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Determination of some organic acids and inorganic anions in beet sugar by ionic HPLC

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This work deals with the identification and quantitative evaluation of sulfates, nitrates, chloride, acetic, lactic and formic acids. Determination was carried out using a Dionex ion chromatograph with a conductivity detector. Different white and raw sugar samples have been analysed with the aim of evaluating their quality. Organic acids production seems related to storage conditions rather than processing. Inorganic anions may be used to determine the crystallization quality.
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INTRODUCTION

When white sugar is used as a raw material in the food industry, it is necessary that its quality be evaluated and compared with international and local standards. In the European Union, the quality standards are given by the so-called 'European Points'. These are based on the determination of three parameters: the visual appearance of white sugar using Braunschweig colour types (method GS2-11 (of ICUMSA (1994)), the solution colour of white sugar obtained at a wavelength of 420 nm and calculated as 1 colour point = 7.5 ICUMSA units; and the ash content determined by conductivity for a solution of white sugar at 28% (w/w) concentration, the correspondence between ICUMSA conductivity C28 and European Ash point being: 1 point = 3.13 × C28 (method GS 2/3–17 (1994)). Three qualities of white sugar are differentiated according to the European standards: grade 1 with less than 8 European points; grade 2 (8–22 points) and grade 3 with more than 22 points.

Depending on the way sugar is used in the food or the beverage, it may be necessary to add some other quality criteria. When sugar is used as such and not submitted to a thermal treatment of sterilization as in some beverages, criteria such as organoleptic value (odour) and microbiological quality, as well as the obvious absence of traces of toxic elements are added. Volatiles seem to be the more critical components. It was recently reported (Marsili *et al.*, 1994) that more than 26 compounds were found using GC and a purge and trap technique. The odour defects in white sugar were assigned to the combination of acetic, butyric and isovaleric acids with geosmin. Butyric acid was present at about 50–250 ppb and isovaleric acid ranged from 20–105 ppb. Godshall *et al.* (1994) confirmed by use of GC and a purge and trap technique the presence in white sugar of acetic and butyric acids, but also propionic acid. Acetic acid ranged from 0.5 to 5.0 ppm. Propionic acid was not always detected. Its average concentration was 1 ppm. Butyric acid was present at about 0.1 ppm. An extraction method was used for acids in soft sugar with a GC/MS detection technique (Godshall and Miranda, 1996). A change in acid composition was found during the storage and 12 compounds were reported among which are lactic, glyceric, malic, tetronic, gluconic, xylonic lactone, benzoic, 4-hydroxy benzoic, vanillic, syringic and 4-hydroxy-cinnamic acids. Colonna *et al.* (1996) have studied some causative agents of odour in beet sugar. They used an ion chromatographic method and found, in white sugar, lactic, acetic, propionic, butyric, hexanoic and heptanoic acids. Foissac and Feinberg (1991) attempted to quantify non-volatile acids in order to establish reference standards for the quality control of beet sugar. Using ion exclusion chromatography, they found in white sugar from a second run crystallization, citric (1 ppm), oxalic (1 ppm), lactic (13 ppm), formic (2 ppm), acetic (4 ppm) and pyroglutamic (23 ppm) acids. In first strike white sugar, only lactate (2 ppm) and pyroglutamate (4 ppm) were detected. In raw sugar, fumarate was also found. As regards mineral anions, Karim *et al.* (1986) studied the presence of chloride and nitrate in highly refined sugars with an ion selective-electrode. Chloride was present at about 1.1 ppb, and nitrate at about 0.38 ppb. Kvasnicka *et al.* (1993) showed, by a capillary isotachophoresis method, the

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presence (in raw sugar) of chloride, sulfate, phosphate and also oxalate and fumarate ions. The concentrations of nitrite, sulfite and nitrate were lower than the threshold detection (< 2ppm).

Ionic HPLC, coupled with a conductivity detector allows detection and quantification of inorganic and organic anions in one injection. This method proves to be easy to perform as no preparation of sample is necessary. The aim of this work is the quantification of organic and inorganic acids by use of ionic HPLC in order to evaluate white sugar quality. The origin of these impurities might be either the process itself, or a contamination during the storage of white sugar. Knowing this can help the user of white sugar to estimate effects on his product and eventually to establish specifications.

MATERIALS AND METHODS

Grades of sugar used based on the European Points (E.P.)

Grade 1 sugar (1A), 4.7 E.P. (visual appearance 0.70, solution colour 2.10, ash 1.89), factory Y; Grade 1 sugar (1B) from factory Y, 5.57 E.P. (visual appearance 1.60, solution colour 2.13, ash 1.84), factory Y; Grade 2 sugar (2A) from factory X, 8.98 E.P. (visual appearance 3.00, solution colour 2.76, ash 3.22; Grade 2 sugar (2B) from factory Y, 9.25 E.P. (visual appearance 2.82, solution colour 3.00, ash 3.43; Grade 2 sugar (2C) from factory X, used by a soft drink industry; raw sugar (3), from factory Y

Ion high-performance liquid chromatography (HPLC) method

The Dionex chromatograph was fitted with a AS11 column; 4 × 250 mm, composed of a 13 μm highly cross-linked polyethylvinylbenzene/divinylbenzene substrate agglomerated with anion-exchange latex that was completely aminated. The ion-exchange capacity was 45 μeq/column. The 85 nm diameter latex particles were permanently bonded to the substrate surface by electronic and van der Waals interactions. The column was used with an AG11 guard column and an ion trap column ATC-1 (4 mm). The eluents used were 1 and 21 mM sodium hydroxide. Detection was made by a conductivity detector (Dionex, USA). The eluent flow rate was 1 ml min^{-1} and temperature was 20°C. The injection loop was 25 μl. An anion self-regenerating suppressor ASRS-1 was used. The chromatograph was monitored by an AI-450 software (Dionex) which allowed processing of the chromatograms.

Table 1. Gradient used for the quantification of organic and inorganic anions. eluent (1) NaOH 1 mM; eluent (2) DI water; eluent (3) NaOH 21 mM

Time (min)	% (1)	%(2)	%(3)
0	7	93	0
15	9	91	0
16	0	98	2
21	0	95	5
30	0	74	26
38	0	72	28
38.5	0	40	60
41.5	7	93	0
50	7	93	0

The calibration was external with the following standard mixture: K_2SO_4 99.5% (SDS, France), NaCl 99% (Prolabo, France), $NaNO_3$ 99% (Prolabo, France), CH_3COONa 99% (Merck, Germany), $C_3H_6O_3$ 99% (Prolabo, France), CH_2O_2 98–100% (Prolabo, France). The sugars were dissolved in deionized water at a concentration of 20 g litre^{-1} for the grade 3 sugar, 400 g litre^{-1} for the grade 2A/B sugars, and 700 g litre^{-1} for the grade 1A/B sugars. The solutions were subsequently filtered on a 0.45 μm membrane (Whatman). Separation was performed with the elution gradient presented in Table 1.

Statistic treatment

Response linearity and detection threshold

Three increasing concentrations of standard acid solution were prepared independently and injected three times. Concentrations of about 0.5 mg litre^{-1} were easily detected. The linearity was obtained with a good correlation coefficient, $r = 0.978$ for formate, 0.988 for chloride, 0.983 for nitrate, 0.981 for sulfate. For lactate, $r = 0.948$ probably because this compound coeluted with acetate ($r = 0.984$).

Repeatability and reproducibility

Each sugar solution was injected five times independently. Analysis was repeated three times. Calculation was based on the French standard AFNOR on DIS5725-2. The Grubb test was used to determine whether certain values were aberrant (threshold 5%). Then an estimation of means and fidelity values was made. Standard concentrations were varied from 1 to 20 mg litre^{-1}; the results are shown in Table 2.

RESULTS AND DISCUSSION

Identification of anions

White beet sugars show comparable ion chromatogram profiles, with seven major peaks which are pyroglutamic,

Table 2. Reproducibility *R* and repeatability *r* of standards

Standards	*R* %	*r* %
Sulfate	0.61–6.30	0.50
Chloride	0.50–4.32	0.07–0.60
Nitrate	0.50–4.32	0.30–0.20
Acetate	0.04–7.71	0.17–3.70
Formate	0.61–2.45	0.10–1.37
Lactate	0.76–10.24	1.20–6.00

lactic, acetic, formic acids, chloride, nitrate and sulfate ions (see Fig. 1 and Table 3). Other components were only detected in some of the studied samples. This was the case for nitrite, isovaleric, valeric, butyric and propionic acids found in raw sugar and sulfites detected in Grade 1 sugar, as may be seen in Table 3.

Immediately after injection, coeluted peaks (A) which remain unidentified appeared (see Fig. 1). Peak 1 was identified as pyroglutamic acid. The loss of ammonia during the calco-carbonic purification of glutamine lead to the formation of pyroglutamic acid which was partially hydrolyzed into glutamic acid (Lodi and Rossin, 1995). This fact could be associated with a poor sugar beet quality because, generally, degraded beets contain excessive amounts of amides (Mac Ginnis, 1982). Peak 2 was identified as lactic acid and peak 3 as acetic acid. Only for the raw sugar, peak 4 was identified as propionic acid. Peak 5 was attributed to formic acid and found in sugar samples. Sugar may contain two microbiological species: thermophilic bacteria and yeast. Experimental results indicated that heating carried out in the sugar factory cannot rid all spores of thermoresistant bacteria (*Bacillus stearothermophilus*). Most bacteria are removed from the crystal surface by the centrifuge phase. However, mother liquor inclusions, which contain these spores, can be formed during the growth of crystals. These spores can grow again in vegetative form if the conditions are favourable. The final sugar stages of production show an increasing secondary contamination of sugar on its surface as a result of a growing number of yeast fungi (Kerekes, 1988). The organic acids production is mainly due to yeast fermentation, which can incidentally provoke other effects, such as gas formation and nitrate reduction. In soft drinks, the presence of yeast in syrups with a high beet sugar content is undesirable because it can result in the fermentation of the product. In the wine industry, acetic acid was found to be directly responsible for the suppression of yeast fermentation (Rasmussen *et al.*, 1995). All of these acids are responsible for unpleasant odours in the final product. For raw sugar, peaks 7, 8 and 9 are identified respectively as butyric, valeric and isovaleric acids. These fatty volatile acids were previously detected in sugar and quantified by a GC/FID method (Marsili *et al.*, 1994, Godshall *et al.*, 1995). However, when sugar has a strong molasses odour as is the case for raw sugar, the amount of acids is high enough to be detected by the conductimeter. Peak 9 is identified as chloride. It was not the only mineral ion. The following peaks are also inorganic anions and number 10 corresponds to nitrites, 11 to nitrates, 16 to sulfites (just for one sample) and 17 to sulfate. Beet sugar contains up to 0.5% of mineral compounds. Chloride and nitrate could have their origin in beet sugar and also occasionally in fertilizers and wash water. The nitrates could be reduced by certain thermophilic bacteria to nitrites (Mac Ginnis, 1982). Sulfate is added as such (or as SO_2) during the sugar processing. It is used mainly during diffusion, where it acts as an acidifier for water to prevent contamination and to harden the beet cells and make water and sucrose diffusion easier. Sulfites are particularly used for the blanching of white sugar, owing to their reducing power. Peaks 12–15 were identified as acids of the tricarboxylic acid cycle: gluconic (peak 12), malic/succinic (peak 13) and malonic (peak 14). Our method of analysis does not allow the total separation of malate and succinate. The oxalate and fumarate attributed to peak 18, were totally coeluted. It was impossible to know if only one or both were present. According to Foissac and Feinberg (1991), only oxalate is found. This compound was an indicator of sucrose and amide degradation. Moreover, oxalates are known to coprecipitate with calcium, as well as sulfates and saccharates on the factory equipment (Carruthers, 1959). The other acids mainly originate from sugar beet degradation.

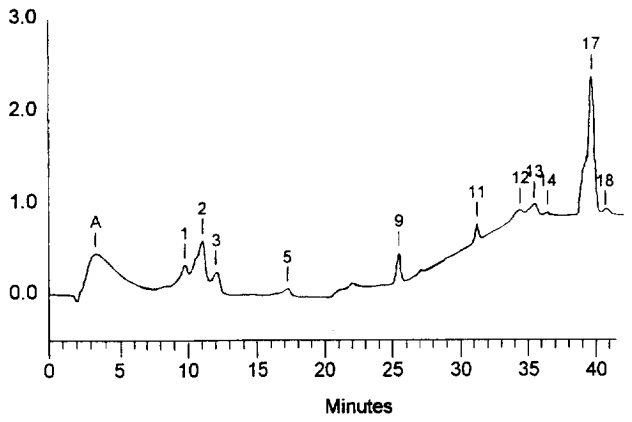

Fig. 1. Ion chromatogram of Grade 2A sugar (400 g litre^{-1}). Column AS11 Dionex. Eluent NaOH 1 and 21 mM. Flow rate 1 ml min^{-1}. Conductivity detector. Peaks: A: unknown, 1 = pyroglutamic, 2 = lactic, 3 = acetic, 5 = formic, 9 = chloride, 11 = nitrates, 12 = gluconic, 13 = malic/succinic, 14 = malonic, 17 = sulfates, 18 = oxalic/fumaric.

Comparison of sugar qualities

To compare the different qualities of sugar, we have quantified some of the identified anions: lactate, acetate, formate, chloride, nitrate and sulfate. The results are given in Table 4. It may be seen that lactic acid is predominant. Its concentration ranged from 6.58 to

Table 3. List of components detected by ionic HPLC

Components detected in Grades 1 and 2 sugars and raw sugar
Pyroglutamic acid, lactic acid, acetic acid, formic acid, chloride, nitrates, gluconic acid, malic/succinic acids, malonic acid, sulfites, oxalic/fumaric acids
Components only detected in raw sugar
Propionic acid, butyric acid, valeric acid, isovaleric acid, nitrites
Component only detected in Grade 1 sugar
Sulfites

Table 4. Components quantified by ionic HPLC in different sugars: sugar 1A: Grade 1, 4.7 E.P.; sugar 1B: Grade 1, 5.57 E.P.; sugar 2A: Grade 2; 8.98 E.P.; sugar 2B: Grade 2, 9.25 E.P.; raw sugar 3. (R = reproducibility)

Sugar	Sulfate (ppm)	R	Nitrate (ppm)	R	Chloride (ppm)	R
1A	6.90 ± 0.79	11.4	1.25 ± 0.10	8.29	1.93 ± 0.11	5.93
1B	4.40 ± 0.06	1.36	1.58 ± 0.16	10.12	1.38 ± 0.04	3.21
2A	6.69 ± 1.29	19.2	1.45 ± 0.08	5.68	2.59 ± 0.37	16.7
2B	11.3 ± 0.10	0.83	1.98 ± 0.06	2.93	2.95 ± 0.20	6.76
2C	5.35 ± 0.42	7.72	0.62 ± 0.01	2.30	1.94 ± 0.01	1.04
3	702 ± 18.0	2.56	142.5 ± 4.6	3.24	196.2 ± 4.7	2.42
Sugar	Acetate (ppm)	R	Formate (ppm)	R	Lactate (ppm)	R
1A	1.36 ± 0.06	4.35	3.67 ± 0.16	4.42	6.94 ± 0.53	6.38
1B	1.91 ± 0.02	4.12	2.98 ± 0.12	1.04	6.58 ± 0.18	2.78
2A	2.39 ± 0.14	4.61	8.08 ± 0.38	5.96	18.6 ± 0.82	4.42
2B	2.42 ± 0.10	3.59	5.88 ± 0.20	4.07	19.4 ± 0.60	3.07
2C	3.23 ± 0.08	2.70	1.36 ± 0.05	2.87	10.8 ± 0.23	2.10
3	150.5 ± 2.0	1.97	322.5 ± 6.4	1.36	643 ± 10.7	1.67

19.40 ppm in white sugars, and 643 ppm in raw sugar. The nitrate content did not change for the various sugars and ranged from 1.25 to 1.98 ppm. Grade 1 sugars contain more than 2 ppm of chloride and acetate while the concentration is higher than 2.50 ppm for Grade 2 (see Table 4). This information may help to track the history of the analyzed product. The common practice for achieving a competitive product quality and for a reasonably efficient sugar recovery is the three-strike scheme in sugar factories. Wash syrup of the white massecuite centrifugal is recycled to avoid raising the raw massecuite purity.

The raw sugar analyzed was taken after the second run of crystallization before the centrifugation. It was humid and subject to microbiological proliferation. The odour was strong and characteristic of beet. We were interested in the comparison of white sugars with this sample which could be considered as an utmost limit. Raw sugar contained more components and their concentrations were generally about one hundred-fold that of white sugars. This was especially true for organic acids, which increased in number, not all of them being identified. Fatty volatile acids such as propionic, butyric, isovaleric and valeric acids were detected. It is possible that traces are present in white sugars but are not detected by the conductivity detector. Raw sugar was characterized by the presence of nitrites, which might be due to the reduction of nitrates by thermophilic bacteria. The reaction of these nitrites with hydrogenosulfites following the sulfitation of thin juices leads to the formation of the dipotassium salt of imidodisulfonic acid, which can precipitate and raise the ash content of sugar. As only nitrates and not nitrites are found in the soil, the reducing power of the thermophilic bacteria of nitrates to nitrites can be used as a measure of their activity (Mac Ginnis, 1982).

Grade 1 sugars are characterized by the presence of sulfites, the concentrations of other anions being not very different except for sulfates. Sugar 1 B contained 4.40 ppm of sulfites and sugar 1 A 6.90 ppm, more than sugar 2A (6.69 ppm) (see Table 4). Sulfites are added during the process to thin juice to catalytically inhibit the browning reaction between traces of invert sugar and amino acids, which form coloured compounds (Mac Ginnis, 1982). They are oxidized to sulfates. This is probably at the origin of sulfate concentration, which is higher in 1A than 1B. Indeed, both factories X and Y added sulfite in clear juice of first carbonation. Sugar factories can thus obtain a better grade based on European points, especially for the visual appearance. However, this does not mean that this sugar is purer since its ash content 1A (1.89), was slightly higher than that at 1B (1.84). Grade 2 sugars do not contain sulfite. Overall, the concentrations of all the components are increased as compared with Grade 1.

There is no significant difference between the sugars 2A and 2B. Both samples contained the same kind of anions. The only difference is sulfate concentration which is lower in sugar 2A, (6.69/ppm) than in sugar 2B (11.28 ppm). Generally, factory Y added 1100 g H_2SO_4/t beet during the diffusion, whereas only 700 g H_2SO_4/t beet were added by factory X. We can use this information to compare sugars 2 A/B and sugar 2 C which was used in a beverage factory. The concentrations of inorganic anions were the same as for Grade 1. Sulfites are present. However, the organic acids were comparable with that of Grade 2. Sugar 2C is characterized by an undesirable odour. When Grade 1 sugar is stored for a long time, it often transpires that its quality is reduced to second grade. This is probably the case for sugar 2C.

CONCLUSION

From the present study, it can be concluded that an ionic chromatographic analysis of the organic and inorganic anions is relevant for establishing of quality criteria for white beet sugar. Although crystallization is known as a purification step, when crystal growth rate is very high, mother liquor drops can be included inside crystals, thus increasing their impurities. Sulfate, which

is added during the process, as well as other mineral anions, may be used to determine the crystallization quality. During storage, the behaviour of sugar depends on temperature and relative humidity. Moreover adsorption of water is higher when the concentration of impurities at the surface of crystals is greater. Water activity may then increase rapidly and this allows yeast development and consequently organic acids production (Bressan and Mathlouthi, 1994). Organic acids production seems more related to storage conditions than to sugar processing.

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