Specific Screening for Color Precursors and Colorants in Beet and Cane Sugar Liquors in Relation to Model Colorants Using Spectrofluorometry Evaluated by HPLC and Multiway Data Analysis

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A comparison was made of the fluorophores in beet thick juice and cane final evaporator syrup, which are comparable in the production of cane and beet sugar; that is, both represent the final stage of syrup concentration prior to crystallization of sugar. To further elucidate the nature of the color components in cane and beet syrup, a series of model colorants was also prepared, consisting of mildly alkaline-degraded fructose and glucose and two Maillard type colorants, glucose−glycine and glucose−lysine. Fluorescence excitation−emission landscapes resolved into individual fluorescent components with PARAFAC modeling were used as a screening method for colorants, and the method was validated with size exclusion chromatography using a diode array UV−vis detector. Fluorophores from the model colorants were mainly located at visible wavelengths. An overall similarity in chromatograms and absorption spectra of the four model colorant samples indicated that the formation of darker color was the distinguishing characteristic, rather than different reaction products. The fluorophores obtained from the beet and cane syrups consisted of color precursor amino acids in the UV wavelength region. Tryptophan was found in both beet and cane syrups. Tyrosine as a fluorophore was resolved in only beet syrup, reflecting the higher levels of amino acids in beet processing. In the visible wavelength region, cane syrup colorant fluorophores were situated at higher wavelengths than those of beet syrup, indicating formation of darker colorants. A higher level of invert sugar in cane processing compared to beet processing was suggested as a possible explanation for the darker colorants.

Keywords: *Sugar; nonenzymatic browning; size exclusion chromatography; fluorescence; chemometrics; PARAFAC*

INTRODUCTION

The development of color impurities during the processing of sugar is a common problem in both the cane and beet sugar industries. Nonenzymatic color formation in the sugar process is caused by thermal degradation reactions of sugars with or without the participation of amino acids, the former also being known as the Maillard reaction (*1*). The color arising during evaporation and crystallization stages and the color developed after the subsequent storage of the processed sugar have been reported to originate mainly from these colorant reactions (*2*, *3*). The reaction mechanisms of the degradation of reducing sugars are very complex with many intermediate products, which may react further to produce larger polymer systems (*4*). This makes it difficult to characterize the color composition of process samples. Often, model colorant systems focusing on one of the browning reactions at a time have been used to achieve more information (*5*, *6*).

Carpenter and Wall (*7*) showed in 1972 that commercial sugars exhibit fluorescence. It has also been reported that fluorescent components are formed from both Maillard (*8*) and mild alkaline fructose degradation reactions (*9*), but the contribution of model fluorophores to the fluorescence in sugar process samples has not been clearly elucidated due to the complexity of the measured fluorescence. Spectrofluorometry combined with chemometric methods has been used in recent years as a screening method to obtain chemical information from various beet sugar process samples (*10*). Bro (*11*) used a multiway decomposition method called PARAFAC (parallel factor analysis) as a form of "mathematical chromatography" to deconvolute pure excitation and emission spectra from three-way data consisting of the fluorescence excitation−emission landscapes of 268 sugar samples collected from a beet sugar factory during a campaign. Four fluorescent components were found to capture the variation in the fluorescence data, and two of them showed spectra with a close similarity to the pure fluorescence spectra of the amino acids tyrosine and tryptophan. Baunsgaard et al. (*12*) confirmed the findings by Bro (*11*) by combining HPLC

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separation and fluorescence measurements of beet thick juice, the final evaporated syrup prior to crystallization of sugar. PARAFAC was used to resolve seven fluorophores from fluorescence landscapes of HPLC fractions of thick juice. Apart from tyrosine and tryptophan, four of the fluorophores were identified as high molecular weight compounds, and three of them were suggested to be Maillard reaction polymers. A recent study by Baunsgaard et al. (*13*) estimated fluorophores from raw cane sugar samples with PARAFAC. Three fluorophores were found: an ultraviolet color precursor and two components showing colorant polymer characteristics, one of them related to the development of color on storage.

The found fluorophores in these beet and cane sugar studies are difficult to identify as individual structures, especially the components in the visible wavelength area, due to the evident continuity between the fluorophores, which characterize growing polymers differing slightly in their molecular weights. Because the polymers are suspected to be products of sugar degradation reactions, a comparison of colorants in model systems with colorants in process samples can be used to associate these polymers with specific kinds of colorant reactions without the need for a complete structural identification. Therefore, in the work presented here, fluorescence excitation−emission landscape measurements decomposed by the multiway PARAFAC model and HPLC size exclusion analyses using a UV−vis diode array detector were used to extract information of chromophores and fluorophores from sugar colorant solutions. Mildly alkaline degradation of glucose and fructose as well as Maillard reactions from glucose and glycine and glucose and lysine were selected as model systems. Similar analyses were performed on final evaporator syrups from beet and cane sugar processing, and the spectroscopic information obtained was used to compare the likeness of colorants from the sugar process with the "synthetic" colorants.

MATERIALS AND METHODS

Materials. Danisco Sugar A/S provided the beet thick juice. Sugar Processing Research Institute, Inc., collected cane final evaporator syrup at a sugar cane factory in Louisiana. Cane final evaporator syrup is the syrup that exits the final evaporator prior to crystallization. The Brix concentrations of beet thick juice and cane final evaporator syrup were 71 and 68 °Brix, respectively.

D-Fructose, D-glucose, L-lysine, and L-glycine were purchased from Sigma (St. Louis, MO). The model colorants were prepared from procedures described by Cookson et al. (*14*) with some modifications. The glucose−glycine and glucose−lysine colorants were prepared from a 2% glucose solution with 0.1 M glycine or lysine refluxing for 2 h after the pH had been adjusted to 8 with sodium hydroxide. The alkaline degradation products of fructose were prepared by refluxing a 2% fructose solution for 2.5 h with the initial pH level at 8. The alkaline degradation products of glucose were prepared using the same procedure as with fructose except that the solution refluxed for 4 h.

The model colorants were all made under initial mild alkaline conditions that would be likely to produce the type of colorants that would form in cane and beet processing during alkaline conditions. The pH level was not maintained at 8 during the course of the reactions but was allowed to change with the development of color to imitate the drop in pH, which is normally observed during the color development in the sugar streams (*15*). Both the glucose−glycine and the glucose−lysine browning solutions had a final pH of ∼7.8 when the reaction was stopped. The final pH of the glucose and fructose browning solutions was ∼4.5.

Color development in the four model colorant systems showed different reaction patterns. The alkaline glucose degradation reaction developed least color of all and had to be boiled for 4 h before a slight yellow color was visible. The alkaline fructose degradation, in comparison, developed color faster and had obtained a brown color after 2.5 h of boiling. The glucose−lysine solution very quickly developed a dark brown color, whereas color formation in the glucose−glycine solution was more moderate. However, the Maillard reactions developed color more quickly than in the alkaline fructose degradation reaction. Absorption at 420 nm was measured of the four colorant solutions to compare their degrees of color. Glucose−lysine, glucose−glycine, and alkaline-degraded fructose solutions of 4.30 g diluted in 30 mL of water had absorptions of 1.22, 0.13, and 0.08, respectively. The alkaline-degraded glucose solution measured without dilution had absorption of 0.08.

Size Exclusion HPLC Analysis. The HPLC method used was originally developed for beet thick juice fractionation (*12*). A Gilson system with a UV−vis diode array detector was equipped with a Waters 250 Ultrahydrogel column. The mobile phase consisted of 0.2 M ammonium buffer, pH 8.9, and water (20:80 v/v) at a flow rate of 0.5 mL/min. The model colorants were diluted 1:1.25 (v/v) and the factory samples 1:10 (v/v) with the mobile phase before a filtered aliquot of 100 μL was injected onto the column. The column dead time was determined as 12 min using Blue Dextran 2000. The adsorptive behavior of the column made it impossible to achieve reliable size calibration of the column using molecular weight standards. Instead, phenylalanine and tyrosine eluting at ∼25 min were used to establish the size exclusion range of the column.

Fluorescence Landscape Measurements. Fluorescence measurements were performed on a Perkin-Elmer LS50 B fluorescence spectrometer. The excitation range was 200−500 nm with an interval of 10 nm, and the emission range was 280−700 nm. Excitation and emission slit widths were set to 10 nm, and the scan speed was 1500 nm/min. The fluorescence concentration quenching levels of the different colorant samples were determined by measuring the fluorescence of dilution series of each sample diluted with ion-exchanged water. The alkaline-degraded glucose, alkaline-degraded fructose, glucose−glycine, and glucose−lysine colorant samples had to be diluted 1:2, 1:8, 1:4, and 1:12 (w/w), respectively, to get below the overall concentration quenching level. Beet thick juice and cane final evaporator syrup had to be diluted 1:200 and 1:320 (w/w), respectively. The fluorescence landscapes were then measured on five dilutions (1:2, w/w) in succession below the found quenching levels for each colorant sample to be used in the PARAFAC modeling. All samples were adjusted to pH 7.

Multivariate Data Analysis. The PARAFAC model (*16*, *17*) can be used to decompose a three-way structure consisting of excitation−emission fluorescence landscapes of several samples (samples × excitation wavelengths × emission wavelengths) into individual excitation and emission profiles of a number of underlying fluorophores. The third dimension in the three-way structure is decomposed as a concentration profile showing the contribution of the resolved fluorophores to each sample in the data set. Bro (*17*) provides a thorough tutorial of the PARAFAC model. For the PARAFAC model to be valid, wavelength areas in the fluorescence landscapes not conforming to true fluorescence, such as the Rayleigh scattering peaks and emission wavelengths less than excitation wavelengths, have to be treated as missing values. An example of a fluorescence landscape of the glucose browning solution diluted 1:2 is shown in Figure 1. The white areas in the plot are the missing data areas. All of the PARAFAC models were estimated under a non-negativity constraint to improve the interpretability of the resolved spectral profiles. Additionally, in some of the models the emission profiles were estimated under a unimodality constraint to avoid the interference of artificial extra peaks in the spectra due to too many missing variables in the data set. Implementation of the PARAFAC model is obtained from the N-way Toolbox for MATLAB (*18*).

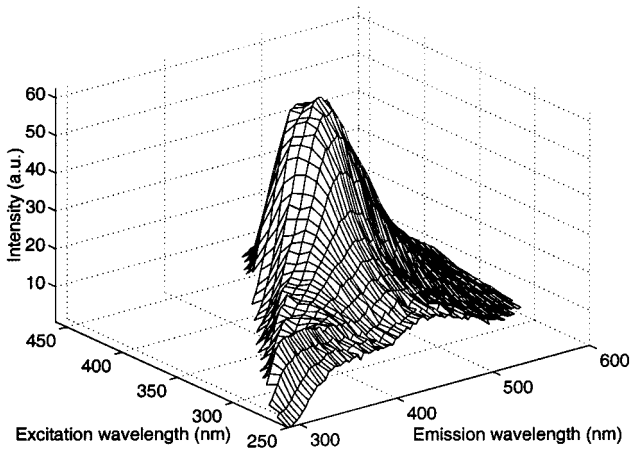

Figure 1. Fluorescence excitation−emission landscape of the alkaline-degraded glucose sample [1:2 (w/w) dilution] with excitation range (250−460 nm) and emission range (288−600 nm). White areas denote missing data due to measurement areas nonconforming to the PARAFAC model.

Principal component analysis (PCA) was used to compare resolved spectra from PARAFAC models (*19*). See Baunsgaard et al. (*13*) for details about software used in the data analyses.

RESULTS AND DISCUSSION

Fluorophores in Model Colorants and Factory Samples. The decomposition model PARAFAC was used to resolve fluorophores from three-way fluorescence data of the four model colorant samples and the beet and cane factory samples. Beet thick juice and cane final evaporator syrup represent comparatively the same stage in the sugar processes between evaporation and crystallization when considerable color already has been formed in the factory. The three-way data array for each sample consisted of excitation−emission landscape measurements of five dilutions, that is, 5 dilutions × 22 excitation wavelengths (240−460 nm, step 10 nm) × 105 emission wavelengths (288−600 nm, step 3 nm). Each data array was then modeled with PARAFAC with varying numbers of components. The optimal number of components was determined for each model by assessing the interpretability of the solution, comparing residual variation to the intrinsic noise level, and using core consistency diagnostic (*20*). The resolved components in a model are represented by estimated excitation and emission spectra and a dilution profile containing the concentration of each component in the five dilutions. In Figure 2 (rows 2−7) the excitation and emission

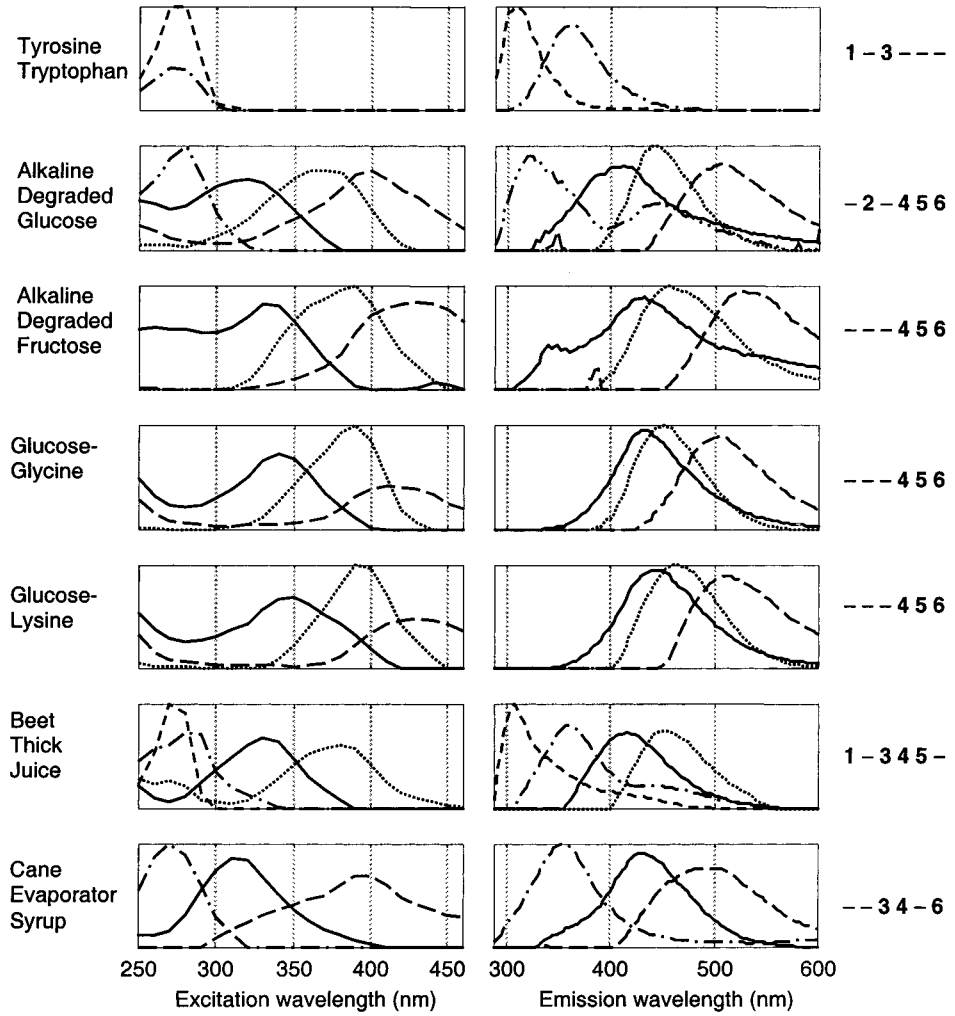

Figure 2. Estimated excitation and emission spectral profiles from PARAFAC models of the fluorescence data of both model colorants and factory samples. All spectra have been normalized to unit length. Excitation and emission spectra of pure tyrosine and tryptophan are shown in the top row. Numbers to the far right are assigned to emission spectra in different models that have comparable spectral profiles and wavelength maxima: 1 (- - -), 2 (- · · -), 3 (- · -), 4 (—), 5 (· · ·) and, 6 (- -). Components 1 and 3 are tyrosine and tryptophan, respectively, component 2 is an ultraviolet fluorophore, and components 4−6 are fluorophores in the visible wavelength area.

Table 1. Correlation Coefficient Matrix between the Emission Spectra from the Factory Samples and the Emission Spectra from the Model Colorant Samples and Pure Amino Acids As Given in Figure 2[a]

	Tyr1	Trp3	Glu2	Glu4	Glu5	Glu6	Fru4	Fru5	Fru6	Gly4	Gly5	Gly6	Lys4	Lys5	Lys6
Can3		0.95													
Can4					0.90		0.95			**0.98**			0.94		
Can6						0.90						0.92			
Bee1	**0.96**														
Bee3		**0.97**													
Bee4				0.94											
Bee5					**0.96**			**0.96**			**0.99**		0.93	**0.97**	

[a] Tyr, tyrosine; Trp, tryptophan; Glu, alkaline-degraded glucose; Fru, alkaline-degraded fructose; Gly, glucose−glycine; Lys, glucose−lysine; Bee, beet thick juice; Can, cane final evaporator syrup. Numbers correspond to numbers assigned to the emission spectra in Figure 2. Only coefficients ≥0.90 are displayed; **boldfaced** values > 0.95.

spectra from the PARAFAC models of model colorant samples and factory samples are shown. A few of the emission spectra display more than one peak, for example, the emission profile farthest to the left in the alkaline-degraded glucose model in row 2. These extra peaks indicate that the fluorescence data do not totally conform to trilinearity, which is a premise of the PARAFAC model (*11*). Dilutions of the samples also make it more difficult to estimate true spectral profiles because the dilution of colorants not only decreases the fluorescence intensity but also shifts the fluorescence toward lower wavelengths. In the top row in Figure 2 the excitation and emission spectra of pure tyrosine and tryptophan are displayed to help the assignment of these amino acid components because they have already been identified in beet thick juice (*12*). The numbers shown to the right of the emission spectra in Figure 2 are used to compare the estimated components in the various models. The same number indicates emission spectra from the different models that show approximately the same spectral profile and wavelength position. The emission spectra were compared because the excitation spectra of fluorophores are in general less specific than their emission spectra; for example, tyrosine and tryptophan have very similar excitation spectra. Components 1 and 3, which are assigned to tyrosine and tryptophan, respectively, are present in beet thick juice as expected from the previous results. Component 3 or the tryptophan component is also present in cane final evaporator syrup. Component 2 resolved from the alkaline-degraded glucose fluorescence data is an ultraviolet component, and components 4−6, which are situated in the visible wavelength area, are estimated in all of the model colorants with minor spectral differences. Beet thick juice does not fluoresce at the high wavelength of component 6, and cane final evaporator syrup lacks an equivalent to component 5.

It is difficult to compare the likeness of the components in the models only by visual inspection of the emission spectra. Consequently, a PCA was performed on all of the emission spectra in Figure 2. The three-dimensional score plot of principal component 1 (PC1) versus principal component 2 (PC2) and principal component 3 (PC3) is shown in Figure 3. Along PC1 the spectra are divided into a group containing the ultraviolet spectra and another group containing the visible spectra. PC3 divides tyrosine, component 2 from alkaline-degraded glucose, and component 1 from the beet thick juice model in their own group separately from the other ultraviolet components. Components 3 of cane final evaporator syrup and beet thick juice are close to pure tryptophan. The visible emission spectra in Figure 2 are also separated in subgroups, especially the group with components 6 along PC2. The score plot thus confirms the approximate assignments made visually in Figure

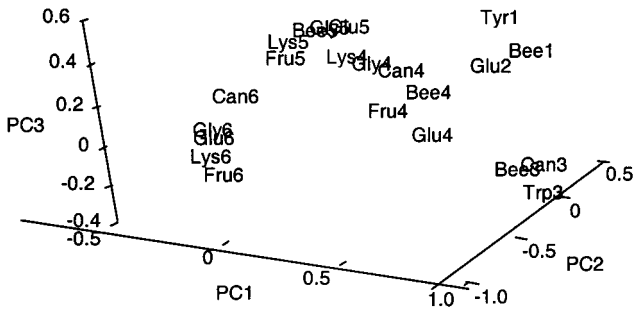

Figure 3. Three-dimensional PCA score plot of the emission spectra from Figure 2 showing PC1 versus PC2 versus PC3 explaining 48, 32, and 13% of the total variance, respectively. Tyr, tyrosine; Trp, tryptophan; Glu, alkaline-degraded glucose; Fru, alkaline-degraded fructose; Gly, glucose−glycine; Lys, glucose−lysine; Bee, beet thick juice; Can, cane final evaporator syrup. Numbers correspond to numbers assigned to each spectrum in Figure 2.

2. However, the PCA cannot be used in a more precise match of the individual fluorophores in the visible wavelength area. Another approach to compare the fluorophores is to calculate a correlation coefficient matrix between the emission spectra from the factory samples and all of the other emission spectra displayed in Figure 2. The correlation matrix is shown in Table 1. A visual estimation of the results indicated that spectra with correlation coefficients <0.90 were too different for a spectral likeness and that correlation coefficients >0.95 represented a spectral match between two components. Coefficients >0.95 are boldfaced in Table 1. The correlations of the ultraviolet components confirm the previous findings. In the visible wavelength area cane component 4 has a close likeness with glucose−glycine component 4 but is also quite similar to glucose−lysine and alkaline-degraded fructose components 4. In contrast, beet component 4 matches alkaline-degraded glucose component 4. Beet component 5 is correlated strongly to alkaline-degraded glucose, alkaline-degraded fructose, glucose−glycine, and glucose−lysine components 5, whereas cane component 6 is weakly correlated to alkaline-degraded glucose and glucose−glycine components 6. Alkaline-degraded glucose component 2 and alkaline-degraded fructose and glucose−lysine components 6 are all three without any reasonable correlations to the factory sample components.

Chromophores in Model Colorants and Factory Samples. The fluorophores in the model systems and factory samples show spectral profiles that make them potential colorants or color precursors, but these findings have to be supported by more information about the color composition in the various samples. Size exclusion chromatography using UV−vis diode array

detection has provided information about colorless constituents and colorants in complex sugar browning samples (*12*). The chromatograms of the four model colorants at 280 and 420 nm are shown in Figure 4. The chromatogram at 420 nm is used to establish the amount of color formed in the browning reactions because this is the wavelength normally employed by the sugar industries for color determination (*21*). All 420 nm chromatograms in Figure 4 have peaks in the beginning of the run between 16 and 21 min. The dead time of the column is 12 min, which implies that the colored components in all of the model solutions have molecular weights well below the 80 kDa capacity of the column. Results reported by Hofmann (*22*) support these findings. He showed that ultracentrifugation on colorants formed in a glucose−glycine model system produced only trace amounts of compounds with a molecular weight >30 kDa, whereas the majority of the compounds had a molecular weight <3 kDa. The chromatograms of the alkaline-degraded glucose and alkaline-degraded fructose samples at 280 nm in parts a and b, respectively, of Figure 4 resemble each other in the overall peak profile up to 30 min, but there are a few distinct peaks at 35.80 min (Figure 4a) and 32.12 min (Figure 4b) that differ. In Figure 5 the absorption spectra shown at 17.75, 18.87, 22.45, and 45.25 min have rather similar profiles in the two chromatograms. The spectra from the alkaline-degraded fructose chromatogram are shifted slightly toward higher wavelengths, which implies a more advanced color formation in the alkaline-degraded fructose sample, which is also supported by the higher absorption intensities. Fructose is known to degrade more quickly than glucose (*23*). Absorption spectra of peaks that differ in the two chromatograms are also displayed in Figure 5a,b. The glucose−glycine and glucose−lysine chromatograms at 280 nm in Figure 4c,d also show a similar peak pattern except for a lower resolution in the glucose−lysine chromatogram. The absorption spectra at the comparative peaks in Figure 5c,d display a difference in the spectral profiles where the spectra from the glucose−lysine sample have maxima at higher wavelengths, which along with the higher absorption intensities and lower resolution indicate that lysine forms more color with higher molecular weights than glycine. According to Parker and Williams (*24*) darker colorants are formed in glucose−lysine systems due to the cross-linking of lysine. A comparison of all four model colorant chromatograms reveals that the comparative peaks at ∼19 min have a spectral maximum around 265 nm related to a well-known but unidentified color precursor (*23*). The spectra of the Maillard colorants (Figure 5c,d) suggest that this color precursors enter into a colorant molecule with a partly intact chromophore even during increased browning.

Size exclusion chromatography was also performed on factory samples from beet and cane sugar processing. Chromatograms of the two factory samples are shown in Figure 6. The absorption intensity level is 4 times higher for the cane sample, which would explain the small peaks at higher retention times in the 420 nm chromatogram compared to the beet sample. Otherwise, the 420 nm chromatograms of the two factory samples have the same peak pattern as the model colorants. The 280 nm chromatograms of the two factory samples display a close resemblance in the peak distribution, which is supported by several peaks showing quite

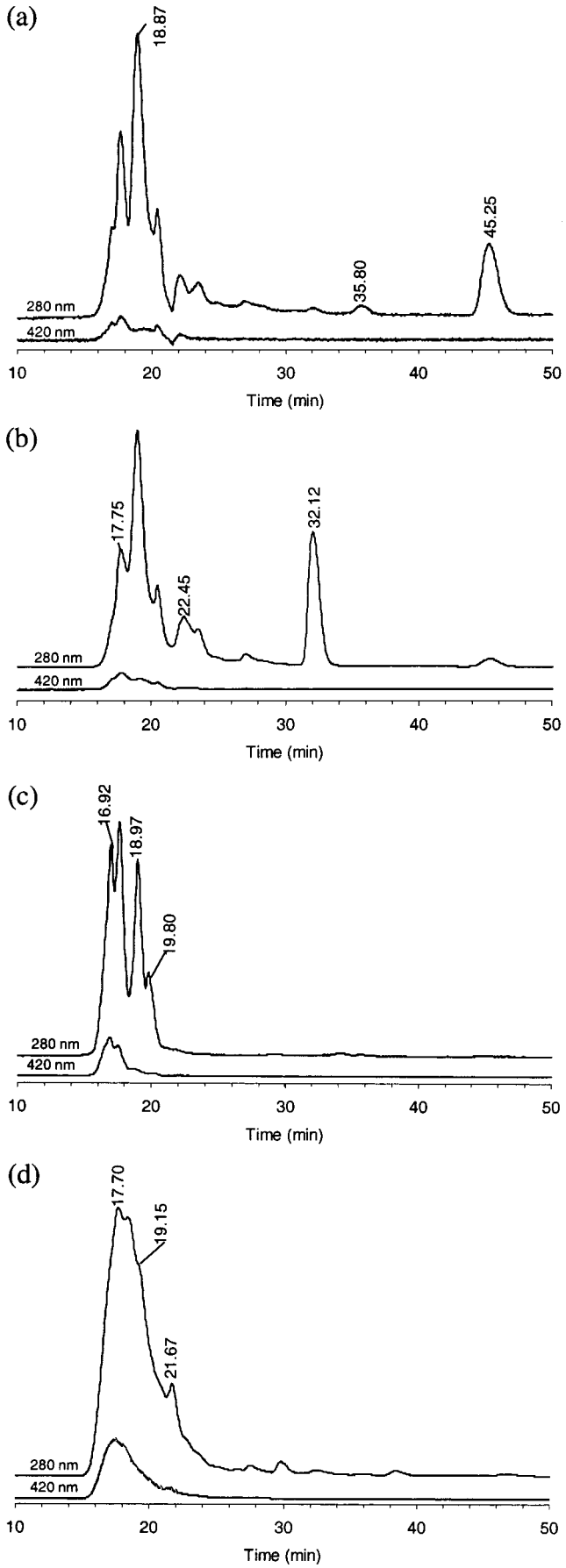

Figure 4. Chromatograms at 280 and 420 nm of the model colorants diluted 1:1.25 (v/v): (a) alkaline-degraded glucose; (b) alkaline-degraded fructose; (c) glucose−glycine; (d) glucose−lysine.

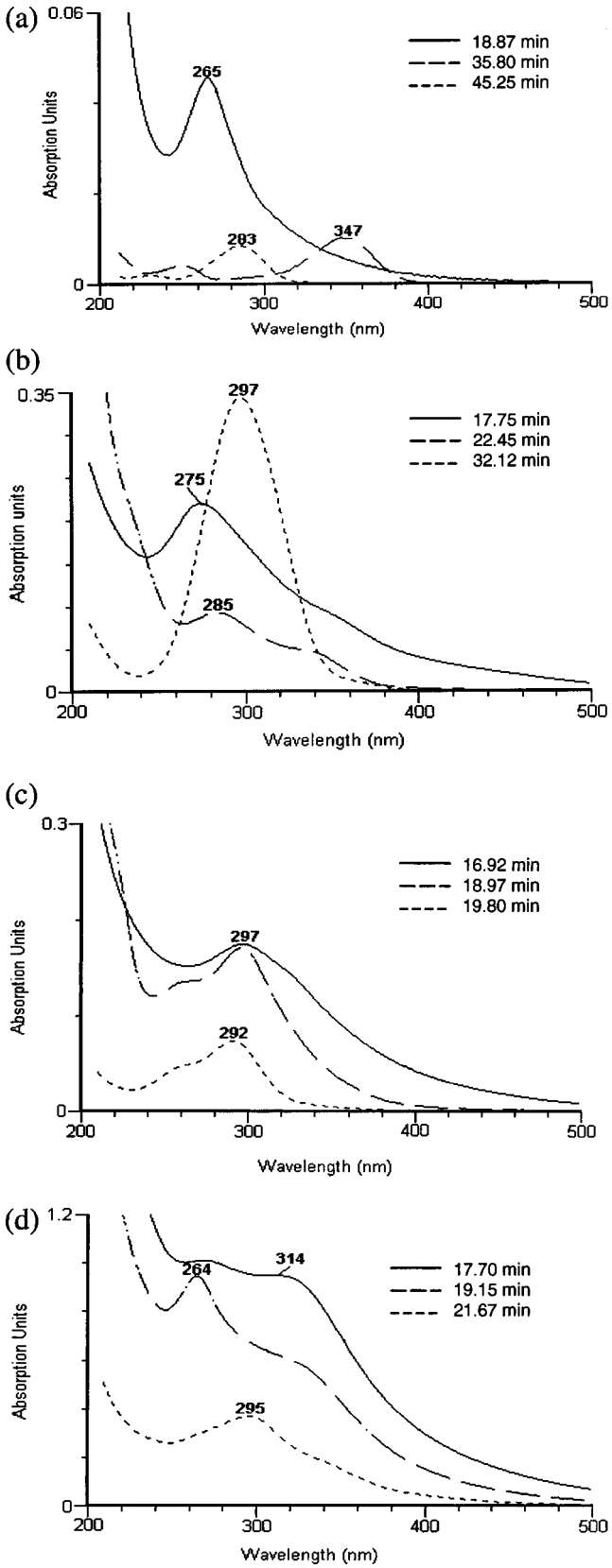

Figure 5. Diode array absorption spectra of the model colorants corresponding to peaks marked in the chromatograms in Figure 4: (a) alkaline-degraded glucose; (b) alkaline-degraded fructose; (c) glucose−glycine; (d) glucose−lysine. Bold numbers denote wavelengths of absorption maxima of the spectra.

similar absorption spectra. In Figure 7 a,b the spectra from peaks at 17.28, 28.35, and 41.20 min in the beet

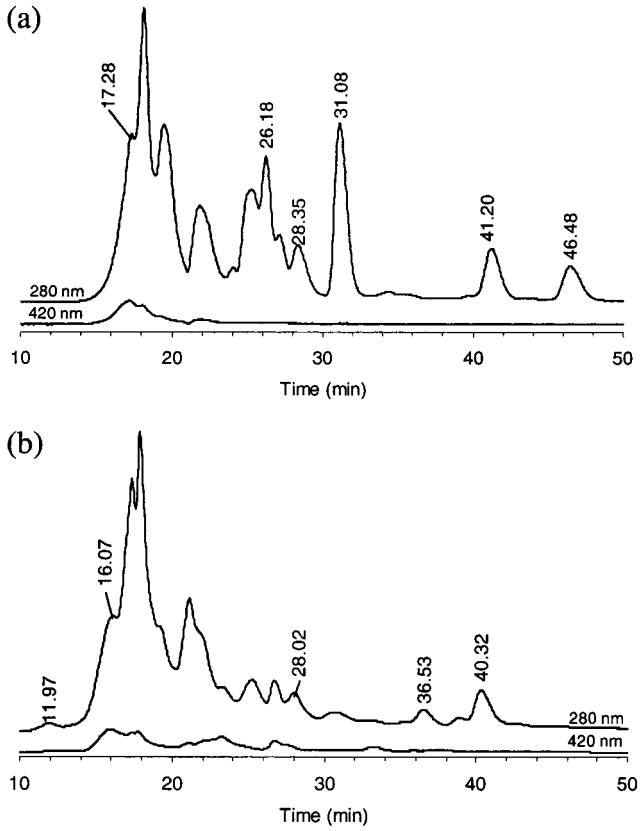

Figure 6. Chromatograms at 280 and 420 nm of the factory samples diluted 1:10 (v/v): (a) beet thick juice; (b) cane final evaporator syrup.

thick juice chromatogram resemble spectra from comparative peaks at 16.07, 28.02, and 40.32 min in the cane final evaporator syrup chromatogram. The two spectra at 28.35 and 28.02 min have a retention time and a spectral form that was previously identified as tryptophan (*12*). Tyrosine was identified as a peak at ~25 min in the beet chromatogram as well (spectrum not shown), but the amino acid was not found in the cane chromatogram. Some spectra from different chromophores in the two chromatograms are shown in Figure 7c,d. Beet thick juice has several compounds with absorption maxima around 265 nm, which are well separated late on the column and are consequently not related to the color precursor at 265 nm in the model colorants. The cane final evaporator syrup chromatogram in Figure 6b has a small peak at 11.97 min that is not retained on the column, and the molecular weight has to be >80 kDa. The beet thick juice chromatogram beginning at 15.5 min does not contain this early peak but has the same chromatogram course as the model colorants. Bento (*25*) separated several groups of colorants from cane and beet factory samples on a sugar crystal column, and a colorant group eluted with an alkaline mobile phase had a UV−vis diode array spectrum very close to the 11.97 min spectrum in Figure 7d. Bento found this group of colorants in cane syrup but not in beet syrup, Maillard reaction products, or alkaline-degraded fructose products, which is consistent with our results. The 11.97 min peak obviously represents a category of colorants that are of very high molecular weight but are not formed in the sugar degradation reactions. These high molecular weight colorants probably originate in the sugarcane plant (*3*).

Colorant and Color Precursor Characteristics in Cane and Beet Liquor Samples. Tyrosine and

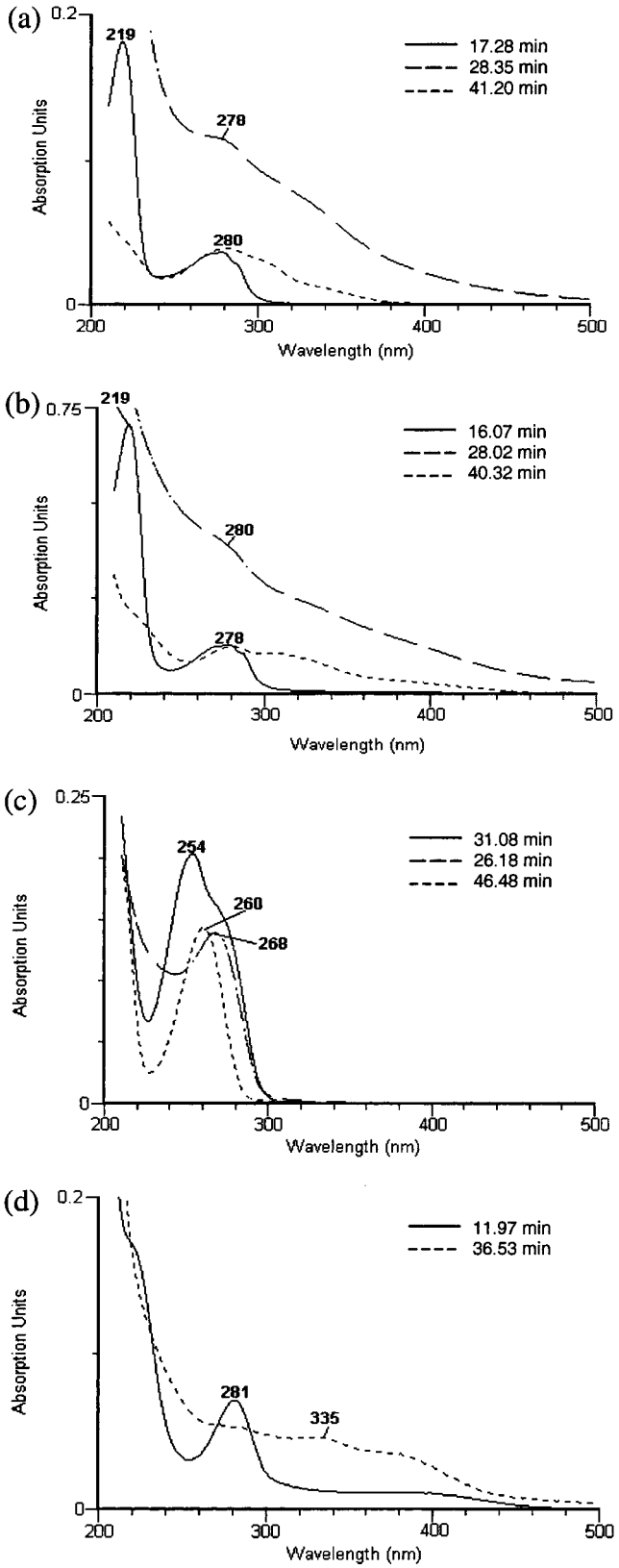

Figure 7. Diode array absorption spectra of the factory samples corresponding to peaks marked in the chromatograms in Figure 6: comparison of similar (a and b) as well as dissimilar (c and d) spectra of beet thick juice and cane final evaporator syrup, respectively. Bold numbers denote wavelengths of absorption maxima of the spectra.

tryptophan have repeatedly been resolved as fluorophores in beet factory samples (*11*, *12*). The separation of a tryptophan peak in the HPLC chromatogram of the cane final evaporator syrup and the close spectral likeness of the cane component 3 with beet component 3 and tryptophan from the PARAFAC models (Figures 2 and 3) confirm that tryptophan is found in cane final evaporator syrup in this study. A tryptophan-like component was also resolved in a PARAFAC model of fluorescence landscapes from raw cane sugars (*13*). Tyrosine, identified as beet component 1, was not resolved as a component in the cane model in Figure 2 or found in the cane chromatogram. A PARAFAC model made on the fluorescence data of tyrosine, tryptophan, and the two factory samples together showed a small contribution of the tyrosine component in the resolved cane sample concentration profile (plot not shown). This suggests that tyrosine is present in cane final evaporator syrup but in too low a concentration to be resolved in the PARAFAC model. There are higher amounts of amino acids in beet juice than in cane juice (*1*), which would explain the difficulty in resolving tyrosine from cane factory samples. Furthermore, a previous study has shown that the fluorescence properties of tryptophan as a functional group are better preserved than those of tyrosine in high molecular weight colorants (*12*). Amino acids are known color precursors in the sugar process samples, and as the precursors are important indicators of potential color, for example in the storage of the produced sugar (*13*), tryptophan is a promising fluorophore marker of Maillard color formation in both cane and beet sugar processing.

The ultraviolet component 2 from the alkaline-degraded glucose sample and a corresponding ultraviolet shoulder on the emission spectra of component 4 of the alkaline-degraded fructose sample (Figure 2) are suggested to be catechols formed in the base-catalyzed degradation of glucose and fructose (*26*). Catechols are fluorescent with excitation and emission maxima at approximately 280 and 320 nm, which are consistent with the spectral characteristics of component 2.

The correlation coefficients between the model colorant fluorophores and the factory colorant fluorophores in Table 1 show that there is a spectral comparability for some of the visible components. The cane fluorophores resemble the model colorant fluorophores at higher wavelengths more than the beet fluorophores. The two color components at the highest wavelengths, Fru6 and Lys6, are poorly matched to cane component 6, but alkaline-degraded glucose and glucose−glycine components 6 show a reasonable correlation with cane component 6. The beet fluorophores are not represented in that higher wavelength area as shown in Figure 2, but there are good correlations of beet component 5 with component 5 of all the model colorants in Table 1 at lower wavelengths.

The UV−vis diode array spectra of higher molecular weight colorants (16−20 min) in the model systems and factory samples (Figures 5a−d and 7a,b) display almost the same spectral behaviors, and it is not possible to confirm the differences between cane and beet colorants in the fluorophores. Fluorescence is a more specific and sensitive sensor than UV−vis absorption, and it is very likely that fluorophore structures are easier to distinguish than chromophore structures in colorants.

Overall, the chromophores and the fluorophores in the four model colorant samples display very similar characteristics, and the color development in the various reactions seems to be more dependent on the reactivity and ratio of the reactants, and thus on the formation of

darker color, than on differences in the reaction products. The main difference found between the colorants in the beet thick juice and cane final syrup was a formation of darker colorants in cane sugar processing. Cane juice contains much more invert sugar than beet juice (*1*). The higher amounts of invert sugars in cane juice would favor alkaline degradation of invert sugar and could be a possible explanation for the formation of the darker colorants in cane sugar processing. However, the colored material in cane and beet sugar processing is complex and, to date, is not completely resolved. There are other sources of colorant that have to be considered. For example, enzymatic browning reactions involving polyphenolics occur when the plant cells are disrupted during the extraction of juice from both sugarcane and sugarbeet, and they may be part of color measured at later processing steps (*27*). Examination of the influence of such types of colorants on the fluorescence measured in beet and cane sugar processing still needs to be undertaken.

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