CHARACTERISATION OF CUBAN FINAL MOLASSES FROM 1999 TO 2008

By

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KEYWORDS: Molasses, Oligosaccharides, True Purity.

Abstract

SUCROSE loss in molasses is a common concern of the sugar industry worldwide as the largest contributor to total losses. In Cuba the loss is just less than 60%. Various factors influence sucrose solubility increase, among which is the nature of impurities present in the molasses. Molasses from different Cuban mills were characterised during the seasons 1999 to 2008, to determine the constituents of greatest influence in increasing the purity. Samples were analysed for soluble solids (brix), conductivity ash, sludge, sucrose crystals, sucrose, glucose, fructose and oligosaccharides. Impurities, water ratio (I/W) and apparent and true purities of samples studied were determined. The average value of true purity in samples was 43.37%. The biggest difference between samples was the impurities content; for example, the percentage of oligosaccharides ranged over an order of magnitude. The correlation between impurities, water ratio and true purity was confirmed, and it was also found that oligosaccharides percent correlated significantly ($p < 0.001$) with the above characters. A total of eighteen oligosaccharides was detected in the molasses studied. The presence of each of these species, both qualitatively and quantitatively, differed from sample to sample. Only one of these oligosaccharides showed significant correlation ($p<0.005$) with molasses sucrose content (pol). The present work highlights oligosaccharide influence on molasses final purity, so these compounds should be taken into account in formulating the Cuban equation for the target purity.

Introduction

In Cuba since the early eighties, the purity of molasses increased well above historical levels. Loss of sucrose in molasses is produced by a number of factors so numerous and in a variety of complex interactions that cannot be studied in a simple manner. Some of these cases probably arise long before the manufacturing process of sugar cane, in its cultivation and harvest (Eggleston *et al.*, 2001). Final molasses provides the largest contribution to the loss of sucrose in the sugar making process. Exhaustion of molasses depends mainly on sucrose solubility, which is influenced by temperature and the composition and concentration of different compounds. It has been established that reducing sugars decrease sucrose solubility and most inorganic salts increase the solubility (Lajos, 2000). It is well known that it is not feasible to remove all the sugar in cane juice entering the factory; however, it is possible to improve sucrose recovery to decrease losses that occur during the manufacturing process; in fact, good sucrose recovery depends on the crystallisation process, which in turn depends on exhaustion of massecuites and molasses.

This has been studied by many researchers in the world looking for impurities that have a higher incidence in sucrose crystallisation, both in the form of crystal (Hormaza, 2003), and crystallisation mechanism (Vaccari, 1998). To find an equation to model the behaviour of molasses purity, it is necessary to calculate values of molasses true purity and other parameters that could influence it, like reducing sugar, ash, etc, and then use data accumulated over time to look for variables with strong correlation with purity to determine the constants of the equation that defines it.

This paper presents a compilation of information in the Cuban Sugar Research Institute on the characterisation of Cuban final molasses of sugarcane over the last decade. In addition, results are shown of statistical data analysis targeting which molasses components influence purity and thereby increase losses of sucrose in the manufacturing process.

Materials and methods

Samples

Final molasses samples produced by different Cuban sugar factories, spread over the country were studied. Samples were constituted by ten-day final molasses samples accumulated at each factory during three harvest periods during the seasons 1999, 2000, 2001, 2002, 2005, 2007 and 2008.

Physical – chemical analysis

Molasses samples were analysed according to ICUMSA methods or Cuban National Bureau standards (2009) according to the following list:

- Brix: refractometric dry substance. ICUMSA method GS4-13
- Apparent sucrose (pol): Perez Sanfiel (2006)
- Conductivity ash: NC 714:2009.
- Sludge (insoluble material): NC 710:2009. It is based on precipitation of insoluble materials contained in final molasses and centrifugation at 3500 r/min, for 10 minutes.
- Sucrose crystals: Total insoluble solids are made up of sludge and sucrose crystals that are suspended, so washing the pellet formed after centrifugation with distilled water will enable these two components to be separated and, and by weight difference, the content of crystals can be determined.

High Performance Liquid Chromatography (HPLC) analyses

Determinations for fructose, glucose, sucrose and oligosaccharides were performed in a Knauer HPLC equipment with cation-exchange column EuroKat Ca 300 × 7.8 mm to 85 °C with a water flow of 0.6 mL/min and refractive index detector. A typical chromatogram is shown in Figure 1.

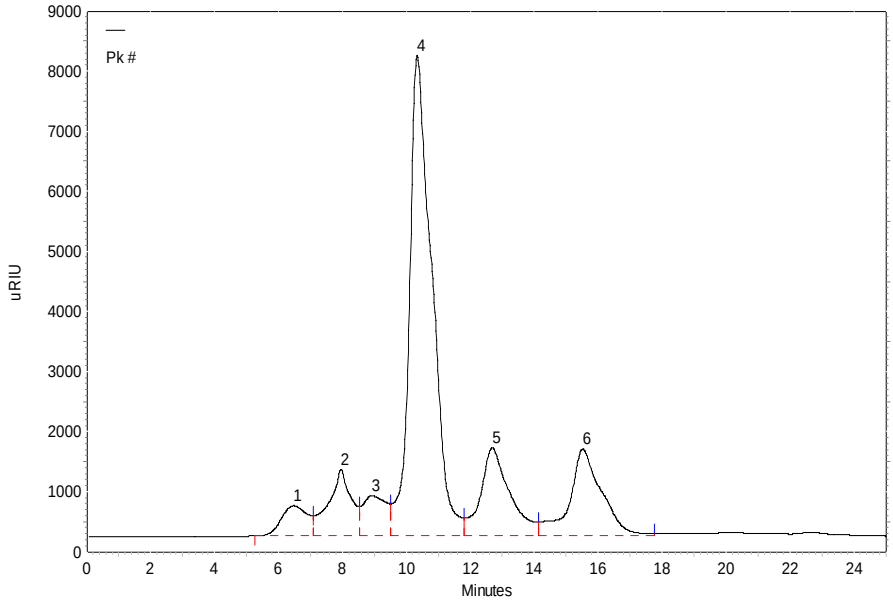

Fig.1—Molasses HPLC cation-exchange characteristic profile. Peaks 1–3 oligosaccharide, 4 sucrose, 5 glucose, 6 fructose.

Determination of oligosaccharide composition on molasses

Determinations of different oligosaccharides present in molasses were made by using an anion-exchange high resolution chromatographic system (HPAEC, Figure 2) with amperometric pulse detector (PAD) Dionex instrument, using a CarboPac 1 column, 300 mm × 7.8 mm with a gradient of 0.05 N sodium hydroxide in 0.1 N sodium acetate to 0.1 N sodium hydroxide in 0.1 N sodium acetate. A hydrolysed dextran was used as a standard to determine the oligosaccharide species polymerisation degree.

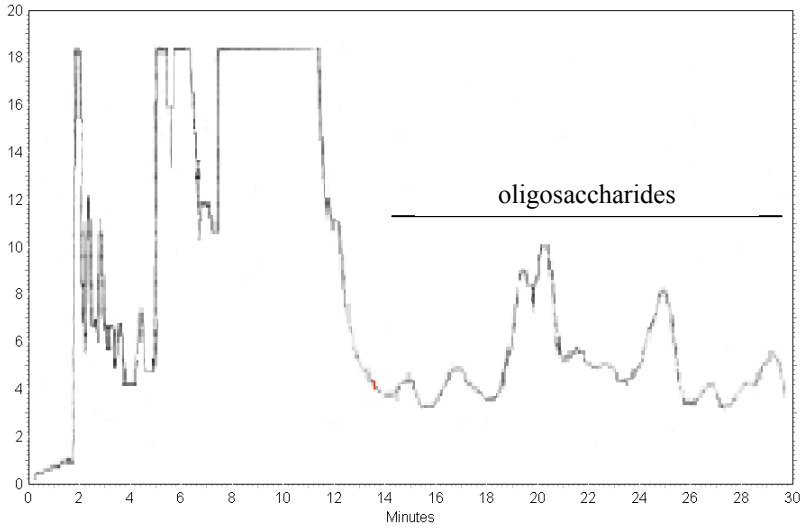

Fig. 2—Molasses HPAEC characteristic profile. Differential fractions considered as oligosaccharides have been underlined.

Statistical analysis

Data obtained from chemical – physical analysis as well as chromatographic analyses were processed using the statistical program Statgraf version 5.1.

Results

Characterisation of Cuban final molasses in the period 1999–2008

An average 39 samples of final molasses were analysed per season. All results are expressed as averages of molasses samples analysed in each of the seasons studied. Table 1 shows the results of brix, ash, apparent and true sucrose, glucose, fructose, oligosaccharide, crystals, sludge and impurity/water ratio in molasses analysed.

Brix has ranged between 91% and 82%, showing a clear tendency to decline over the last decade, probably due to extra water addition to molasses after the centrifugal to reduce viscosity.

Sucrose crystal content ranged between 4.3 and 11.5%. A possible explanation for the increase in sugar crystal percentage was the increment of crystal fragility due to crystal habit deformations by a high concentration of oligosaccharide in final molasses (Hormaza, 2003). In 2001, the highest contents of crystals in Cuban molasses were reported which coincided with the highest content of oligosaccharides detected that year. Sludge content in the molasses remained below 2%. Impurity/water ratios ranged from 2.8 to 6.9% respectively while ash content ranged from 9.8 to 15.5%.

Apparent sucrose in Cuban molasses showed an increase of five percentage points over the last decade. Furthermore, sucrose content in final molasses in the period 1999–2008 has averaged between 30% in 2000 to 46% in 2007. Reducing sugars on the other hand maintained little variability over time. As for the rest of the non-sucrose carbohydrates, oligosaccharide net content fluctuated more between harvests.

Table 1—Cuban molasses composition during 1999–2008. Results expressed as % by mass.

Year	Brix	Ash	App. sucrose	True sucrose	Glucose	Fructose	Oligos	Crystals	Sludge	I/W
1999										
Avg	90.62	11.20	34.10	35.25	6.09	6.98	6.13	3.99	0.49	6.32
Min	86.05	8.60	23.50	27.07	3.14	3.27	2.82	0.15	0.01	3.74
Max	95.36	16.80	40.70	42.67	9.03	10.5	10.13	12.06	2.05	13.42
2000										
Avg	90.79	12.13	31.98	29.81	6.23	7.59	7.4	2.88	0.75	6.94
Min	88.75	9.56	25.91	26.51	3.16	5.23	4.85	1.12	0.07	4.19
Max	95.46	31.34	44.12	36.84	9.67	12.03	9.72	12.17	2.50	13.66
2001										
Avg	89.20	11.77	34.7	36.5	5.88	7.33	19.58	11.2	0.32	5.22
Min	85.20	9.66	28.78	30.17	3.70	4.77	15.41	4.72	0.12	3.61
Max	92.00	14.70	41.08	43.11	13.72	17.50	30.10	18.23	1.63	7.28
2002										
Avg	88.16	14.77	34.68	35.66	7.17	9.46	13.94			5.39
Min	82.76	11.05	29.57	24.21	4.47	6.05	5.33			2.60
Max	96.76	22.15	42.44	46.12	13.07	16.13	44.20			19.21
2005										
Avg	86.24	9.2	37.5	34.5	5.76	6.97	14.18	4.70	0.44	3.63
Min	68.8	0.28	32.52	28.17	3.83	4.73	12.41	0.34	0.01	1.00
Max	101.44	17.9	44.4	41.11	13.90	16.40	27.10	19.01	3.79	8.51
2007										
Avg	87.00	11.2	36.36	49.09	9.21	5.58	13.15			3.67
Min	77.74	9.00	31.08	38.15	5.51	2.47	4.91			1.89
Max	94.20	13.27	50.22	70.62	11.28	10.11	15.62			5.89
2008										
Avg	83.86	4.80	39.40	34.11	4.39	5.05	15.98	4.28	0.83	2.76
Min	66.48	2.40	31.40	16.02	3.00	0.00	0.00	0.59	0.10	1.04
Max	89.40	7.90	46.90	56.86	12.61	9.05	33.91	9.19	1.67	5.22

The purity of final m olasses in the last d ecade has shown an upward trend, reaching th e lowest values (34 true and 36 apparent) in the harvest of 2002. The m aximum value of true purity was 50% in 2007 whereas the highest apparent purity was 46% in the harvest of 2008.

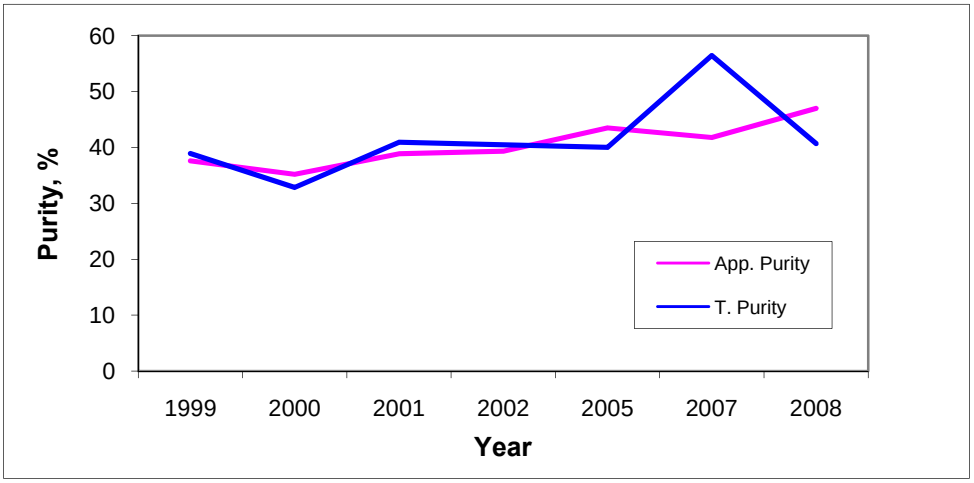

Fig. 3—Trend of true and apparent purities of final molasses in the period 1999–2008.

Determination of oligosaccharide species in Cuban molasses

Using high resolution anion-exchange chromatographic techniques with pulse amperometric detector, a total of 18 different oligosaccharides with degree of polymerisation between three and seven units were determined.

Some of those oligosaccharides were identified as gentibiose, leucrose, raffinose, melicitose, neo-kestose, 6-kestose and maltotriose. A total of eleven unidentified species were detected, five of these comprised three monosaccharide units and the remainder with a degree of polymerisation between four and seven. The oligosaccharide composition and frequency of the molasses studied were very heterogeneous, finding between two and ten of these species in them (Table 2).

Table 2—Oligosaccharides detected in cane molasses. Minimum and maximum expressed as relative percent of total oligosaccharides.

Oligosaccharide	Relative percent		Frequency (%)
	minimum	maximum	
gentibiose	1.05	55.13	33
melicitose	0.98	12.76	25
leucrose	1.40	1.45	8
neo kestose	0.98	1.00	8
1-kestose	0.27	70.80	67
6-kestose	58.70	58.90	8
maltotriose	1.20	3.60	17
unknown 1	19.10	19.40	8
unknown 2	14.68	63.15	83
unknown 3	9.68	9.70	8
unknown 4	11.37	79.80	33
unknown 5	1.40	15.00	17
unknown 6	20.53	20.60	8
unknown 7	2.00	85.00	33
unknown 8	7.16	17.25	25
unknown 9	4.75	21.25	17
unknown 10	2.70	4.20	17
unknown 11	0.30	3.00	17

Statistical analysis

All data obtained from the characterisation of final molasses during the past decade were used for statistical analysis looking for correlations between these variables and the true and apparent purities so that, once localised, it would be useful as a tool for a better understanding of the manufacturing process, providing guidance on what aspects to consider for further development of an equation to predict target purity.

Table 3 shows the Pearson product moment correlations between pairs of variables. These correlation coefficients range from −1 to +1 and measure the strength of the linear relationship between variables.

It also shows the number of pairs of data used to calculate each coefficient. The third row is a P-value which confirms the importance of the statistic of the estimated correlations.

P-values below 0.05 indicate statistical significance of non-zero correlation for a confidence level of 95%.

Table 3—Correlation analysis between apparent and true purities and some variables.

	Fructose	Glucose	Reducing sugars	Ash	Oligosaccharides	I/W	
Apparent purity	–0.3356	–0.2492	–0.3080	0.0896	0.1946	–0.2168	c. coefficient
	100	100	100	100	100	100	data
	0.0006*	0.0124*	0.0018*	0.3755	0.0523	0.0303*	p-value
True purity							
	–0.044	–0.0262	–0.0373	0.0338	0.4018	–0.2453	c. coefficient
	100	100	100	100	100	100	data
	0.6641	0.7961	0.7129	0.7388	0.0000*	0.0139*	p-value

Influence of variables in real and apparent purities

Multiple regression analysis was performed with true purity as the dependent variable and as independent variables those in which there was a linear non-zero correlation with the first. It was determined that the percent of oligosaccharides and the impurity/water ratios present a statistically significant relationship with true purity at the 99% confidence level with these independent variables responsible for 17.7% of the true purity variability. However, no correlation was found between ash content and reducing sugars with true purity (Table 4).

Table 4—Multiple regression analysis results with true purity as the dependent variable.

Dependent variable: True purity				
Parameter	Estimation	Standard error	T statistic	P-value
Constant	42.98490	1.80582	23.80350	0.00001
I/W	–1.02952	0.23148	–4.44743	0.00002
Oligosaccharide	0.17830	0.06576	2.71115	0.00740

R^2=0.176781

Using the same method, the existence of a statistically significant relationship ($p < 0.01$) exists between the apparent purity and the two independent variables of glucose and reducing sugars that accounts for 26.5% of the variability of the apparent purity (Table 5).

Table 5—Multiple regression analysis results with apparent purity as the dependent variable.

Dependent variable: Apparent purity				
Parameter	Estimation	Standard error	T statistic	P-value
Constant	45.82190	1.74982	26.18660	0.00003
Reducing sugars	–1.53387	0.31665	–4.84405	0.00002
Glucose	1.86792	0.62203	3.00290	0.00330

R^2=0.265387

Influence of oligosaccharides on pol

To determine which oligosaccharides have more influence on the pol in the molasses, all oligosaccharides detected were analysed for Pearson´s product moment correlations and non-zero correlations were obtained between pairs of variables for a confidence level of 95%. A linear regression analysis was undertaken using pol as the dependent variable to determine those independent variables with a linear non-zero correlation with pol.

It was determined that from the oligosaccharides present only one of them (Unknown 8), with polymerisation degree between four and seven, had a non-zero correlation with the pol ($p < 0.01$) so this oligosaccharide, identified with retention time of 26.88 minutes, is responsible for 30 percent of the variability of pol data (Table 6).

Table 6—Multiple regression analysis results with pol as the dependent variable.

Dependent variable: Pol				
Parameter	Estimation	Standard error	T statistic	P-value
constant	98.3500	1.34081	25.1676	0.00005
Unknown 8	0.0500	0.21635	–4.74205	0.00003

R^2=0.300027

Discussion

Results of analyses of Cuban molasses during the last decade have shown that oligosaccharides showed greater variability, reaching a maximum value in 2001 season and resulted in an increment of sucrose crystal content in molasses that year high above average values.

In general, there was a tendency for the oligosaccharide content to increase probably as a result of delays between cut and crush (Ramos, 2002). A similar trend was observed for true sucrose content (Figure 3) due to problems caused by these compounds in the crystallisation process.

Ash and reducing sugars showed little variability in the samples which could explain why no correlation was observed between these variables and purity of final molasses unlike those reported for other regions (Gil *et al*., 2001; Miller *et al.*, 1998; Smith, 1995). It appeared there was a correlation, with 95% confidence, between the total oligosaccharide content and I/W with true purity (Table 4). Similar results have been reported previously by Rein (2002).

The qualitative and quantitative composition of oligosaccharides in sugarcane juice and in final molasses depends on a lot of causes, both agricultural and industrial. It is characteristic of the variety and age of the plant and tends to increase after harvesting, often by the action of microorganisms (SMRI, 1992). The oligosaccharide composition results found heterogeneous mixtures of between two and ten different oligosaccharides in the molasses samples studied.

In early works on molasses oligosaccharides, Binkley (1964) identified 1 kestose, 6-kestose, raffinose, nistose, planteose and fructosil 1 kestose. Later Morel du Boil (1995) reported different mixtures of sugarcane oligosaccharides, neo kestose, 6-kestose and 1- kestose according to different months of harvesting. The present study detected a total of 18 different oligosaccharides (Table 2), including neo kestose, 6 kestose and 1-kestose as has been reported, but the oligosaccharide species most frequently detected was another unidentified with a similar degree of polymerisation.

The present study showed that one of these species, Unknown 8, correlated with the reading of pol contributing 30% of the variability of the pol. This oligosaccharide, together with the glucose and reducing sugars in the molasses, introduces another source of variability to be considered in determining the apparent purity of Cuban molasses.

Conclusions

Cuban molasses produced in different sugar factories, spread throughout the country, for seven seasons within the period from 1999 to 2008 has been characterised. The analyses have found variables that influence the pol and purity of Cuban molasses. It was demonstrated that, from all of the oligosaccharides detected only one, Unknown 8, was a source of variability in the pol reading. It was also shown that the level of glucose and reducing sugars influenced the apparent purity of molasses and the total content of oligosaccharides and that the I/W affects the true purity of the molasses.

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CARACTÉRISATION DE LA MÉLASSE FINAL CUBAINE DE 1999 À 2008

Par

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MOTS-CLEFS: Mélasse, Oligosaccharides, Pureté.

Résumé

LA PERTE de saccharose dans la mélasse est une préoccupation commune de l' industrie du sucre dans le monde entier comme le plus important contributeur des pertes totales. À Cuba, la perte est au dessous de 60%. L'augmentation de la solubilité du saccharose est influencée par divers facteurs, parmi lesquels est la nature des impuretés présentes dans la mélasse. Les mélasses des différentes usines cubaines ont été caractérisées durant les saisons 1999–2008, afin de déterminer les composantes avec la plus grande influence pour augmenter la pureté. Les échantillons ont été analysés pour les solides solubles (brix), la cendre (conductivité), boues, cristaux de saccharose, saccharose, glucose, fructose et oligosaccharides. Le rapport impuretés / eau (I/W) et les puretés apparentes et réelles des échantillons étudiés ont été déterminés. La valeur moyenne des puretés réelles dans les échantillons était 43.37%. La plus grande différence entre les échantillons était la teneur en impuretés ; par exemple, le pourcentage d' oligosaccharides variait par un ordre de grandeur. La corrélation entre I/W et la pureté réelle a été confirmée, et on a également constaté que la concentration des oligosaccharides corrélés significativement ($p < 0.001$) avec les caractères ci-dessus. Un total de dix-huit oligosaccharides a été détecté dans la mélasse étudiée. La présence de chacune de ces espèces, tant qualitativement et quantitativement, diffère d'un échantillon à l'autre. Un seul de ces oligosaccharides a montré une corrélation significative ($p < 0.005$) avec le contenu de saccharose mélasse (pol). Ce travail rehausse l'influence des oligosaccharides sur la pureté de la mélasse finale ; ces composés devraient donc être pris en compte dans la formulation de l'équation cubaine de la pureté cible.

CARACTERIZACIÓN DE LAS MIELES FINALES EN CUBA DESDE 1999 HASTA 2008

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PALABRAS CLAVE: Mieles, Oligosacáridos, Pureza Real.

Resumen

LAS PÉRDIDAS de sacarosa en miel final son una preocupación de la industria azucarera a nivel mundial por ser las que más contribuyen a las pérdidas totales. En Cuba la pérdida es un poco menos del 60%. Varios factores influencian el incremento de solubilidad de la sacarosa, entre los cuales está la naturaleza de las impurezas presentes en las mieles. Se caracterizaron las mieles de diferentes ingenios cubanos durante las zafras de 1999 a 2008, para determinar los constituyentes de mayor influencia en el incremento de pureza. Las muestras se analizaron para sólidos solubles (brix), cenizas por conductividad, lodos, cristales de sacarosa, sacarosa, glucosa, fructosa, y oligosacáridos. Las impurezas, la razón con el agua (I/W) y las purezas real y aparente se determinaron para las muestras estudiadas. El valor promedio de la pureza real fue de 43.37%. La mayor diferencia entre muestras estuvo en el contenido de cenizas; por ejemplo el porcentaje de oligosacáridos varió por encima de un orden de magnitud. La correlación entre impurezas, la razón de agua, y pureza real fue confirmada, y se encontró también que el porcentaje de oligosacáridos correlacionó significativamente ($p < 0.001$) con los factores anteriores. Se identificaron 18 oligosacáridos en las mieles estudiadas. La presencia, tanto cuantitativamente como cualitativamente, varió entre muestras. Solamente uno de los 18 mostró correlación significativa ($p < 0.005$) con el contenido de sacarosa en las mieles (Pol). El presente trabajo señala la influencia de los oligosacáridos en la pureza de las mieles, por lo que estos compuestos deben ser tenidos en cuenta en la formulación de la ecuación cubana para la pureza objetivo.