice purified by successive treatment with activated carbon and ultrafiltration had minimum levels of coloration. Their corresponding enzymatically hydrolyzed products presented colors of a little more than 100 IU or less, which appeared to be colorless when subjectively appreciated. Increases between 600 and 800 IU in the values of this feature due to the heat treatment were subjectively observed as a light straw color, which will be explained later on. Table 2 shows the results, with t-values, of the statistical analysis for the means, comparing color increments from the effect of heat treatment at pH 7 for *guarapo* and clarified juice samples, after the different purification processes and after the enzymatic reaction of sucrose hydrolysis. The double asterisk in t values shown indicates significant differences at $p < 0.05$.

Storage color stability of fructose syrups from *guarapo* and clarified cane juice

Purified and hydrolyzed sugar cane juices were the basis for the development of fructose syrups of *guarapo* (GS) and clarified juice (CJS), which were subject to color stability testing during storage. Some

of the physicochemical characteristics of these syrups were: 56°Brix, 3.65 pH units and 25.44 ICUMSA color units for *guarapo* syrup and 56°Brix, 3.78 pH units and 76.33 ICUMSA color units for clarified juice syrup. The pH behavior of the fructose syrup samples stored at ambient conditions and under refrigeration showed that the pH of the samples at a room temperature of about 22°C remained around the initial pH (3.65-3.78) during the first 4 weeks of storage; then, gradually declined to more acidic levels (around 3.4-3.6). Not so for the samples kept under refrigeration, whose pH values were maintained throughout the test. The behavior of ICUMSA color for the samples of clarified juice syrup (CJS) and *guarapo* syrup (GS), measured at the current pH of syrups for the two storage conditions showed that the color increased its levels to around 1000 IU (CJS) and 500 IU (GS) under environmental conditions after 8 weeks for both syrups. Not so for the syrup stored in refrigeration, which showed a clear stability in color over the 10 weeks of storage. GS had 63.3 IU (measured at the syrup pH) and CJS increased to 116.3 IU (measured at the syrup pH) at the end of the test period. For a better overview of the results, Figure 1 shows a comparison of the levels of color reached, measured as optical density and related with the corresponding subjective assessment of color for the studied materials, before and after heat treatment: Raw juice *-guarapo-* and clarified sugar cane juice treated with AC&U (G1 and CJ1), their samples, hydrolyzed with invertase (HG1 and HCJ1), and their heat-treated samples (G2, CJ2, HG2 and HCJ2). Likewise, before and after storage at room and refrigeration temperatures of fructose *guarapo* and

Table 2—Different purification processes and enzymatic reactions of sucrose hydrolysis t-values for comparing the effect of heat treatment on the color of *guarapo* and clarified juice samples

F&C ^a		U	AC	AC&U
G ^b		5.09334	17.77735**	18.86896**
CJ		-0.214526	6.396635	7.880186
HG		4.092891	8.941565	7.786075
H CJ		-1.69786	3.20622	2.83169
Dependent samples				
G vs HG	-7.0184	-4.3470	41.3203**	-24.1144**
CJ vs H CJ	-1.2408	-0.9239	-33.8154**	-7.6263
Independent samples				
G vs CJ	11.33285**	1.88277	14.38928**	1.42265
HG vs H CJ	7.1807**	1.7057	-10.4822**	6.7577**

^aF&C: Filtration and centrifugation; U: Ultrafiltration; AC: Activated carbon treatment; AC&U: Combined treatment of activated carbon plus ultrafiltration

^bG: *Guarapo*; CJ: Clarified juice; HG: Hydrolyzed *guarapo*; H CJ: Hydrolyzed clarified juice

**Marked differences are significant at $p < 0.05$

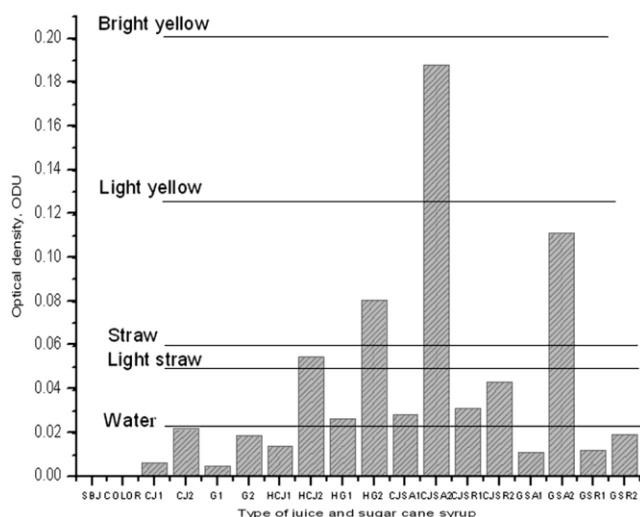


Fig. 1—ICUMSA color development (measured at current pH) in *guarapo* and clarified juice syrups stored at room temperature ($\approx 22^{\circ}\text{C}$) and under refrigeration (8°C)

SBJ color: subjective color of the juice or the syrup; G1 and CG1 are *guarapo* and clarified juice purified through activated carbon plus ultrafiltration; HG1 and HCJ1 are the hydrolyzed ones before thermal treatment and G2, CJ2, HG2 and HCJ2 are these samples after thermal treatment. GSA1, CJS1 and GSR1, CJR1 correspond to syrups made with the purified *guarapo* and clarified juice before storage at ambient and refrigeration conditions, and GSA2, CJS2, GSR2 and CJSR2 correspond to same samples after storage

clarified juice syrups (GSA1 and CJS1, and GSR1 and CJSR1) compared with these after 8 and 10 weeks of storage: GSA2 and CJS2, and GSR2 and CJSR2. It can be seen that the *guarapo* and clarified juice purified with AC&U showed minimum levels of color, whose optical density is related to the subjectively perceived color of pure water. The heat treatment at 100°C for 60 minutes produced slight increases in color levels. The samples of these juices after hydrolysis showed higher levels of color as a result of high temperature treatment, showing, however, an optical density characteristic of a light straw color in the clarified juice and a straw color in the hydrolyzed one. The samples of syrups made from *guarapo* and clarified sugar cane juice, purified with AC&U, hydrolyzed with invertase and concentrated to values greater than 50°Brix showed a color similar to those of pure water, in the first case, and a light straw color, in the second one. This appearance was maintained during 10 weeks of storage at temperatures of 8°C , but samples stored at room temperature (about 22°C) developed a color close to that of light yellow straw (*guarapo* syrup) and strong yellow (clarified juice syrup) at the end of the 8 weeks.

Conclusions

The laboratory purification treatments carried out to remove the chromophore components present in the juices obtained from the sugarcane process for sucrose production, achieved a significant removal of color, which depended on the type of treatment and the original color charges, related to the type of juice. The combined treatment with activated carbon plus ultrafiltration was able to remove over 99% of the original color charge in both *guarapo* and clarified sugarcane juice. After this treatment the remaining amounts of color were in the order of 100 IU. The heat treatment of purified juices caused increases in the remaining levels of coloration, which depended on the type of juice, the pH during heating and the presence or absence of enzymatic treatment for the hydrolysis of the sucrose present in them. The color levels generated by heating in the juices of higher purity were about 1000 IU of color. This color level is subjectively valued as the straw color. Syrups made from the purified juice, enzymatically hydrolyzed and concentrated to more than 50°Brix had an ICUMSA color lower than 100 IU. They showed color levels and optical densities that corresponded to a subjective assessment of color similar to that of pure water. This appearance was maintained for 10 weeks of storage at temperatures of 8°C . The color stability of fructose sugarcane syrups was higher during storage under refrigeration than at room temperature.

Acknowledgements

The authors thank the Fondo Mixto CONACYT-Gobierno del Estado de Veracruz, Mexico, for the financial support to carry out this work under Project 37672.

References

- Smith P & Paton N H, Sugarcane flavonoids, *Sug Technol Rev*, **12** (1985) 117-142.
- Baunsgaard D, Nørgaard L & Godshall M A, Specific screening for color precursors and colorants in beet and cane sugar liquors in relation to model colorants using spectrofluorometry evaluated by HPLC and multiway data analysis, *J Agric Food Chem*, **49** (2001) 1687-1694.
- Carpenter F G & Roberts E J, Colorant formation under refining conditions in *Proceedings of the Technical Session Cane Sugar Refining Research* (Cherry Hill, N J USA), (1974) 106-115.
- Chen J C P, *Manual del azúcar de caña para fabricantes de azúcar de caña y químicos especializados* (Limusa, México), 1991.
- Davis S B, The chemistry of colour removal, a processing perspective. *Proc S Afr Sug Tech Assoc*, **75** (2001) 328-336.

- 6 Godshall M A, Clarke M A, Dooley C D & Roberts E J, (1988) High molecular weight color in refineries in *Proceedings of the Sugar Processing Research Conference* (New Orleans, L A USA), (1988) 75-93.
- 7 Kelly F H C & Brown D W, Thermal decomposition and color formation in aqueous sucrose solutions. *Sugar Technol Rev*, **6** (1978/1979)1-48.
- 8 Prey V & Anders H, Secondary reactions during juice purification, *Zuckerind*, **21**(1971) 267-271.
- 9 Shore M, Broughton N W, Dutton J V & Sissons A, Factors affecting white sugar colour, *Sugar Technol Rev*, **12** (1984) 1-99.
- 10 Clarke M A, Blanco R S, Godshall M A & To T B T, Color components in sugar refinery processes, *Proc Sug Ind Technol*, **44** (1985) 53-87.
- 11 Clarke M A, Edye L A & Eggleston G, Sucrose decomposition in aqueous solution, and losses in sugar manufacture and refining, *Adv., Carbohydr Chem Biochem*, **52** (1997) 441-470.
- 12 Keramat J & Nursten H E, The relationship between the coloured compounds present in the pressed liquor of cane sugar manufacture and those formed in Maillard reactions, in alkaline degradation of sugars, and in caramelisation, *Food Chem*, **51** (1994) 417-420.
- 13 Rossi J P, La combinación de los azúcares con las biomoléculas desde la cocina al organismo, *Medicina* (Buenos Aires, Argentina), **67** (2007) 161-166.
- 14 Solís-Fuentes J A, Calleja-Zurita K & Durán-de-Bazúa M C, Desarrollo de jarabes fructosados de caña de azúcar a partir de guarapo, *Tecnol Ciencia Ed (IMIQ)*, **25** (2010) 53-62.
- 15 Solís-Fuentes J A, Guzmán-Flores L F & Durán-de-Bazúa M C, Kinetic behavior of invertase in the hydrolysis of sucrose in complex sugarcane juice, *Int Sugar J*, **115** (2013) 488-495.
- 16 Solís-Fuentes J A, Raga-Carbajal E, & Durán-de-Bazúa, M C, Direct sucrose hydrolysis in sugarcane juice with immobilized invertase: Multi-response optimization using desirability function on conversion and reactor volumetric productivity, *Sugar Tech*, **17** (2015) 266-275.
- 17 Pancoast H M & Junk W R, *Handbook of sugars* (AVI, Westport Conn), 1980.
- 18 Guzmán-Maldonado S H, *Optimización de un procedimiento enzimático para la licuefacción y sacarificación del almidón mediante la metodología de superficie de respuesta*, Master Thesis. I P N, México, 1992.
- 19 ICUMSA, *ICUMSA Libro de Métodos (2005)* (Verlag, Berlin, Germany) 2006.
- 20 Hernández J P, Rodríguez A S L & Bello P L A, Obtención de jarabe fructosado a partir de almidón de plátano (*Musa paradisíaca* L.). Caracterización parcial, *Interciencia*, **33** (2008) 372-376.