Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 86 (2004) 421–433
Food Chemistry
www.elsevier.com/locate/foodchem

Study of coloured components formed in sugar beet processing

Mónica Coca, M. Teresa García *, Gerardo González, Mar Peña, Jorge A. García

Departamento de Ingeniería Química, Universidad de Valladolid, Paseo Prado de la Magdalena s/n Valladolid 47011, Spain

Received 23 May 2003; received in revised form 11 September 2003; accepted 11 September 2003

Abstract

The main characteristics of the most troublesome colourants formed throughout sugar beet manufacture are summarised. A review of the reaction mechanisms, structures and factors affecting their formation is presented to enhance understanding of the nature of coloured impurities and to propose effective measures for controlling their formation through the process. The main colourants formed in sugar beet processing were synthesised, analysed by gel permeation chromatography (GPC) and characterised by their molecular weights and UV–Vis spectra. The GPC analysis of a sugar beet thin juice showed the presence of coloured compounds with molecular weights ranging from 0.4 to >100 kDa. Spectral analysis confirmed the presence of melanoidins and alkaline degradation products of hexoses in the juice. There is a substantial increase in colour after the evaporation step. Therefore, the coloured impurities of thin juices have to be removed before subsequent stages to produce white sugar of high quality.

Keywords: Melanoidin; Hexoses alkaline degradation products; Caramels; Sugar colourants; Molecular weight

1. Introduction

Juices and syrups formed during sugar beet processing contain compounds that impart yellow or brown colour to the white sugar. The coloured compounds are polymers with different molecular weights, structures and properties. These compounds are formed in the process as a result of sugar degradation reactions, pH changes, thermal effects and reactions between amino compounds and carbohydrates. These impurities may be occluded within sugar crystals, causing a negative effect on both the quantity and quality of the white sugar. Therefore, colour formation is an important problem in sugar manufacture. Colour formation in sugar beet juices is also a complex process. The mechanisms are very complicated, the parameters are many and the possibilities of developing effective treatment schemes are large. As the coloured compounds found in sugar beet juices remain throughout the sugar process, it is important to know the parameters that contribute to the colour formation in order to develop suitable technologies that allow the removal of colourants from sugar beet juices, preventing their further formation at subsequent steps.

Studies on sugarcane colourants have provided comprehensive information about their nature (Bento, 1995, 1999; Bento & Sá, 1998a, 1998b; Godshall, 1996). However, the knowledge of the colour formation processes throughout beet sugar manufacture and the properties of the colourants formed is less. It is known that beet colouring matter is mainly produced during processing from alkaline degradation of invert sugars and melanoidin formation, whereas cane colourants are mainly plant pigments associated with polysaccharides (Godshall, Vercellotti, & Triche, 2002). There are studies that attempt to characterise colourant–polysaccharide complexes that come from the sugar beet plant and can travel through all processing steps, ending up in the final sugar product (Clarke, Godshall, & SPRI group, 1990; Godshall, 1992a). However, there are few studies concerning colour formation during beet processing (Godshall et al., 2002; Shore, Broughton, Dutton, & Sissons, 1984), and these studies do not attempt to identify coloured impurities.

This study aims to present an overview of the properties of the most harmful colourants present in sugar

* Corresponding author. Tel.: +34-983-42-32-37; fax: +34-983-42-36-16.
E-mail address: maite@iq.uva.es (M. Teresa García).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.09.017

beet juices. Most literature available today seldom deals with all types of colourants or explains the essential differences among them. The compilation is essential to increase understanding of beet colouring matter and the fundamental mechanisms of colour formation. After a literature survey concerning the main coloured compounds, a study of the colourants present in sugar beet juices, analysing their molecular weights and UV–Vis spectra, follows. The objective was to characterise the colourants and to examine the influence of sugar processing steps on the formation and concentration of colourants. The technique selected to carry out the study of beet colourants was gel permeation chromatography (GPC). GPC is a suitable analytical technique for separating the coloured constituents in sugar juices and for determining their molecular weights. The beet colouring compounds can be characterised by their UV–Vis spectra, using a photodiode array (PDA) as detector. PDA enables the characterisation of the spectral nature of colouring matter. By global colour measurement is not possible to distinguish the different characters of colourants. By comparing the spectra of colourants in juices with those of synthetic colourants, previously prepared, the identification of colouring impurities is possible.

2. Colorants formed in sugar beet processing

2.1. General

The formation of coloured compounds in sugar beet processing is mainly due to sugar degradation reactions. Monosaccharides, mainly glucose and fructose, are regarded as among the most important compounds in colour formation reactions. Heating monosaccharides under acidic or basic conditions leads to degradation reactions, forming highly reactive intermediates, which can undergo further condensation and polymerisation reactions to form coloured polymers. The monosaccharides, glucose and fructose, are formed from hydrolysis of sucrose during the entire production process. The colourants formed from sucrose can be divided into enzymatic colourants, such as melanins and non-enzymatic colourants, such as melanoidins, alkaline degradation products of hexoses (HADPs) and caramels. Melanins are precipitated during the purification step. Therefore, such a colourant is not considered a great technological problem.

2.2. Melanoidins

The amino compounds present in juices do not lead to any colour formation reaction, but in the presence of monosaccharides and other carbonyl compounds formed from them, the Maillard reaction takes place and browning products appear. The chemistry of the Maillard reaction is very complex, encompassing a whole network of consecutive and parallel chemical reactions. However, the reaction mechanisms are generally divided into three stages:

- *First stage*. The Maillard reaction is initiated by a condensation reaction between a compound possessing a free amino group and a α-hydroxyl carbonyl moiety of a reducing sugar. Aldoses, such as glucose, give an N-substituted aldosylamine. The condensation product rapidly loses water and is converted into a Schiff base. The Schiff base then cyclises into the aldosylamine. The Amadori rearrangement follows, to form a ketosamine. Ketoses, such as fructose, react with amino groups to form aminoaldoses in the Heyns reaction. Aminoaldoses are not stable intermediates and readily react, forming the Amadori compounds. No browning reactions occur at this stage.
- *Second stage*. The subsequent degradation of the Amadori compounds is dependent on the pH. At pH above 7, the degradations involve mainly 2,3 enolisation, where reductones and a variety of fission products are formed (Martins, Jongen, & Van Boekel, 2001). All these compounds are highly reactive and take part in further condensation reactions, which result in the incorporation of nitrogen into the melanoidin structure. Dicarbonyl compounds will react with amino acids to form aldehydes and α-aminoketones. This reaction is known as the Strecker degradation and it is characterised by the release of CO_2.
- *Third stage*. The formation of melanoidins is the result of polymerisation reactions of highly reactive intermediates formed during the Maillard reaction. A wide range of reactions takes place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations, which lead to the formation of brown nitrogenous polymers and copolymers, known as melanoidins. The molecular weight of coloured compounds increases as browning proceeds.

A scheme of the Maillard reaction, adapted from Martins et al. (2001), is shown in Fig. 1. The complexity of the Maillard reaction has been extensively studied during recent years and new important pathways and key intermediates have been established (Martins et al., 2001). Melanoidins are recognised as being acidic compounds with a charged nature. With increasing reaction time and temperature, the total carbon content increases, thus promoting the unsaturation. The colour intensity increases with the polymerisation degree. The degree of browning, usually measured via absorbance at 420 nm, is often used to follow the extent of the Maillard reaction. Heating of melanoidins (90 °C) under aerobic conditions not only causes decolourisation but also produces a development of fluorescence. A heat treatment at 90 °C, under anaerobic conditions, causes an increase in both colour and reductone content. The de-

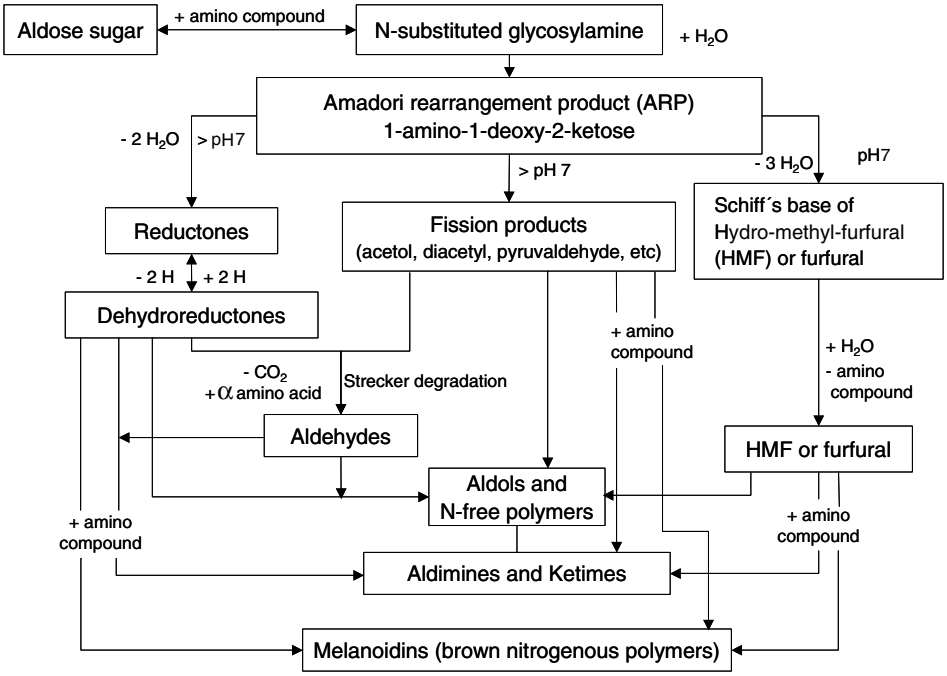

Fig. 1. Scheme of the Maillard reaction.

velopment of fluorescence does not take place under these conditions. The chemical changes of melanoidins by heating were studied by Gomyo, Kato, Udaka, Horikoshi, and Fujimaky (1972).

The elucidation of the chemical structure of melanoidins is difficult due to the complexity of the Maillard reaction. Kato and Tsuchida (1981) proposed a major repeating unit for melanoidins prepared from glucose and butylamine (pH 5.0–6.5). The structure is useful for explaining the great increase of the reductone content of melanoidins on heat treatment under anaerobic conditions. This structure was later confirmed by NMR analysis (Hayase, Kim, & Kato, 1986). However, changing reaction conditions play an important role in the fundamental structure of melanoidins. This means that it cannot be assumed that melanoidins have a regular composition with repeating units. For this reason, Cämmerer and Kroh (1995) proposed a general structure for melanoidins prepared from monosaccharides and glycine. The basic structure is formed by α-dicarbonyl Maillard reaction intermediates, partially branched by amino compounds and with many reactive centres that make possible further decarboxylation and dehydration reactions. The structure of the real melanoidins is likely to be a result of different reactions from the basic framework. Yaylayan and Kaminsky (1998) isolated a brown nitrogen-containing polymer formed in the Maillard mixture. The structure was consistent with that proposed by Cämmerer and Kroh (1995). This polymer exhibited a strong absorption band at 1607 cm^{-1} in the FTIR spectrum, attributed to extensive conjugation. Pyrolysis of the isolated polymer produced typical Amadori products, such as pyrazines, pyrroles, pyridines and furans. Cämmerer, Jalyschkov, and Kroh (2002) have recently suggested a new model of a basic skeleton for melanoidins formed from carbohydrates and glycine.

The composition of melanoidins depends on the reaction conditions, mainly temperature, heating time, pH, water content and the nature of reactants. An increase in pH or temperature leads to an increase in the reactivity between the sugar and the amino group (Martins et al., 2001). The rate and extent of the Maillard reaction increase with increasing pH. The optimum pH values range from 6 to 8 (Heitz, 1995). At pH <3 and >9 other non-enzymatic reactions may compete with the Maillard reaction. The rate of browning increases with temperature. An increase in temperature of about 10 °C causes a change of two- to threefold in reaction rate. The rate of melanoidin formation increases in proportion to the square of the length of heating at a given temperature (Heitz, 1995). The rate of Maillard reaction is also dependent on the amount of free water available. Maximum browning occurs at Brix values of about 80–90% (Heitz, 1995). Sugars present different reactivities toward the amino acids (Cämmerer & Kroh, 1995). Reducing disaccharides are considerably less reactive than their corresponding monomers. Glucose and fructose are regarded as among the most important monosaccharides in sugar beet processing. Glucose and fructose have different chemical reactivities with the amino acids present in sugar solutions, depending on the reaction conditions (Reinefeld, Bliesener, Brandes, & Borrass, 1982). According to these findings, fructose is more

reactive than glucose in weakly alkaline aqueous medium. Under these conditions, degradation products of the hexoses, such as glyceraldehyde, methylglyoxal and deoxyhexosuloses are formed at first. In this case, melanoidins may be formed from the reaction of amino acids with both the monosaccharides and the degradation products of hexoses. In concentrated systems, with lower water content, glucose was found to be more reactive than fructose. No degradation products are formed. Under these conditions, glucose and fructose react directly with amino acids to form melanoidins. The following reactivity of amino acids participating in colouring reactions has been found (Reinefeld et al., 1982):

γ-amino butyric acid, lysine > aspartic acid, proline, phenyl alanine > leucine, glutamic acid, glycine > alanine, valine.

The most common amino acids, found in sugar beet juices are aspartic acid and glutamic acid. Both are acidic α-amino acids, formed from asparagine and glutamine, respectively. γ-Amino butyric acid is a highly reactive compound in browning reactions that can be formed in beet juices from degradation reactions of glutamine conversion products, e.g., pyrrolidone carboxylic acid, via 2-pyrrolidone. The concentration of γ-amino butyric acid in thick juices may be twice higher than that found in the corresponding raw juice (Reinefeld et al., 1982).

2.3. Alkaline degradation products of hexoses

The HADPs together with the melonidins are responsible for up to 80% of colour in sugar beet juices (Heitz, 1995). Monosaccharides in aqueous alkaline solutions undergo both reversible and irreversible transformations (De Bruijn, 1986). The reversible reactions include: (1) ionisation, (2) mutarotation and (3) enolisation and isomerisation, resulting in the formation of the enediol anions which are generally considered as common intermediates in isomerisation reactions of monosaccharides. The enolisation reaction is known as the Lobry de Bruyn–Alberda van Ekenstein rearrangement. The initial production of the enediol anion species is followed by a subsequent chain of irreversible reactions known as the alkaline degradation reactions, which can ultimately lead to organic acid products. Enediol species are considered to be intermediates in the isomerisation of monosaccharides as well as starting intermediates in the alkaline degradation reactions. The enediol anions may undergo β-elimination reactions. The resulting α-dicarbonyl compounds can undergo either a benzylic acid rearrangement or a α-dicarbonyl cleavage. The products formed upon benzylic acid rearrangements are α-hydroxycarboxylic acids, such as lactic acid and saccharinic acids. Dicarbonyl cleavage gives a carboxylic acid, such as formic acid, acetic acid and an aldehyde, e.g., formaldehyde and acetaldehyde. Aldolisation and retroaldolisation of carbonyl compounds result in an elongation and fragmentation of the carbon chain, respectively. The products of alkaline degradation reactions can be divided into two groups, according to the number of carbon atoms:

$\leqslant C_6$ carboxylic acids. The major part of the monosaccharides is converted into carboxylic acids with six or smaller number of carbons. They are colourless compounds, mainly lactic acid but saccharinic acid, formic acid, acetic acid and oxalic acid are also formed.

$>C_6$ carboxylic acids. The amounts of carboxylic acid products containing more than six carbon atoms may be substantial and depend on the conditions of the degradation reactions. The products other than $\leqslant C_6$ can polymerise to form high molecular weight compounds, which are in relation to the colour formation during the alkaline degradation reactions. The nature and structure of the $>C_6$ compounds have not been elucidated. Formation of polymers is most probably due to extensive aldolisation of intermediate (di) carbonyl compounds present in the alkaline degradation solution. It is know that $>C_6$ carboxylic acids contain carboxylate, CH_3, CH_2, CH_2 OH, CHOH, and (enolised) β-dicarbonyl moieties (De Bruijn, 1986).

In Fig. 2, a simplified scheme of degradation reactions (with the kind permission of de Bruijn) is depicted. The rate and course of the alkaline degradation reaction can be influenced by several reaction parameters, such as the hydroxyl ion concentration, the temperature, the nature of the base used and the concentration of monosaccharides (De Bruijn, 1986). Variation of the hydroxyl ion concentration influences both the composition of the $\leqslant C_6$ carboxylic acid products and the relative amounts of the $\leqslant C_6$ and $>C_6$ products. The maximum production of $>C_6$ products takes place at pH 11–12. Degradation rate depends on the reaction temperature according to the Arrhenius equation. However, the composition of reaction products seems to be independent of temperature. The presence of divalent cations, such as calcium and magnesium, accelerates the decomposition of monosaccharides and influences the final product composition. Alkaline degradation of very dilute monosaccharide solutions results in an almost complete conversion of the monosaccharides into $\leqslant C_6$ carboxylic acids. Concentrated solutions show the formation of higher amounts of $>C_6$ carboxylic acids. D-Fructose shows a higher enolisation rate than D-glucose and D-mannose.

2.4. Caramels

Caramels are thermal degradation products of sugars. They are colloidal compounds with a tendency to remain preferentially on the crystal surface, affecting to the

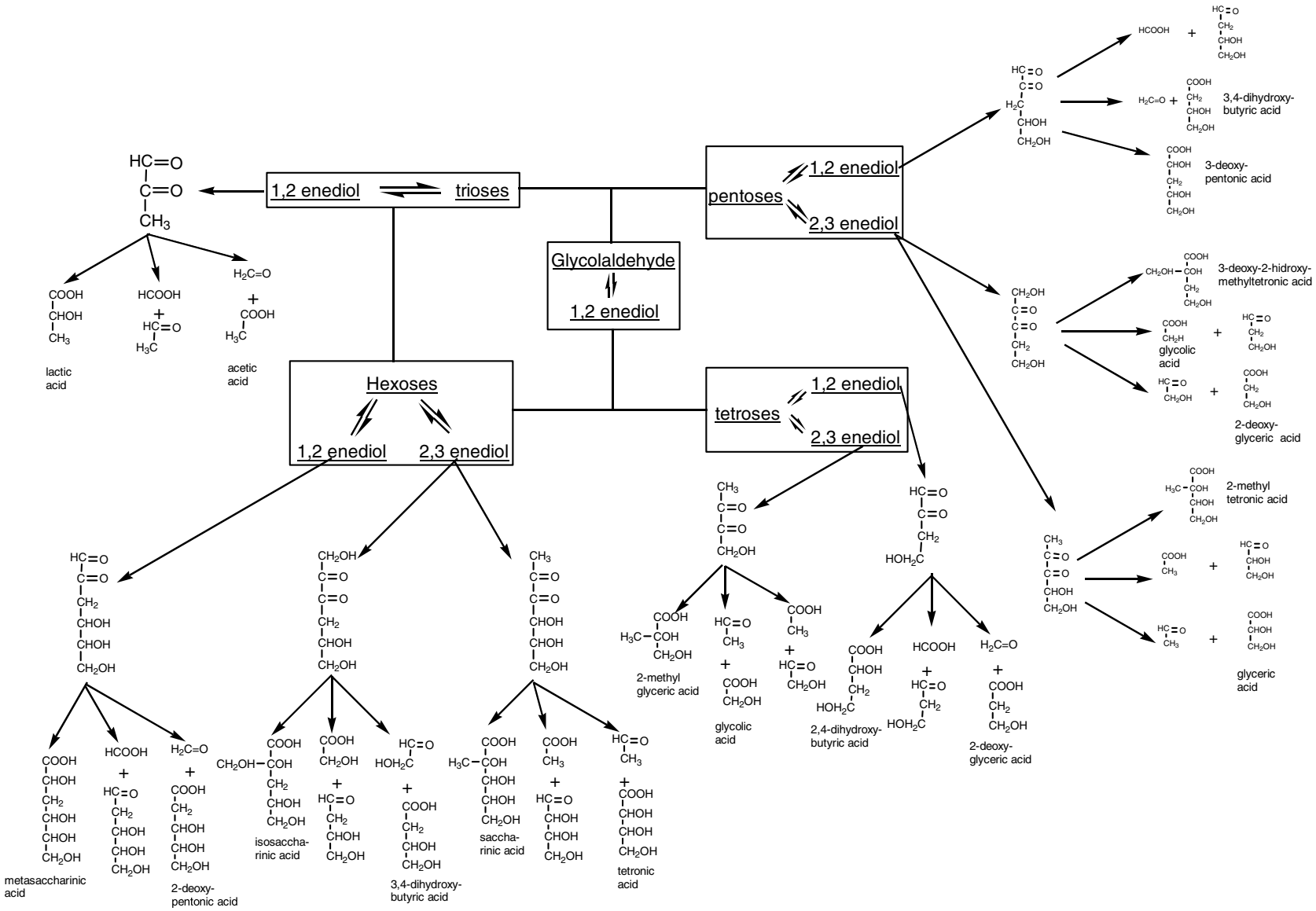

Fig. 2. Scheme of the alkaline degradation reactions adapted from De Bruijn (1986).

quality of white sugar. Caramels are formed by heating concentrated sucrose syrups at temperatures above 210 °C. The generation of colour in caramelisation requires that sugars, normally monosaccharide structures, should first undergo intramolecular rearrangements (Kroh, 1994). Depending on the time and temperature, yellow or brown solutions are obtained. The reaction causes the release of H^+ and the pH of the solution decreases with time up to values of about 4–5. In the sugar degradation reactions described above, osuloses are formed, which are considered to be intermediates of the caramelisation. The osuloses are α-dicarbonyl compounds, such as 3-deoxyhexosulose. Osuloses lead to the formation of typical components of caramel colour and caramel flavour. The osuloses are involved in the formation of three typical *O*-heterocyclic compounds: hydroxymethylfurfural (HMF), hydroxydimethylfuranone (HDF) and hydroxyacetylfuran (HAF) from D-glucose. During thermally induced caramelisation, transglycosidation and the formation of oligomers by polymerisation are important. The formation of anhydro sugars, via intramolecular dehydration, is known (Kroh, 1994). Although the number of reactive intermediates in caramelisation is much lower in comparison with those formed in the Maillard reaction, the structure of these polymeric products is not satisfactorily known. HMF, furfural and HAF are considered to be precursors of regular polymers with the structure reported by Kroh (1994). HMF is a relatively stable compound and it is unlikely that HMF polymerises regularly when its precursors, such as deoxyhexosuloses, are much more reactive and better-suited to form such polymers. Yaylayan and Kaminsky (1998) isolated two non-nitrogen containing intermediates formed during the Maillard reaction that were classified as caramels. Their origin is assigned to glucosone and/or 3- or 1-deoxyglucosones. A pyrolysis treatment of the isolated polymers produces furanoid species similar to sucrose, indicating the presence of glycosidically linked sugar derivatives. Defaye and García (1995) proposed that the composition of non-volatile caramels is a mixture of five dianhydrides of D-fructose (~20% of the total) with the α,β-1,2′:2,1′-difuranose dianhydride being predominant, along with a similar proportion of such dianhydrides glycosylated at the primary hydroxyl position with 1–6 glucose residues.

2.5. Formation of colourants in beet processing

The concentration of nitrogen compounds in sugar beet juices is higher than in cane sugar juices and, therefore, the formation of melanoidins is more important in sugar beet processing. The melanoidin formation reactions may start during slicing of beets into cossettes in spite of the mild temperature conditions (Mersad, Lewandowski, Heyn, & Decloux, 2001). The reaction rate increases during the diffusion step, due to the higher temperatures (69–73 °C). During the purification step, the pH increases up to strong basic values (pH 11–12) and the temperature also increases (80–90 °C). These conditions favour the formation of melanoidins. However, evaporation is the step of beet processing in which the formation of colour, due to the Maillard reaction, is more important because of the high temperatures, up to 120 °C. Polymerisation also proceeds throughout crystallisation. In the purification and evaporation stages, 95% of the glutamine and about one third of the asparagine present in the juices are saponified (González & García, 1996). The saponification of glutamine yields ammonia and pyrrolidone carboxylic acid whereas asparagine forms ammonia and aspartic acid. The liberation of ammonia produces a decrease in the pH value of the juice, causing an increase in the rate of sucrose inversion, thus increasing the concentration of reducing sugars that participate in browning reactions. The presence of impurities and the recycle of low quality products also favour the browning reactions. The following measures can be taken into account in order to prevent the formation of melanoidins throughout the sugar beet processing (Heitz, 1995; Shore et al., 1984):

- Maillard reaction proceeds much faster at high temperatures and basic pH conditions. Browning also increases with longer reaction times. In concentrated juices, the Maillard reaction also proceeds faster. Therefore, reducing the temperature and retention times, at both evaporation and crystallisation stages, where juices are more concentrated, is useful for limiting the formation of melanoidins.
- Control of the invert sugar concentration is important through the process, in order to keep the concentration of carbonyl compounds as low as possible. The formation of coloured compounds can be avoided (up to 70–80%) by controlling the sucrose inversion. The addition of lime to the diffusion juice aims at destruction of the invert sugars by transforming them into thermostable components, preventing their further reaction with the amino acids present in juices.
- Some cations such as copper and iron catalyse the browning reaction. The utilisation of equipment made of stainless steel is recommended in order to avoid corrosion problems.
- Avoid the recycle of low quality products.
- The colour of purified juices can be reduced by the addition of sulphur dioxide. This compound is used in the factory processes to inhibit non-enzymatic browning reactions. SO_2 forms β-sulphonated aldehydes, which are of low reactivity in Maillard reactions. Effective control of nitrite concentration is essential since nitrite reacts with SO_2, reducing the available concentration.
- Use specific decolourisation processes such as activated carbon and resins.

Alkaline degradation reactions take place at the common pH of a beet sugar factory (8–11). The degradation rate in the presence of calcium cations is about fourfold faster than in the presence of sodium cations, but the colour intensity is more than threefold higher in the presence of sodium cations. The formation of degradation products takes place mainly during the purification step where temperature increases up to 85 °C and pH increases up to basic values (11–12). Formaldehyde is added to juices to inhibit the growth of microorganisms in the diffusion step. The produced formose sugars will be degraded under the liming conditions in the same way as the reducing sugars. Regarding HADPs, the following considerations can be taken into account throughout the sugar beet processing in order to prevent and control their formation (Heitz, 1995):

- Avoid the inversion of sucrose. Sugar degradation results in the loss of sugar and colour formation.
- Removal of invert sugars at temperature as low as possible. The elimination of invert sugars is recommended during liming; in presence of calcium it is better than after decalcification, when the concentration of sodium is much higher.
- Copper and iron catalyse the degradation reactions. The utilisation of stainless steel equipment is recommended in the evaporation step, where the highest temperatures are reached.
- The addition of sulphur dioxide or oxygen is recommended to inhibit sugar degradation reactions. SO_2 also reacts with formaldehyde that is regarded as a colour precursor.
- Removal of coloured compound should be done by using decolourisation resins or adsorption into $Ca_3(PO_4)_2$ crystals. Calcium carbonate precipitate does not adsorb HADPs efficiently.

Caramelisation is defined as the degradation of solid sucrose. Caramelisation requires temperatures over 200 °C and pH from 3 to 9. However, the presence of impurities in low concentration, mainly iron, reduces the caramelisation temperature up to 40 °C. Caramelisation occurs in beet sugar production when sugar surfaces are heated strongly, e.g., evaporation. The following measures are listed below, in order to limit the formation of caramels (Heitz, 1995; Shore et al., 1984):

- Avoid any projection of sugar on hot metal surfaces, especially during the crystallisation step. The feed of syrups into equipment and the factory shutdown have to be designed to avoid these projections.
- Production of uniform crystals should be achieved during crystallisation, free from conglomerates, which contain trapped, highly coloured mother liquor.
- Excessive temperatures should be lowered as well as residence times in evaporation and crystallisation stages.
- Avoid the presence of impurities such as iron salts.

2.6. GPC chromatography applied to sugar colourants

GPC has been applied to examine the coloured compounds in both sugar cane and sugar beet juices (Bento & Sá, 1998a, 1998b; Bento, 1999; De Bruijn, Bout-Diederen, & Huijbregts, 2002; Godshall, Clarke, Miranda, & Blanco, 1992b; Shore et al., 1984). GPC studies on sugar beet juices are scarcer and they do no attempt to identify beet colourants. GPC is a suitable technique for determining the molecular weight distribution of compounds in juices and can be used to follow coloured compounds through the sugar beet processing. The separation is based on the distribution of different sized molecules between the mobile phase and the pore volume. GPC columns allow large molecules to pass through more rapidly than smaller molecules. Therefore, GPC columns separate the compounds present in juices according to their molecular weight. A PDA detector allows the identification of UV absorbing compounds. In this way, compounds can be characterised by their UV–Vis spectra. A refractive index detector allows the detection of coloured compounds that do not absorb UV radiation.

A wide range of procedures for the preparation of synthetic melanoidins has been described. Wedchida and Kaputo (1992) provided a complete compilation that illustrates the diversity of systems tried and the purpose for which they were studied. Formation of colourants in beet sugar factories proceeds when the pH is on the basic side. For this reason, the synthesis procedures have to reproduce reaction conditions more closely resembling to those existing in the beet factories. Shore et al. (1984) synthesised sugar colourants by the following procedure:

- *Melanoidins*. Glucose (72 g) and glycine (30 g) in water (60 ml) were incubated at 50 °C for 72 h. The mixture was maintained at pH 11 by addition of concentrated NaOH.
- *Alkaline degradation products of hexoses*. Glucose solution (200 ml, 20 g/l) was adjusted to pH 9.5 and boiled under reflux for 5 h while maintaining pH 9.5.
- *Caramels*. Sucrose (180 g) and water (20 ml) were incubated at 105 °C for 66 h.

After a GPC analysis, Shore et al. (1984) found that melanoidin-type colourants were of relatively high molecular weight (from 1 to 5 kDa) whereas the HADPs and caramels were of considerably lower molecular weight (mostly up to 1 kDa). The colourants of higher molecular weight have a tendency to be occluded into sugar crystals whereas lower molecular weight colourants reside on the crystal surface. Bento and Sá (1998b) synthesised the colourants as described by Shore et al. (1984). However, the molecular weights obtained were rather different from those found by Shore et al. (1984). Melanoidins from 5.7 to 21.1 kDa, HADPs of 6.9 kDa and caramels of 5.5 kDa were found. Differences are

likely to be due to the type of GPC column used and the analysis conditions. Shore et al. (1984) used a cross-linked dextran column (Sephadex G-25), whereas Bento and Sá (1998b) used a cross-linked agarose-based medium column (Superose 12). Other procedures have been proposed to synthesise colourants under conditions closer to those existing in the sugar factories (Mersad et al., 2001; Rafik, Mas, Elharfi, & Schue, 1997).

3. Materials and methods

3.1. Synthetic colourants

Sugar colourants were synthesised according to Shore's method, as described above. Previous to GPC analysis, samples were adjusted to pH 7 and diluted in deionised water, 1:50 (v/v), in order to reduce their strong absorbance at 420 nm, the wavelength proposed by the official methods (ICUMSA, 1994) for measuring colour in sugar juices. Samples were filtered through a 0.22-μm membrane before being injected.

3.2. Preparation of samples

Sugar beet juices were taken from a sugar beet factory. The pH of the sugar beet samples was adjusted to 7. Contents of colour and dry matter were calculated according to ICUMSA methods. Samples were filtered through a 0.22-μm membrane before injecting onto the column.

3.3. GPC analysis

The molecular weights of the coloured compounds were estimated by GPC. Separation was achieved in an Ultrahydrogel^{TM} 250 column (exclusion limit 1–80 kDa) in series with an Ultrahydrogel^{TM} 120 column (exclusion limit 0.2–5 kDa), both columns being packed with hydroxylated polymetacrylated-based gel. A guard column of the same material was also used. Deionised water (Millipore Q system) was used as mobile phase at a flow rate of 0.7 ml/min at 30 °C. Identification was achieved by using a Waters 996 Photodiode Array Detector, coupled in series with a refractive index detector.

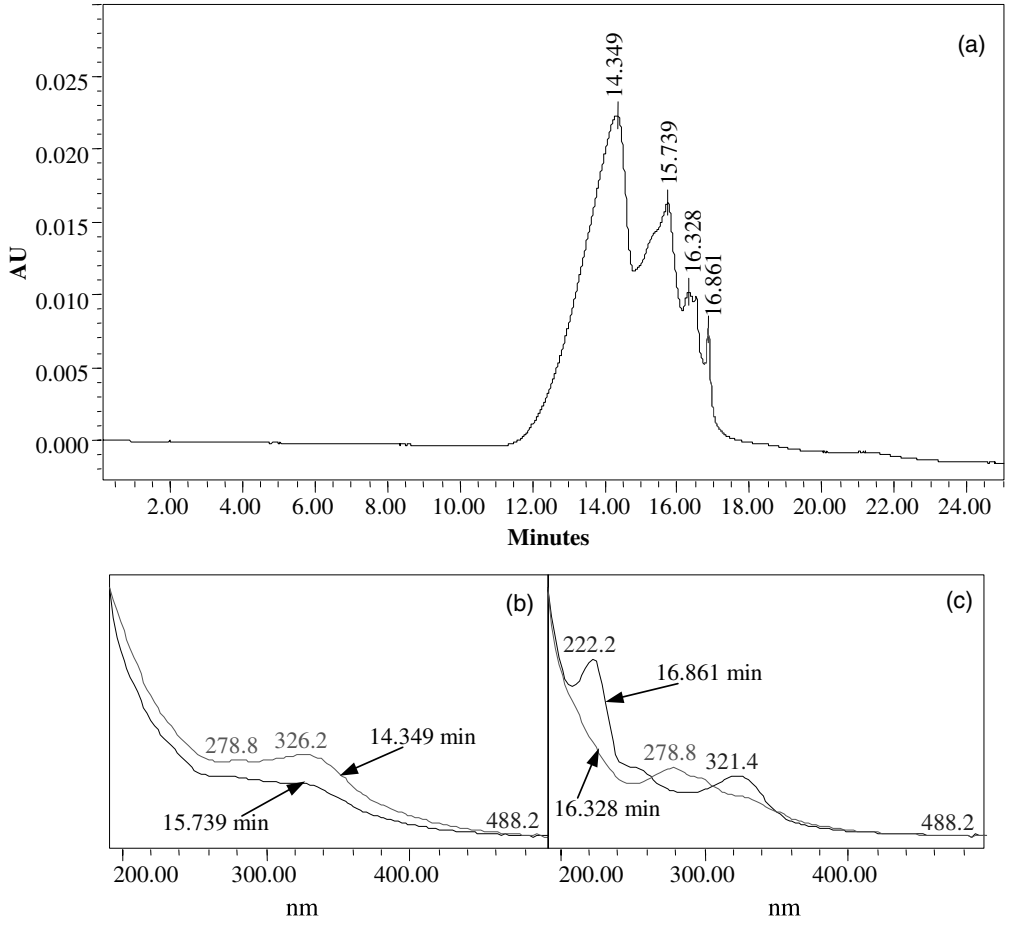

Fig. 3. Synthetic melanoidins; (a) chromatogram extracted at 420 nm; (b) spectra of peaks with retention times of 14.349 (>100 kDa) and 15.739 min (80 kDa); (c) spectra of peaks with retention times of 16.328 (48 kDa) and 16.861 min (31 kDa).

Pullulans (non-branched polymeric sugars) and polyethylene glycol polymers were used as standards for the estimation of molecular weights. Data were processed with the software Millenium 32 Chromatography Manager.

4. Results and discussion

4.1. Melanoidins

Fig. 3(a) shows the chromatogram, extracted at 420 nm, corresponding to the synthetic melanoidin. This wavelength is the official one to measure colour in juices. Melanoidins were divided into various peaks with different molecular weights, corresponding to their peak apices. The first peak, appearing at 14.349 min, corresponds to a compound with molecular weight above 100 kDa. The second peak, with a retention time of 15.739 min, corresponds to a compound whose molecular weight is about 80 kDa. The last two peaks correspond to compounds of lower molecular weight, 48 and 31 kDa, respectively. The molecular weights found were considerably higher than those found by Bento and Sá (1998b) using Shore's method. The differences are likely to be due to the chromatographic system used. Regarding the spectra of the first two peaks (Fig. 3(b)), they are very similar, with a stable absorbance region between 278.8 and 326.2 nm, characteristic of melanoidins. This trend was also described by other authors for melanoidin-type colourants (Guimaraes, Bento, & Mota, 1996; Rafik et al., 1997). The spectra of the lower molecular weight peaks have quite different shape (Fig. 3(c)). The 48 kDa peak showed a single peak at 278.8 nm and the 31 kDa peak showed maxima at 222.2 and 321.4 nm. During the Maillard reaction, most amino acids and sugars also undergo independent degradation reactions. Products formed from the decomposition of sugars, amino acids and Amadori compounds can further interact to form polymeric compounds (Yaylayan, 1997). The spectra of the compounds of lower molecular weights may correspond to these polymeric compounds formed in the above mentioned degradation reactions.

4.2. Alkaline degradation products of hexoses

Fig. 4(a) shows the chromatogram at 420 nm that corresponds to HADPs. The synthetic colourant was separated into four peaks. The peaks with retention times of 14.277 and 15.238 min have molecular weights corresponding to their peak apex above 100 kDa. The peaks eluted at 16.365 and 17.811 min have molecular

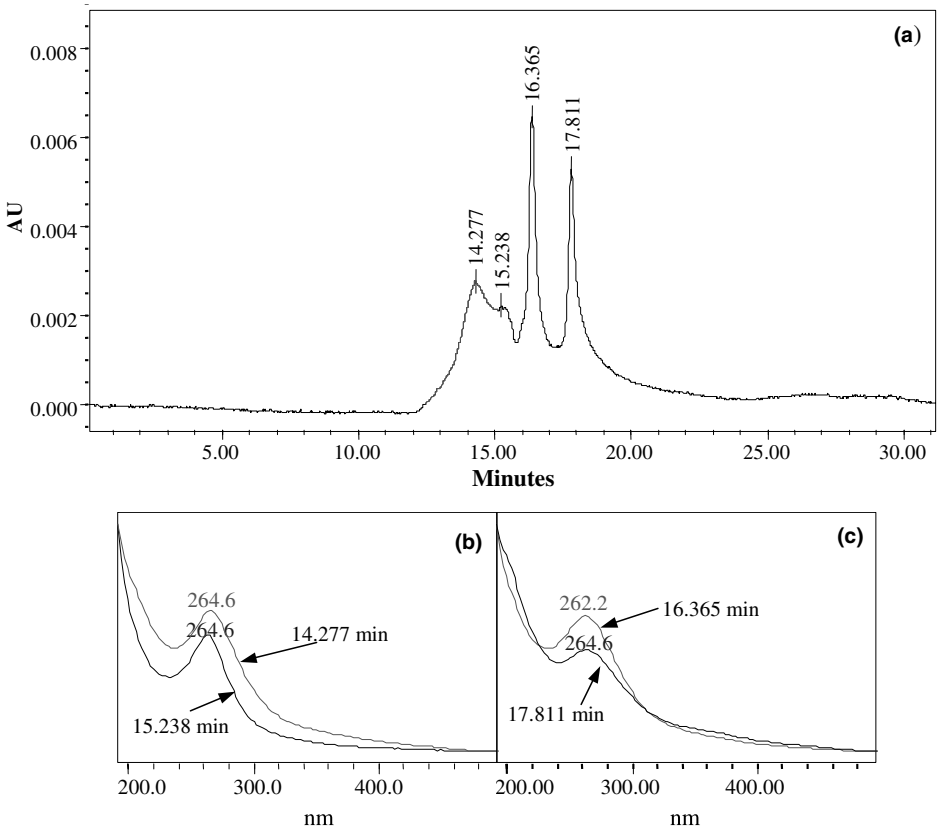

Fig. 4. Synthetic HADPs; (a) chromatogram extracted at 420 nm; (b) spectra of peaks with retention times of 14.277 and 15.238 min (>100 kDa); (c) spectra of peaks with retention times of 16.365 (47 kDa) and 17.811 min (14 kDa).

weights of 47 and 14 kDa, respectively. The molecular weights found were much higher than those found by other workers (Bento & Sá, 1998b) who used the same procedure to synthesise colourants, but from fructose as reagent instead of glucose. The spectra of the higher molecular weight compounds (above 100 kDa) are shown in Fig. 4(b). Spectra presented a strong absorption peak at 264.6 nm, a wavelength very close to that described previously by other researches (Guimaraes et al., 1996; Mersad et al., 2001; Rafik et al., 1997). HADPs show conjugated enol carbonyl moieties, which present UV absorption at 265 nm (De Bruijn, 1986). The spectra of the lower molecular weight peaks (47 and 14 kDa) are shown in Fig. 4(c). The spectra were very similar to those of the compounds of higher molecular weight. Therefore, all these peaks are likely to correspond to more or less polymerised HADPs.

4.3. Caramels

As seen in Fig. 5(a), two compounds with molecular weights of 98 and 51 kDa (retention times of 15.513 and 16.275, respectively) were obtained as a result of thermal degradation reactions from sucrose. The spectra of the 51 kDa peak showed an absorption maximum at 250.4 nm (Fig. 5(c)). According to other authors, caramels show sharp maxima at 226 and 283 nm (Guimaraes et al., 1996), 220 nm (Rafik et al., 1997) or 228 and 285 nm (Mersad et al., 2001). The UV spectrum found for caramels does not resemble those reported earlier for the same colourant. The UV spectrum of the 98 kDa compound (Fig. 5(b)) did not show any characteristic maximum and it is rather different from the spectra of caramels previously described.

4.4. GPC analysis of beet juices

A sample taken after the purification step (thin juice) was selected to determine the type of colourants present in sugar beet juices and to study their spectral nature. As can be seen in Fig. 6(a), GPC corroborated the presence of four groups of coloured compounds in the thin juice whose molecular weight depends on the elution time. There are colourants of high molecular weight, above 100 kDa (35.6%) and 34 kDa (30.3%) and of much lower molecular weight, 1 kDa (18.6%) and 0.4 kDa (15.5%). All of them are responsible for the juice colour because of their absorbance at 420 nm. The peak area percentages were calculated at 420 nm. The molecular weights found are in accordance with the results of other workers. Godshall et al. (2002) reported molecular weights for sugar beet thin juices ranging from <12 to

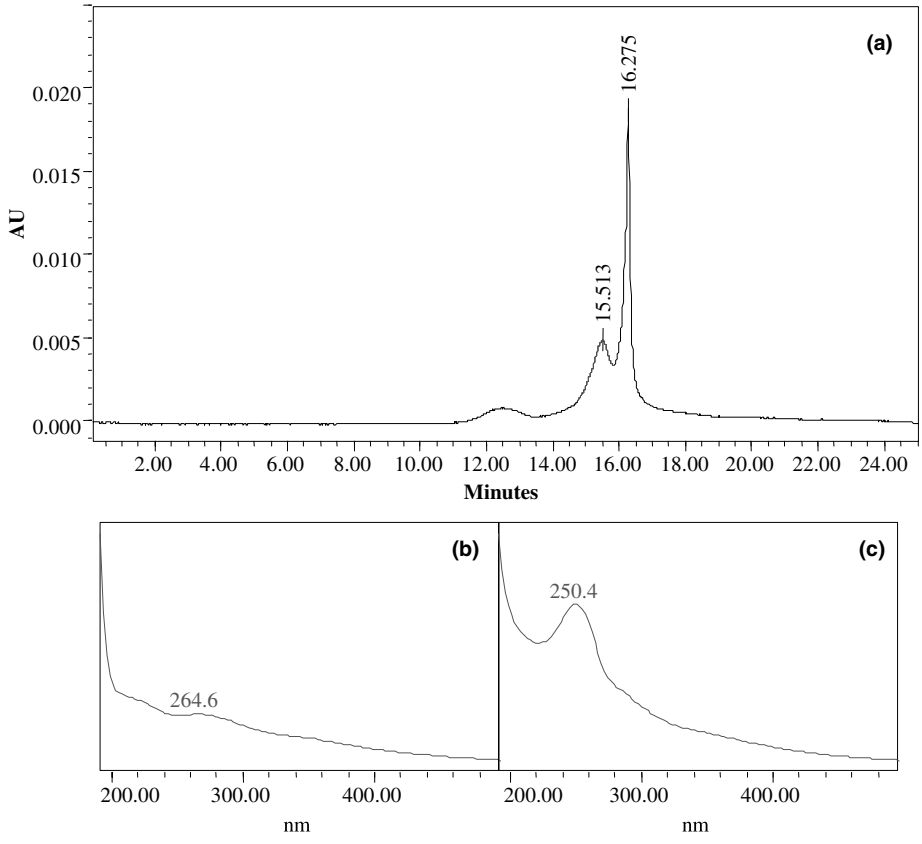

Fig. 5. Synthetic caramel: (a) chromatogram extracted at 420 nm; (b) spectra of the peak with retention time of 15.513 min (98 kDa); (c) spectra of the peak with retention time of 16.275 min (51 kDa).

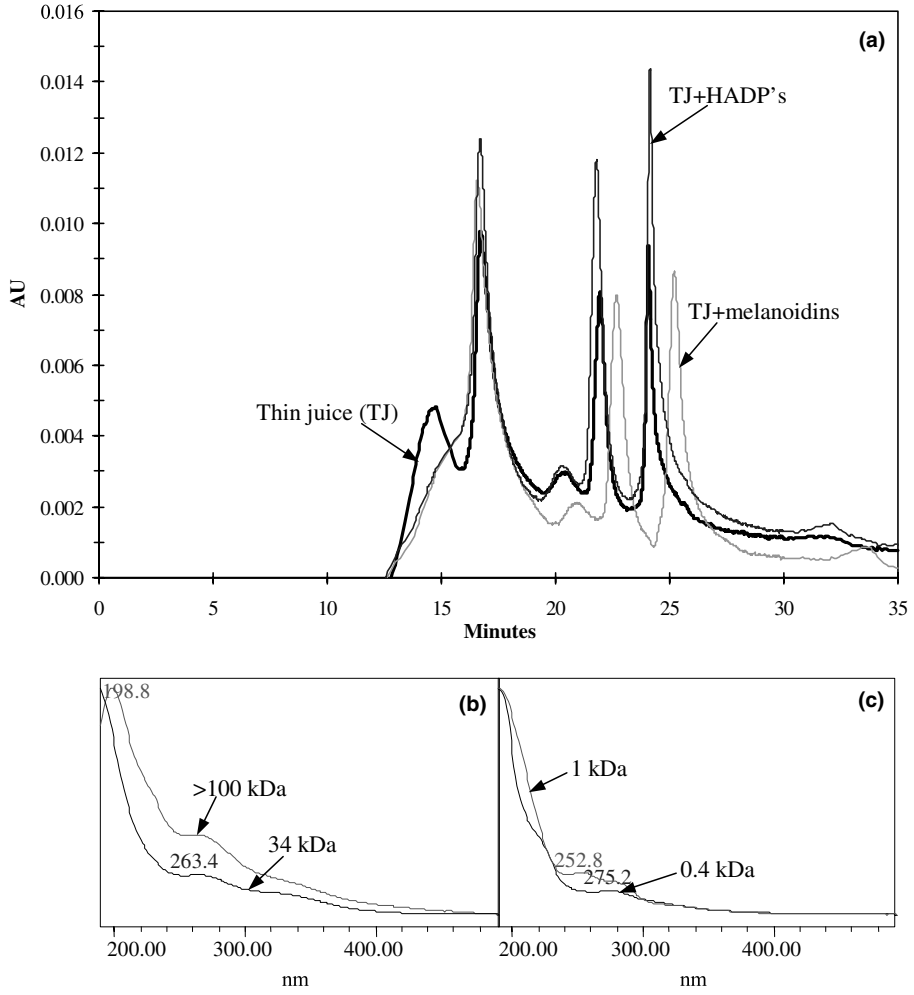

Fig. 6. Thin juice: (a) chromatogram at 420 nm corresponding to the thin juice before and after being spiked with HADPs and melanoidin; spectra of the peaks with molecular weights of (b) >100 and 34 kDa, respectively; (c) 1 and 0.4 kDa, respectively.

900 kDa. Colorants of <12 kDa represented 36% of the total, whereas coloured compounds for 20 to 49 kDa represented 28% of total. The different origin of the analysed juices, the different experimental conditions and the great variation in column sizes affect the pattern of separation obtained.

The spectra of the higher molecular weight compounds (34 and >100 kDa) are quite similar. Spectra are shown in Fig. 6(b). By comparing the spectra of these compounds with those of synthetic melanoidins (>100 and 80 kDa) it can be supposed that melanoidins are associated with these groups of colouring compounds. Millenium 32 PDA software was used to match spectra of the higher molecular weight compounds with those of synthetic colourants. Spectral contrast is the technique that the software uses to compare spectral shapes. PDA Match Angle is a measure of the difference in spectral shapes between an acquired spectrum and a library spectrum. Match Angle ranges from 0° to 90°. Small values indicate that spectra are similar. Large values indicate greater degrees of spectral difference. The PDA Match Angles calculated by matching the spectra of the 34 and >100 kDa peaks with that of synthetic melanoidin (retention time of 15.739 min) were 12.0 and 7.9, respectively. The software did not find any match between the spectra of these peaks and the other synthesised colourants. Small differences between the spectrum shapes may be caused by factors other than those due to the absorbance properties of compounds, e.g., the different conditions of temperature and reactants between the Shore's method and the sugar beet refinery. The method uses glycine as reagent whereas glutamic acid and aspartic acid are the most common amino acids in sugar beet juices. Moreover, the beet processing is a much more complex system of interactions. Many amino acids are present in beet juices and many sugar degradation products may be formed. Therefore, there are almost limitless possibilities for formation of melanoidins and more than one polymeric compound could be formed under specific conditions.

The spectra of the lower molecular weight compounds (1 and 0.4 kDa) were not similar to any of the

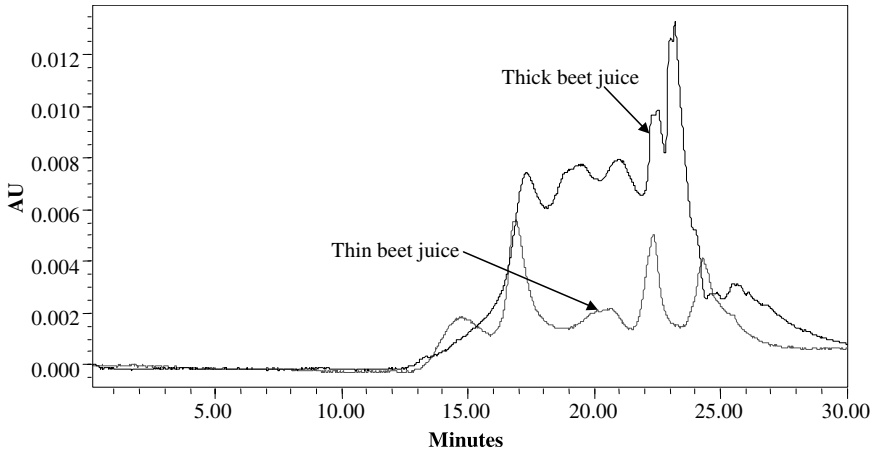

Fig. 7. Chromatograms at 420 nm, corresponding to the thin beet juice and thick beet juice.

synthesised colourants (Fig. 6(c)), with maxima at 252.8 and 275.2 nm, respectively. The lower molecular weight components are likely to be intermediate compounds of degradation reactions or they are likely to be composed of mixtures of different compounds. In this case, the UV/Vis absorption spectrum is a combination of the individual absorption peaks. In order to determine the type of colourants corresponding to the lower molecular weight peaks, the thin juice was spiked with synthetic melanoidin and synthetic HADPs. The results are also depicted in Fig. 6(a). The addition of HADPs produced a slight increase in the height and area of the lower molecular weight peaks. For this reason, it is likely that these groups are composed of HADPs. Moreover, the purification conditions favour the degradation reactions of reducing sugars to form HADPs. Other workers have also found that HADPs are compounds of much lower molecular weight than melanoidins (Bento & Sá, 1998b; Shore et al., 1984). The addition of both melanoidins and HADPs produced an increase in the area of higher molecular weight components. It is likely than HADPs were also coeluting with these groups of compounds. The presence of caramels was not tested because purification conditions do not favour the formation of caramels that are likely to be formed during evaporation and crystallisation steps.

The chromatogram extracted at 420 nm, corresponding to the thin juice, was compared with that corresponding to a thick juice. The colour of the thick juice (2780 IU) is considerably higher than that of the thin juice (1300 IU). As can be seen in Fig. 7, the absorbance of the coloured compounds increased after the evaporation stage, indicating increase in the concentration of chromophore groups. There is a sharp increase in the concentration of compounds with molecular weights of 3–1.5 kDa (retention times about 19–21 min). The concentration of lower molecular weight colourants (1–0.4 kDa) also increased substantially during evaporation. The Brix content is also much higher in the thick juice (77%) than in the thin juice (17%), a condition that favours the formation of melanoidins at subsequent stages.

5. Conclusions

This paper is a survey of the literature data concerning coloured compounds formed during sugar beet processing. The formation pathways of the different colourants are closely related. GPC chromatography, using a PDA as a detector, is the analytical method proposed to study the nature of coloured impurities and to characterise the stages of the process where colourants are generated. After the purification stage, colouring matter with high colour intensity and molecular weights up to 100 kDa are present in sugar beet thin juices. A PDA analysis indicated the presence of melanoidins and HADPs. The pH and temperature reached during the evaporation step, together with the higher content of dry matter, favour the increase in the concentration of coloured impurities in the thick juice. These compounds have a tendency to remain in sugar crystals during the crystallisation process, negatively influencing the white sugar quality. Due to their persistence through the sugar manufacture, it is necessary to find suitable methods for removing colourants before crystallisation. Conventional technologies, such as dioxide sulphur addition, retard the colouring reactions but they do not remove the coloured impurities. In future investigations, the influence of decolourisation processes on molecular weight distribution of colourants will be studied. However, it is important to apply GPC previously to know what kind of colourants will need to be eliminated before decolouring the juices.

Acknowledgements

The authors acknowledge the financial support of this research by the Ministerio of Ciencia y Tecnología

(MCyT, PPQ2000-0270-P4-02) and the company Azucarera Ebro, S.L. for its contribution to the development of the project. Mónica Coca thank the Ministerio de Educación, Cultura y Deporte for the financial support to her PhD grant.

References

Bento, L. S. M. (1995). Application of UV spectrophotometry to study sugar colorants throughout the refining process. In *Proceedings of the sugar industry technologists* (paper no. 683).

Bento, L. S. M., & Sá, S. (1998a). Study of high molecular weight compounds in sugar using gel-permeation chromatography with an evaporative light scattering detector. *Carbohydrate Polymers, 37*, 257–261.

Bento, L. S. M., & Sá, S. (1998b). Study of HMW compounds in sugar liquors from carbonation and ion-exchange resins using gel permeation chromatography with an evaporative light scattering detector. In *Proceedings of sugar processing research conference* (pp. 233–247).

Bento, L. S. M. (1999). Study of colour formation during carbonation in cane sugar refining using GPC with a ELS detector. In *Proceedings of the AVH Association* (pp. 23–27), Reims, France.

Cämmerer, B., & Kroh, L. W. (1995). Investigations of the influence of reaction conditions on the elementary composition of melanoidins. *Food Chemistry, 53*, 55–59.

Cämmerer, B., Jalyschkov, V., & Kroh, L. W. (2002). Carbohydrate structures as part of the melanoidin skeleton. *International Congress Series, 1245*, 269–273.

Clarke, M. A., Godshall, M. A., & SPRI group (1990). Polysaccharide and colorant: A progress report. In *Proceedings of the sugar processing research conference* (pp. 322–336), San Francisco, USA.

De Bruijn, J. M. (1986). Monosaccharides in alkaline medium: Isomerization, degradation and oligomerization. PhD Thesis, University of Technology, Delf.

De Bruijn, J. M., Bout-Diederen, M., & Huijbregts, S., (2002). Characterization of colorants in sugarbeet processing using GPC with ELSD and diode array detection. In *Proceedings of the sugar processing research conference* (pp. 279–291), New Orleans, USA.

Defaye, J., & García, J. M. (1995). The oligosaccharide components of caramel. *Zuckerindustrie, 120*, 700–703.

Godshall, M. A. (1992a). Isolation of a high molecular weight colorant from white beet sugar. In *Proceedings of the conference on sugar processing research* (pp. 312–322), New Orleans, USA.

Godshall, M. A., Clarke, M. A., Miranda, X. M., Blanco, R. S. (1992b). Comparison of refinery decolorization systems. In *Proceedings of the conference on sugar processing research* (pp. 281–305), New Orleans, USA.

Godshall, M. A. (1996). Recent progress in sugar colorants. In *Proceedings of the conference on sugar refining research* (pp. 262–305), New Orleans, USA.

Godshall, M. A., Vercellotti, J. R., & Triche, R. (2002). Comparison of cane and beet sugar macromolecules in processing. *International Sugar Journal, 104*, 228–233.

Gomyo, T., Kato, H., Udaka, K., Horikoshi, M., & Fujimaky, M. (1972). Chemical studies on melanoidins. Part III. Effects of heating on chemical properties of melanoidin prepared from glycine–xylose system. *Agricultural and Biological Chemistry, 36*, 125–132.

González, G., & García, M. T. (1996). Ammonia elimination from beet sugar factory condensate streams by a stripping-reabsorption system. *Zuckerindustrie, 121*, 721–726.

Guimaraes, C., Bento, L. S. M., & Mota, M. (1996). A study of sugar colourants through ion exchange and salt regeneration. *International Sugar Journal, 98*, 584–587.

Hayase, F., Kim, S. B., & Kato, H. (1986). Analyses of the chemical structures of melanoidins by ^{13}C NMR, ^{13}C and ^{15}N CP-MAS NMR spectrometry. *Agricultural and Biological Chemistry, 50*, 1951–1957.

Heitz, F. (1995). Les colorants en sucrerie. *Actualités techniques et industrielles*, 538–544.

ICUMSA methods book (1994). ICUMSA (Ed.), England.

Kato, H., & Tsuchida, H. (1981). Estimation of melanoidins structure by pyrolysis and oxidation. *Progress Food Nutritional Science, 5*, 147–156.

Kroh, L. W. (1994). Caramelisation in food and beverages. *Food Chemistry, 51*, 373–379.

Martins, S. I. F. S., Jongen, W. M. F., & Van Boekel, M. A. J. S. (2001). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science and Technology, 11*, 364–373.

Mersad, A., Lewandowski, R., Heyn, B., & Decloux, M. (2001). Formation des colorants de fabrication en sucrerie: Reconstitution au laboratoire et analyses par spectrométrie. *Industries Alimentaries et Agricoles, 118*, 42–51.

Rafik, M., Mas, A., Elharfi, A., & Schue, F. (1997). Decoloration de solutions sucrees par ultrafiltration sur une membrane a base de poly(organocyclophosphazene). *European Polymer Journal, 33*, 679–690.

Reinefeld, E., Bliesener, K. M., Brandes, E., & Borrass, V. (1982). Contribution to the knowledge of colour formation in sugar beet juices. *La sucrerie belge, 101*, 333–345.

Shore, M., Broughton, N. W., Dutton, J. V., & Sissons, A. (1984). Factors affecting white sugar colour. *Sugar Technology Reviews, 12*, 1–99.

Wedchida, B. L., & Kaputo, M. T. (1992). Melanoidins from glucose and glycine: Composition, characteristics and reactivity towards sulphide ion. *Food Chemistry, 43*, 359–367.

Yaylayan, V. A. (1997). Classification of the Maillard reaction: A conceptual approach. *Trends in Food Science and Technology, 8*, 13–18.

Yaylayan, V. A., & Kaminsky, E. (1998). Isolation and structural analysis of Maillard polymers: Caramel and melanoidin formation in glycine/glucose model system. *Food Chemistry, 63*, 25–31.