

THEANDEROSE – A CHARACTERISTIC OF CANE SUGAR CRYSTALS

PG MOREL DU BOIL

Sugar Milling Research Institute, University of Natal, King George V Avenue, Durban, 4001

Abstract

High performance anion exchange chromatography (HPAEC) work has established that theanderose, a glucosyl-sucrose, was present in all cane sugar products screened at the Sugar Milling Research Institute (SMRI). Several beet sugars from various countries were also examined using this technique and no trace of theanderose was found. Raffinose is often used as a criterion for the identification of beet sugar. It is proposed that the presence of theanderose is a better indicator for distinguishing cane from beet white sugars than is the absence of raffinose. Screening for both these oligosaccharides provides useful complementary information for indicating the authenticity of beet or cane sugar.

Keywords: Theanderose, raffinose, cane sugar identification

Introduction

Cane and beet sugars differ in the types of impurities found in the refined product. The presence of beet sugar or beet-derived syrup is often based on the detection of raffinose. Higher raffinose levels are associated with beets grown in cooler climates, although considerable varietal and seasonal differences are observed. Raffinose is also a common deterioration product in stored beet. Tsang *et al.* (1991) used ion chromatography to differentiate between beet and cane white sugars by measuring the raffinose content. They could not detect raffinose in any cane sugar or cane molasses samples. Clarke *et al.* (1992) state that raffinose concentrations in the range 300 to 1 200 mg/kg are indicative of beet sugars since no cane sugar tested contained observable raffinose. The typical b-axis elongation observed in beet sugar crystals has been attributed to the chemisorption of raffinose (Vaccari *et al.*, 1986).

The presence of raffinose in raw and refined cane sugars was first reported by Gross *et al.* (1962) and it was later isolated from cane molasses by Binkley (1964). Although the raffinose contents of cane products are generally much lower than the corresponding beet products, raffinose has been measured in several cane products including sugars (Gross *et al.*, 1962; Binkley, 1964; Schiweck and Büsching, 1970; Gross, 1970). Recently, concern has been expressed that raffinose was incorrectly identified in some of these early studies (Bichsel, 1990). Work carried out at the SMRI, using seven different chromatographic systems [two thin layer (tlc), three high performance liquid chromatography (hplc), two gas chromatography (gc)], confirmed the presence of raffinose in cane refinery molasses (Anon., 1991).

Oligosaccharide profiling has been used to check the authenticity of food products (Swallow and Low, 1990; Swallow *et al.*, 1991). However, such fingerprinting needs cautious interpretation (White and Cancalon, 1992; Wudrich *et al.*, 1993; Cancalon, 1993; Swallow and Low, 1994; Low and South, 1995), and generally needs augmenting with more sophisticated isotope or nuclear magnetic resonance (NMR) techniques since the oligomer composition of a solution does

not depend only on the botanical origin of the sugar. Inversion, by acid or invertase, gives rise to a family of oligosaccharides (mainly kestoses) and the proportions of the different sugars depend on reaction conditions and results from the extent of reversion and hydrolysis (Bourzutschky and Mauch, 1969; Straathof *et al.*, 1986; Manley-Harris and Richards, 1991; Cancalon, 1993).

Measurements of oligosaccharides in cane sugar have included those reported by Tu (1968), Staker (1968), and Nurok and Reardon (1975). The kestoses and nystose were the main sugars reported. Tu (1968) found only one oligosaccharide in cane juice, but isolated several from cane final molasses (raffinose, 1-kestose, 6-kestose, planteose and nystose). He extracted the oligosaccharides from sugar crystals and used paper chromatography to show the presence of at least one reducing trisaccharide. Staker (1968) used analytical carbon-celite columns to measure oligosaccharides in most cane products, including raw and refined sugars. The major components in raw sugar were an unidentified oligosaccharide (0/5-1, probably theanderose) and neo-kestose. Nurok and Reardon (1975) used a two-phase enrichment procedure to prepare TMS derivatives for gc separation on OV-17 and found similar amounts of 1-kestose and 6-kestose in affinated sugars. Theanderose elutes well after the internal standard (melezitose), [R_{Mz} (Raff) = 0,84; R_{Mz} (Th) = 1,15 (Morel du Boil, 1992)] and might not have been detected with their gc programme.

More recently, high performance anion exchange chromatography (on pellicular quaternary anionic stationary phases with alkaline eluents) (HPAEC) coupled with sensitive pulsed amperometric detection (PAD) has been the preferred analytical procedure for sugars and oligosaccharides (Anon., 1993(a), (b); Thielecke *et al.*, 1989; Lee, 1990; Paskach *et al.*, 1991; Tsang *et al.*, 1991). The major advantages include the simplicity of sample preparation and the high detector sensitivity which is particularly suitable for trace analysis. This technique has been used in the SMRI laboratories for some time (Morel du Boil and Schäffler, 1990; Morel du Boil, 1991) and conditions have been optimised so that theanderose can be distinguished from 1-kestose with isocratic elution of the oligosaccharides found in cane materials (Anon., 1994; Morel du Boil, 1995).

It has been shown that theanderose occurs in cane products and is implicated in the c-axis elongation typical of cane sugar crystals (Morel du Boil, 1992). The oligosaccharides in sugar were measured using HPAEC/PAD. The results of the survey are presented in this paper.

Experimental

The HPAEC methodology has been described previously (Morel du Boil, 1995). In this study two different solvents were used for isocratic elution of the oligosaccharides:

- Solvent A: 15 mM sodium acetate/100 mM sodium hydroxide (de-gassed with helium)

- Solvent B: 10 mM sodium acetate/60 mM sodium hydroxide (de-gassed with helium)

Sugar solutions (0,2%) were filtered (0,45 µm) and aliquots (20 µl) were injected for liquid chromatographic analysis. Freeze drying was used to concentrate aliquots (200 µl) of a sugar solution (25%) prior to derivatisation and gc analysis using a splitless technique (Morel du Boil, 1995).

Results and discussion

Sugars were screened for oligosaccharide content using HPAEC with two different solvents. Solvent A was the same as that described previously (Morel du Boil, 1995). This solvent has been found useful for monitoring oligosaccharides, since most of the peaks observed in *cane* products are isocratically eluted in less than 30 minutes. The separation of 1-kestose and theandrose is not good, but it could not be further improved by adjustment of temperature or solvent composition. The use of two columns in series or of a sodium acetate gradient gave only marginal improvement. The resolution of the 1-kestose-theandrose doublet was comparable to that found with 10 mM sodium acetate/100 mM sodium hydroxide (Morel du Boil, 1992), and was considered adequate for both identification and measuring purposes. With these conditions raffinose co-elutes with iso-maltotriose. Solvent B readily resolves raffinose and iso-maltotriose (but iso-maltotriose then co-elutes with 1-kestose and theandrose), allowing the presence of raffinose to be verified. These effects are illustrated in Figure 1. The overall analysis time is increased with solvent B. This is acceptable when 'clean' products such as sugar are being analysed. The sucrose 'overload' was judged to be acceptable using 0,2% sugar solutions (20 µl aliquots). This allowed maximum oligosaccharide sensitivity without totally obscuring minor peaks (eg raffinose) eluting on the sucrose tail. The detection limit for raffinose is about 2 ng. A raw sugar was chromatographed using the two solvents. It can be seen from Figure 2 that an unknown component (probably Peak 4 with solvent B) is included in the 'raffinose' peak using solvent A. While this illustrates the usefulness of using two solvents, such behaviour was not

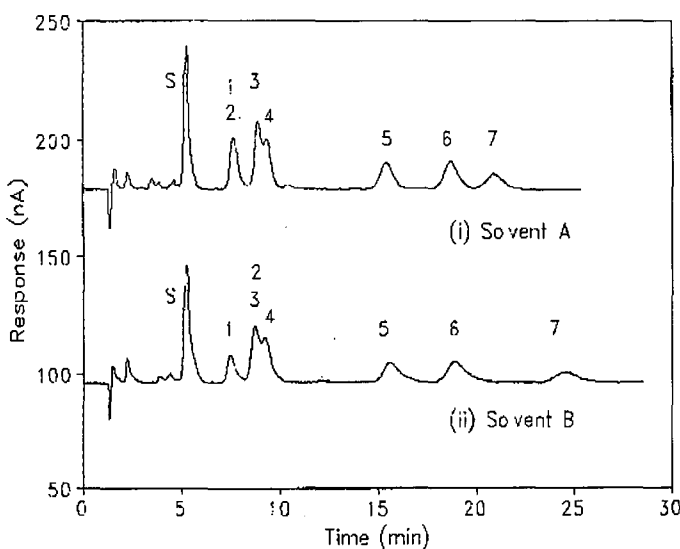


FIGURE 1: HPAEC chromatogram of known oligosaccharides using different solvents for elution (approximately 40 ng of each). (i) Solvent A, (ii) Solvent B. S = sucrose; 1 = raffinose; 2 = iso-maltotriose; 3 = 1-kestose; 4 = theandrose; 5 = 6-kestose; 6 = neo-kestose; 7 = panose.

shown by most of the sugars. Although quite variable response factors have been reported for different types of oligosaccharide (Lee, 1990; Swallow and Low, 1990), raffinose was used to calibrate the hplc. Some precautions and limitations have been highlighted (Morel du Boil, 1995).

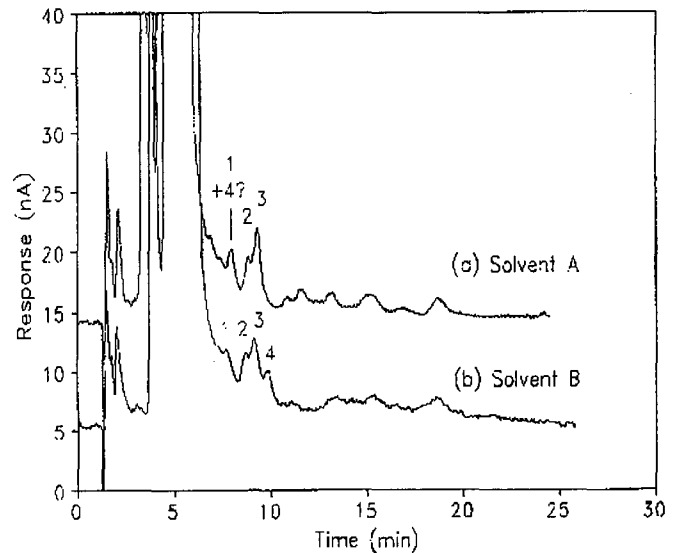


FIGURE 2: PAD responsive components separated from cane raw sugar using two different solvents. 1 = raffinose; 2 = 1-kestose; 3 = theandrose; 4 = unknown (not iso-maltotriose).

Table 1
Oligosaccharide concentration in typical cane raw sugars

Source	Oligosaccharide (ppm)			
	Theandrose	1-Kestose	6-Kestose	neo-Kestose
South Africa	135	45		60
South Africa	160	105	45	50
South Africa	155	95		115
Swaziland	165	0	0	125
Swaziland	165	70	110	190
South Africa	170	105	0	120
South Africa	170	55	0	55
Malawi	190	0	0	80
Reunion	195	70	65	115
South Africa	200	140	50	130
South Africa	200	150	70	125
South Africa	210	85	50	150
South Africa	220	120	105	205
Swaziland (affinated)	190	70	40	85
Swaziland (unaffinated)	220	115	75	110
Zimbabwe (affinated)	220	100	0	75
Zimbabwe (unaffinated)	240	70	0	95
South Africa	230	160	135	190
Reunion	275	150	55	140
Reunion	275	180	125	240
Swaziland (affinated)	290	110	0	135
Swaziland (unaffinated)	300	195	90	165
Australia (affinated)	295	50	0	55
Australia (unaffinated)	320	100	45	70
Malawi	315	270	0	125
USA (Florida)	260	220	0	225
Brazil	350	180	0	240

Oligosaccharides were analysed in over 90 cane raw sugar samples. Some of these results are presented in Table 1. All of the raw sugars examined contained significant amounts of theandrose (135 to 350 ppm), while many showed trace amounts (<50 ppm) of a peak corresponding to raffinose. Kestose concentrations were fairly high. It has been shown that sucrose strongly adsorbs theandrose during crystallisa-

tion (Morel du Boil, 1995) and it is interesting to note the similarity of theandrose concentrations in the unaffinated and affinated raw sugars.

There was no obvious link between theandrose and dextran concentrations (Table 2). Once again, theandrose was the predominant oligosaccharide. Crystals which contained more kestose than theandrose were generally made when processing extremely deteriorated cane (Anon., 1994). Theandrose apparently does not increase significantly during cane deterioration (Anon., 1987; Morel du Boil, 1995).

Table 2
Oligosaccharide and dextran concentrations in raw sugars

Sugar	Dextran (ppm) (Haze)	Oligosaccharide (ppm)			
		Theandrose	1-Kestose	6-Kestose	neo-Kestose
1	0	350	40	0	170
2	55	270	150	55	120
3	130	355	160	50	135
4	290	185	250	160	125
5	420	270	120	90	70
6	480	325	170	145	145
7	500	230	155	135	190
8	590	270	190	155	145

Gc analysis (Morel du Boil, 1995) confirmed that the peak being monitored was, in fact, theandrose. Theandrose is well resolved from the raffinose/kestoses cluster (Figure 3). The comparison between the two procedures is shown in Table 3.

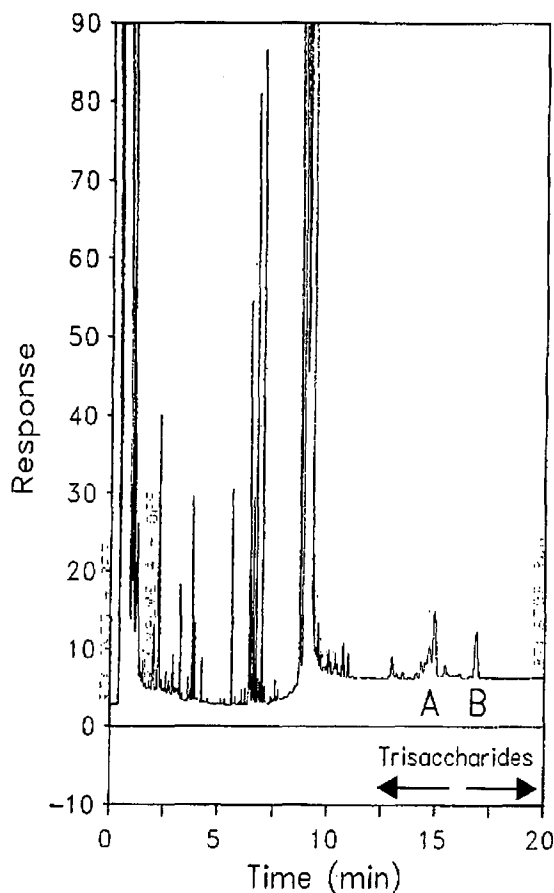


FIGURE 3: Gas chromatogram of affinated raw sugar on HP-5 (25 m x 0,32 mm x 0,52 µm) at 290°C, using the splitless mode and H₂ as carrier. A = raffinose and kestoses (1-, 6- and neo-); B = theandrose.

Table 3
Comparison of HPAEC and GC results for theandrose in raw sugar

Sample	Theandrose (ppm)		
	HPAEC	GC	Difference (GC-HPAEC)
1	205	215	10
2	205	220	15
3	230	260	30
4	245	245	0
5	280	255	-25
6	295	245	-50
7	210	175	-35
8	225	200	-25
9	215	205	-10
10	235	240	5
11	220	190	-30
12	195	230	35
13	250	240	-10
14	275	245	-30
15	155	145	-10
16	200	215	15
17	200	200	0
18	170	145	-25
19	150	130	-20
20	220	180	-40
21	210	210	0
Mean	219	209	-10
SD	37	38	-

Affinated sugars from several factories were analysed at different times during the season. The seasonal variation in theandrose concentration is not large (Table 4).

Table 4
Theandrose (ppm) in affinated very high pol (VHP) sugars during the season

Month	Factory				
	NB/UC 93	NB 91	UC 91	AK 91	SZ 91
May	350	260			
June	-	300	200		235
July	295	270	-	180	-
Aug	305	320	210	195	220
Sept	335	-	240	240	195
Oct	310	280	225	255	245
Nov		275	250	-	-
Dec				295	275

Theandrose was also observed in all the white cane sugars screened (15 samples, 55 to 350 ppm) (Table 5). Some white sugars contained as much theandrose as was found in raw sugars. Generally, the kestose concentrations in white sugar samples were negligible, while some showed traces of a peak corresponding to raffinose (<50 ppm).

In summary, *all* of the cane sugars contained theandrose at readily discernible levels. By contrast, all the sugars known to originate from beet showed a total absence of theandrose and significant levels of raffinose (Table 6). The different oligosaccharide profiles obtained with beet or cane white sugars are shown in Figure 4a and 4b. Two samples, supposedly of beet origin, showed distinct evidence of both raffinose and theandrose (Figure 4c). However, this refinery indicated that

both cane and beet sugars were being refined at the time (personal communication).

Table 5
Oligosaccharide concentration in cane white sugar

Source	Oligosaccharide (ppm)			
	Theandrose	1-Kestose	6-Kestose	neo-Kestose
Mauritius	55			
South Africa (HR)	85			
Sucrose 1*	90			
Cuba	105		70	5
USA (Texas)	110			
South Africa (TSB)	120			75
UK	130			10
Brazil	135	190		
UK	140		105	140
UK	145			80
South Africa (HR)	145			95
South Africa (NB)	185			
South Africa	220		80	90
Sucrose 2*	245			115
Malaysia	345		75	

* From various chemical supply houses

Table 6
Oligosaccharide concentration in beet white sugar

Source	Oligosaccharide (ppm)	
	Raffinose	Theandrose
Sucrose 3*	45	
Italy	150	
Italy	270	
Sucrose 4*	370	
USA (Idaho)	385	
Europe	565	
Europe	880	
Portugal	315	120

* From various chemical supply houses

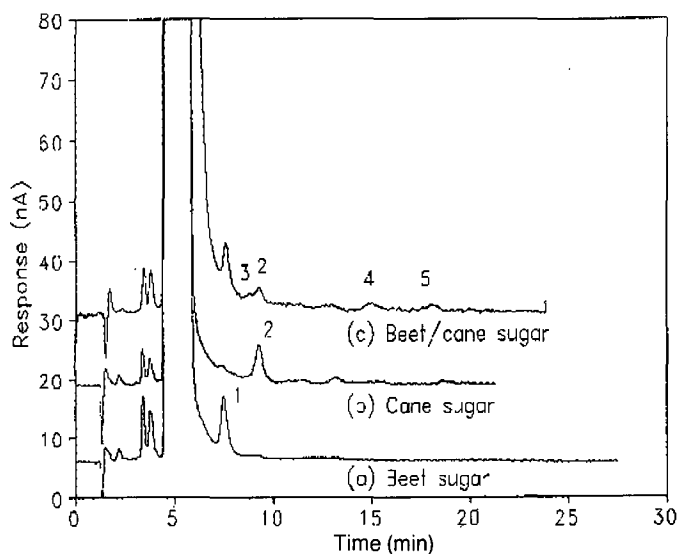


FIGURE 4: Chromatograms obtained with Solvent A for (a) beet white sugar (370 ppm raffinose), (b) cane white sugar (345 ppm theandrose), (c) white sugar from a refinery processing both beet and cane sugar (315 ppm raffinose; 120 ppm theandrose). 1 = raffinose; 2 = theandrose; 3 = 1-kestose; 4 = 6-kestose; 5 = neo-kestose.

¹ M de Campos Vidal, Alcantara Refinery, Portugal

Conclusions

Theandrose has been observed in all the cane sugar samples examined and has not been detected in any of the beet sugar samples. The range of concentrations observed in raw sugar is relatively small, as though adsorption might reach a limiting level. No attempt has been made to explain the origin of theandrose, other than to mention that it does not increase markedly with deterioration, indicating that it could be a natural constituent of cane. Theandrose can be regarded as the trace oligosaccharide typical of cane sugar as raffinose has been considered typical of beet sugar. The HPAEC analysis of sugars for both raffinose and theandrose provides useful complementary information for indicating the authenticity of beet or cane sugar.

REFERENCES

- Anon (1987). *Sugar Milling Res Inst Ann Rep (1986-1987)*: 6-7.
- Anon (1991). *Sugar Milling Res Inst Ann Rep (1990-1991)*: 7.
- Anon (1993a). Analysis of carbohydrates by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD). *Dionex Tech Note*: 20 (7/93).
- Anon (1993b). Optimal settings for pulsed amperometric detection of carbohydrates using Dionex pulsed electrochemical and amperometric detectors. *Dionex Tech Note*: 21 (6/93).
- Anon (1994). *Sugar Milling Res Inst Ann Rep (1993-1994)*: 6-7.
- Bichsel, SE (Referee) (1990). Subject 18: Sucrose. *Proc Int Commn for Uniform Methods of Sugar Analysis* 20: 352-357.
- Binkley, WW (1964). The isolation of raffinose from cane final molasses. *Int Sug J* 66: 185-187.
- Bourzutschky, H and Mauch, W (1969). Die Bildung von Kestosen und anderen Sacchariden bei der Säurehydrolyse der Sukrose. *Z Zuckerind* 19(12): 661-664.
- Canalon, PE (1993). Oligosaccharide generation in acidic sugar media. *J Ass Off Analyt Chemists* 76(3): 584-590.
- Clarke, MA, Cargel, GLR, Blanco, RS and Tsang, WSC (1992). Oligosaccharide analysis by ion chromatography. *Proc Sug Processing Res Inc*: 220-231.
- Gross, D (Referee) (1970). Subject 15: Raffinose, other oligosaccharides, polysaccharides and glycosides. *Proc Int Commn for Uniform Methods of Sugar Analysis* 15: 148-159.
- Gross, D, Gardiner, FJ and Butters, RW (1962). The presence and estimation of raffinose in cane sugar products. *Int Sug J* 64: 69-71.
- Lee, YC (1990). Review: high performance anion-exchange chromatography for carbohydrate analysis. *Analyt Biochem* 189: 151-162.
- Low, NH and South, W (1995). Determination of honey authenticity by capillary gas chromatography. *J Ass Off Analyt Chemists* 78(5): 1210-1218.
- Manley-Harris, M and Richards, GN (1991). Formation of trisaccharides (kestoses) by pyrolysis of sucrose. *Carbohydrate Res* 219: 101-113.
- Morel du Boil, PG (1991). The role of oligosaccharides in crystal elongation. *Proc S Afr Sug Technol Assoc* 65: 171-178.
- Morel du Boil, PG (1992). Theandrose – a contributor to c-axis elongation in cane sugar processing. *Int Sug J* 94: 90-94.
- Morel du Boil, PG (1995). Cane deterioration – oligosaccharide formation and some processing implications. *Proc S Afr Sug Technol Assoc* 69: 146-151.
- Morel du Boil, PG and Schäffler, KJ (1990). Ion chromatography: a comparison between anion and cation exchange HPLC for carbohydrates. *Proc Sug Processing Res Inc*: 397-413.
- Nurok, D and Reardon, TJ (1975). Quantitative determination of sugars in factory products by gas chromatography using open tubular columns. *Proc S Afr Sug Technol Assoc* 49: 94-98.
- Paskach, TJ, Lieker, H-P, Reilly, PJ and Thielecke, K (1991). High-performance anion-exchange chromatography of sugars and sugar alcohols on quaternary ammonium resins under alkaline conditions. *Carbohydrate Res* 215: 1-14.
- Schiweck, H and Büsching, L (1970). Raffinose in Zuckerrüben- und Zuckerrohrprodukten. *Zucker* 23(14): 405-409.
- Staker, R (1968). An analytical method for oligosaccharides in sugarcane products. *Proc Int Soc Sug Technol* 13: 1848-1856.
- Straathof, AJJ, Kieboom, APG, van Bekkum, H (1986). Invertase catalysed fructosyl transfer in concentrated solutions of sucrose. *Carbohydrate Res* 146: 154-159.
- Swallow, KW and Low, NH (1990). Analysis and quantitation of the carbohydrates in honey using high-performance liquid chromatography. *J Agric Food Chem* 38: 1826-1832.
- Swallow, KW and Low, NH (1994). Determination of honey authenticity by anion-exchange liquid chromatography. *J Ass Off Analyt Chemists* 77(3): 695-702.

- Swallow, KE, Low, NH and Petrus, DR (1991). Detection of orange juice adulteration with medium invert sugar using anion-exchange liquid chromatography with pulsed amperometric detection. *J Ass Off Analyt Chemists* 74(2): 341-345.
- Thielecke, K, Lieker, HP and Paskach, T (1989). Analytical determination of technically produced oligosaccharides by high performance ion chromatography. *Zuckerind* 114(12): 953-961.
- Tsang, WS, Cargel, GLR and Clarke, MA (1991). Ion chromatographic analysis of oligosaccharides in beet sugar. *Zuckerind* 116(12): 1058-1061.
- Tu, CC (1968). The occurrence of oligosaccharides in cane products. *Proc Int Soc Sug Cane Technol* 13: 374-379.
- Vaccari, G, Mantovani, G and Sgualdino, G (1986). The raffinose effect on sucrose morphology and kinetics. *Sug Technol Rev* 13: 133-178.
- White, DR and Cancalon, PF (1992). Detection of sugar adulteration of orange juice by liquid chromatography/pulsed amperometric detection with column switching. *J Ass Off Analyt Chemists*: 75(3): 584-587.
- Wudrich, GG, McSheffreys and Low, NH (1993). Liquid chromatographic detection of a variety of inexpensive sweeteners added to pure orange juice. *J Ass Off Analyt Chemists*: 76(2): 342-354.