Progress in Beet Sugar Colorant Research

M. A. Godshall, M. A. Clarke, C. D. Dooley and R.S. Blanco
Sugar Processing Research Institute, Inc.
P.O. Box 19687
New Orleans, LA 70179

ABSTRACT

Beet sugar colorant is composed of a mixture of colorant types, distributed between the surface of the crystal and the inner crystal structure. This study describes the distribution of phenolic, amine and high molecular weight (HMW) components of colorant in several white beet sugars and some of the changes that may occur on storage. Very high molecular weight (VHMW) colorant (greater than 12,000 daltons) can represent a significant proportion of the colorant, especially after color development on storage. Gel permeation chromatography (GPC) was used to examine the VHMW colorant, most of which fell in the range of 12,000 to 30,000 daltons in white sugars and 30,000 to 50,000 in molasses.

Additional Key Words: *Beta vulgaris*, sucrose, sugarbeet processing, amino nitrogen, phenolics.

Much of the white beet sugar produced in the United States, as elsewhere, is manufactured within a fairly short period of time, corresponding to the harvesting season. Even in places where the beets are deep-frozen for an extended period after the harvest, once the beets are defrosted, the production of sugar must proceed without delay. This means that a large proportion of the sugar produced will

be stored for months before it is sold or used. Although the sugar, as produced, usually is of low color (10-20 ICU units), the presence of trace organic and inorganic constituents, under certain conditions, can lead to the development of color.

Sugar colorant has been studied for over a century (Broughton, et al., 1987a). Questions about sugar color include: The type of colorant, origin of colorant, location of color in the crystal, and physical characteristics of the colorant, such as molecular weight, charge and sensitivity to pH changes.

Beet sugar colorant includes several chemical types: Phenolic, caramel, melanoidin, melanin and alkaline degradation products, summarized in Table 1. Color originates either from the sugarbeet plant or during processing. Beet juice contains many colorless precursors, such as phenolics and amino acids, that react during processing to form color. Melanoidins result from the "browning reaction" of amino acids with reducing sugars, and melanins result from the reaction of phenolic acids with amino acids or proteins (Godshall and Roberts, 1983), and are also described as the black enzymatic oxidation products of phenolics (Madsen, et al., 1978/79; Godshall, et al., 1987). Degradation reactions of sucrose during processing produce caramel and alkaline degradation products.

Sugarbeet phenolics and flavonoids have been extensively investigated in relation to the formation of enzymatic and non-enzymatic color (Gross and Coombs, 1976; Winstrom-Olsen, et al., 1979; Winstrom-Olsen, 1981; Rhinefeld, et al., 1984; Vukov, et al., 1984; Maurandi, 1988) and are reported to be a major factor in crystal color by several European workers (Madsen, et al., 1978). The purple-

Table 1. Types of sugar colorants.

Phenolic	Colorless to light yellow precursors; darken at high pH; auto-oxidize to form yellow and brown polymers; react with polyphenol oxidase to form light yellow to dark brown colorants.
Caramel	Thermal degradation products of sucrose
Alkaline degradation	Alkaline degradation products of sugars; similar to caramels; fructose is most reactive.
Melanoidin	Reaction products of amino acids with reducing sugars.
Melanin	Reaction procucts of amino acids with phenolics; very dark; also, black enzymatic oxidation products of phenolics.

black color of beet juice is due to the enzymatic reaction of phenolics, especially catecholamines (Winstrom-Olsen, et al., 1979).

No single test exists to differentiate the various types of colorants, which have different coloring properties, ease of removal, and tendency to enter the crystal on crystallization. Russian workers have proposed measuring the optical density at various wavelengths — 250 nm for alkaline degradation products, 282 nm for caramelization products, and 300 nm for melanoidins (Huda, et al., 1980; Bugaenko and Sapronov, 1981).

Australian workers (Smith and Gregory, 1971) working with cane sugar, showed that the various colorant types have different degrees of pH sensitivity, and developed a measure of the indicator value (I.V.), which is the ratio of 420 nm color at pH 9 to that at pH 4. Indicator values of colorant types are summarized in Table 2. A group of simple tests was also developed for phenolic and factory-derived colorants, which was adopted and modified by Sugar Processing Research, Inc., (S.P.R.I.) for use with cane sugar products (Clarke, et al.,1985, 1986). These tests are here applied to determine the chemical composition of beet sugar colorant.

The molecular weight distribution also is of interest in a study of sugar beet colorant, especially the colorant that is greater than 12,000 daltons, or very high molecular weight (VHMW) colorant.

MATERIALS AND METHODS

White and raw beet sugars and molasses were obtained from sponsoring companies of S.P.R.I. for this study.

White sugars were washed with ethanol/water mixtures, as described by Shore, et al., (1984) to remove 1.5-2.5% of the crystal. Alternately, crystals were washed with 100% methanol. Phenolics and amino nitrogen were measured by the procedures described by Clarke, et al. (1986).

The high molecular weight colorants were determined by comparing the normal ICU color (filtered through a 0.45μ filter) to that filtered through a 20,000 MW cut-off membrane ultrafilter.

Table 2. Range of Indicator values (I.V.) for sugar colorants.

Colorant Type	Indicator Value
Melanoidin	1.0 - 1.2
Caramel	1.0 - 1.5
Alkaline degradation products of fructose	1.5 - 3.2
Phenolics and Flavonoids	5.0 - 14

VHMW colorant was physically isolated from the sugar by dialysis through regenerated cellulose bags with a nominal cut-off of 12,000 daltons. The material isolated (the tenate) was separated by low pressure gel permeation chromatography (GPC) on a 2.6 cm × 70 cm glass column filled with Sephacryl S-500 to a depth of 38-40 cm, using water at a flow rate of 2.8 ml/min as the eluent (Godshall, et al., 1987). Peaks were detected by their UV absorbance at 214 nm. Sephacryl S-500 has a separation range of 20,000 to 20,000,000 daltons. Columns were calibrated with Pharmacia dextrans.

RESULTS AND DISCUSSION

Distribution of Color and Color Precursors in the Crystal. Table 3 shows the phenolics, amino nitrogen and color in the crystal as a percent of the total for ten white beet sugars. Color within the crystal averaged 47.7% of the total color. This value is considerably lower than the 75-80% figure reported for U.K. white sugars (Shore, et al., 1984) after 2-20% of the crystal was washed away, but is in better agreement with a subsequent study by the same workers using a larger sample that showed 57% of color was inside the crystal (Broughton, et al., 1988).

Table 3. Distribution of color and colorant precursors in white beet sugars (1.5-2.5% of crystal surface washed off).

	% Inside Crystal		
Sugar	Phenolics	Amino N	Color
1	22.0	70.2	37.5
2	35.5	74.2	20.7
3	25.0	19.0	85.2
4	66.7	16.2	71.8
5	63.0	67.3	27.1
6	60.7	21.8	40.0
7	92.9	19.9	59.2
8	59.1	19.1	64.9
9	62.4	36.8	12.0
10	57.5	19.6	58.1

Summary of distribution of color and color precursors inside crystals of production white sugars:

Phenolics	Range: 22.0 - 92.9%	Avg. 56.5%
Amino N	Range: 16.2 - 74.2%	Avg. 36.4%
Color	Range: 12.0 - 85.2%	Avg. 47.7%

A significant proportion of the colorant precursors are found in the film around the crystal — 63.7% of the total amino nitrogen and 43.5% of the total phenolics, on average. In the presence of oxygen, moisture and heat, these will react to form storage colorant.

Table 4 shows the whole and washed color of 18 sugars (including sugars from Table 3 and 8 others). The average percentage of color inside the crystal for this set was 46.0%; thus, about 54% of the total color was in the film surrounding the crystal. Sugars with a percentage of color significantly above 50% in the film will have a greater tendency to develop color over time. The sugars in this set with greater than 50% of the total color in the film had begun to show color development on storage in the laboratory.

Very High Molecular Weight Colorant. In this study, VHMW colorant has been defined as colorant with a molecular weight greater than 12,000 daltons. The VHMW colorant was studied in two ways

Table 4. Whole and washed color of beet white sugars.

Whole color	Washed color	% Color in crystal
7	2	28.6
8	3	37.5
10	7	40.0
18	5.5	30.6
23	7	30.4
24	14	58.3
24	7.5	31.3
25	18	72.0
25	3	12.0
27	23	85.2
29	6	20.7
30	20	66.7
33	11	33.3
37	24	64.9
39	28	71.8
43	25	58.1
59	16	27.2
98	58	59.2

Whole color range: 7-98; Avg. = 31
Washed color range: 2-58; Avg. = 15
Correlation of whole to washed color: R = 0.87
Average % color in crystal = 46.0%

— by membrane ultrafiltration, which gives the distribution of color above and below 20,000 daltons; and by GPC of the nondiazlyzable material greater than 12,000 daltons, separated into its component fractions on Sephacryl S-500 columns.

The results of ultrafiltration are shown in Table 5A for several beet raws and in Table 5B for several beet white sugars. The raw sugars had significant quantities of colorant >20,000 MW, and the proportion increased as the whole color decreased, indicating a greater tendency of VHMW color to be included in the crystals of low color raw sugars. Cane raw sugar shows a similar tendency (Godshall, et al., 1987).

For white sugars, the findings were more variable (Table 5B). The VHMW colorant fraction in beet sugars has not received much attention until recently. British workers found that in well washed, high quality U.K. sugar, the maximum molecular weight of colorant was about 5000 daltons (Shore et al., 1984) and was preferentially occluded within the crystal. They later reported finding colorants up to 40,000 daltons (Broughton, et al., 1987b). This study shows that some white sugars do have color >20,000 daltons but also confirms that not all beet white sugars have VHMW colorant.

Dialysis is a way to isolate colorant physically from sugar. In addition to colorant, the nondialyzable material (the tenate) contains the other high molecular weight components present, including polysaccharides, proteins, colloidal salts and "turbidity." Table 6 shows typical

Table 5A. Distribution of high molecular weight color in beet raw sugars.

Sugar	Whole color	Color <20,000	% Color >20,000
High raw, U.S	1666	1095	34.3
Raw, Europe	445	280	37.1
Raw, Europe	207	44	78.7
Raw, Europe	171	42	75.4

Table 5B. Distribution of high molecular weight color in beet white sugars.

Sugar	Whole color	Color <20,000	% Color >20,000
White, U.S. (Good)	17	18	0
White, Chile (Good)	23	24	0
White, Chile (Poor)	29	15	48.3
White, U.S. (Poor)	59	13	78.0

quantities of nondialyzable material in several types of beet products. The total polysaccharide values are also given to show that a major portion of the tenate is non-polysaccharide. This is in marked contrast to cane sugars, in which the polysaccharide in raw sugar represents at least 30-50% of the total tenate and increases to 80-95% of the total in white sugars (Godshall, et al., 1989). Polysaccharide levels are very low in white beet sugars. It is evident that much of the polysaccharide is eliminated to the molasses.

The colorant isolated by dialysis and separated by GPC is shown in Figures 1-3, for white sugar, raw sugar and molasses. These figures represent the chromatographic profile of colorants, detected at 214 nm, with molecular weight greater than 12,000 daltons.

Figure 1 is representative of white beet sugars: The major VHMW colorant elutes as a single, sharp peak in about 85 min, is in the range of 12,000-20,000 daltons, and has a clear yellow color. This peak was present in the VHMW colorant profiles of 4 white sugars, differing only in relative peak height and, therefore, concentration. The other peak (MW approximately 800,000, eluting in about 38 min) was buff colored. This peak was more variable and did not always appear in white sugar colorant profiles.

The profile shown in Figure 1 was from a sugar that had developed color on storage (discussed below). Chromatograms of sugars without storage color development had less of the buff-colored 800,000 MW peak.

The profile of colorant in molasses (Figure 3) was also fairly typical. In all the molasses tenates we have examined so far, the major pigment shows a broad peak in the 30-50,000 dalton range. The colorants in molasses represent colorants that have been eliminated in process.

A Case Study. The detailed chemical composition of a U.S. sugar that developed severe color on storage is presented here as a case study in the application and interpretation of the various tests discussed in this paper.

Table 6. Typical quantities of non-dialyzable material in beet products.

Product	Tenate (ppm)	Polysaccharide (ppm)
Molasses	6,000 - 15,000	4447 - 6911
Raw Sugar	342	54
White, Europe	145	9
High Color White	197	27
Low Color White	97	None detected

Table 7 lists the composition of the whole and washed sugar, and Table 8 shows the effect of temperature (40° C for 24 hours) on the color.

This sugar was unusual in that most of the color occurred in the film surrounding the crystal, and most of the color was VHMW, as confirmed by both ultrafiltration and dialysis. The tenate (material over 12,000 MW) contained approximately 84% of the total color. The low I.V. in both whole and washed color indicated that the phenolics were not a major factor in the color of this sugar. This means that the colorant, which developed on storage, is likely to be one of the process colorants, such as caramel or melanoidin.

Significant color increase occurred upon heat treatment, with colorant below 20,000 MW doubling while the VHMW color increased 24%. The presence of color precursors (amino nitrogen and

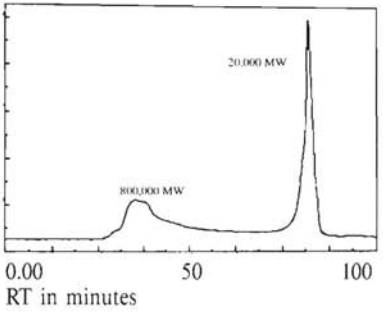

Figure 1. Profile of VHMW colorant from white beet sugar by GPC. (Represents colorant from 25 g of sugar.)

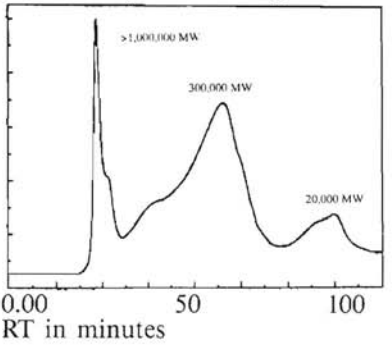

Figure 2. Profile of VHMW colorant from raw beet sugar by GPC. (Represents colorant from 20 g of raw sugar.)

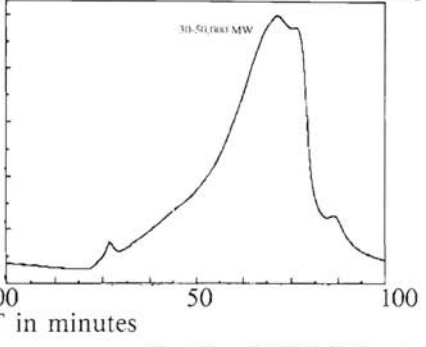

Figure 3. Profile of VHMW colorant from beet molasses by GPC. (Represents colorant from 1.0 gram of molasses.)

phenolics) and catalysts (ash and moisture) in the film contributed to the formation of color. Common ash constituents, such as sodium and magnesium, have been implicated in increasing color formation under certain conditions (Carpenter and Roberts, 1975; Richards, 1988).

CONCLUSIONS

This study of the chemical nature of colorants in beet sugars, and their behavior under storage conditions, is in the preliminary stages, but it appears that the tests applied to cane sugar will be useful for beet sugars also. The colorant in the film around the crystal is an important contributor to development of color on storage, especially at higher temperatures. Temperature is a major determinant of color development in stored sugar, but color precursors must also be present to cause darkening.

Table 7. Chemical characterization of colorant in a sugar with an increase in color on storage.

Parameter	Whole	Washed	% in Film	% in Crystal
Color, Total	59	16	72.9	27.1
Color, <12-20,000	14	14	0-12.5	87.5-100
Color, >12-20,000	46	2	87.5-95.7	4.3-12.5
Amino N (ppm)	12	8	33.3	67.7
Phenolics (ppm)	27	17	37.0	63.0
Ash (%)	.0146	.0059	59.6	40.4
Indicator Value	1.35	1.65	—	—
Polysaccharide (ppm)	27	Not done	—	—
Nondialyzable (ppm)	197	Not done	—	—

Table 8. Effect of heat treatment on color development in a white beet sugar.

Parameter	Before heat	After heat	% Increase
Total color	59	83	40.7
Color, <20,000	13	26	100
Color, >20,000	46	57	23.9

This study has shown that a significant quantity of the color and color precursors that cause darkening on storage are present in the syrup film that surrounds the crystal, rather than inside the crystal.

Several of the sugars in this study had VHMW colorant, which also may increase on the application of heat due to the continued reaction of colorants already present and formation of new colorant from the precursors.

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