Determination and Classification of Added Caramel Color in Adulterated Acerola Juice Formulations

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Four batches of acerola juice concentrate were suspected of being adulterated with caramel color from a specific caramel color raw material. Analyses of both the caramel color raw material and the finished acerola juice products were conducted. The detection of the caramel marker compounds 5-HMF and 4-MeI in the raw material was consistent with its classification as a class IV caramel color. A distinctive four-peak sequence in the HPLC−UV profile of the raw material was used to match the caramel color to its commercial source. The same four-peak sequence was used to detect and quantify added caramel color in the finished acerola juice products. This is the first report of an adulterated acerola juice product and the first report in which added caramel color has been matched to its commercial source.

Keywords: *Caramel color; HPLC profiling; acerola; juice adulteration*

INTRODUCTION

Fruit juice adulteration is estimated to be at least a billion dollar per year "enterprise" in the United States alone (Brause, 1995). Adulterated forms of orange juice (Petrus et al., 1984; Brause et al., 1984), apple juice (Coppola, 1984), and cranberry juice (Hong and Wrolstad, 1986) have all been detected on the U.S. market. The incentive for adulteration is simple economics: selling products which demand premium prices while reducing or eliminating the overhead in bringing the authentic products to the market.

Acerola is a small, red fruit which is native to the West Indies, but is also grown in South and Central America, Florida, and Texas (Leung and Foster, 1996). Acerola is the richest known natural source of vitamin C, with a content of 1000−4500 mg of vitamin C per 100 g of fruit or up to 90 times the vitamin C content of oranges (Leung and Foster, 1996). Methods for commercial production of acerola were developed in the 1950s (Ag and Food News, 1954), and processing of the fruit into a dry juice concentrate was patented in the U.S. in 1961 (Morse, 1961). Authentic acerola fruit juice concentrate is frequently used to fortify fruit juices and other nutritional products with "natural vitamin C". Despite the fact that acerola juice has been commercialized for more than 30 years, there are no reports of acerola juice adulteration in the literature. The present paper reports on a case of acerola juice adulteration in which caramel color was used to color the adulterated acerola juice product.

The detection and quantification of added caramel color in finished products is not a trivial analytical problem. Caramel color is produced by the controlled heating of carbohydrates and comprises a complex mixture ranging from low molecular weight compounds to colloidal aggregates. Despite the fact that significant progress has recently been made in the chemical characterization of caramel color (Licht et al., 1992a,b; Myers and Howell, 1992), the major components of caramel color remain unidentified. Several minor components of caramel color have been identified and are considered "caramel markers" (Licht et al., 1992a,b), but these components are often too dilute in finished products. The detection of added caramel color in finished products is further complicated by the fact that "caramelization" can occur during the heat processing of carbohydrate-containing products, producing some of the same compounds which are produced during the manufacture of caramel color. Thus, the presence of caramel markers in the finished product may not prove that caramel color was added to the product.

Historically, chemists relied on wet chemical tests for the detection of caramel color in finished products (Tomasik et al., 1989). These tests typically involved color formation as a result of reaction between a component of caramel color and a reagent and were generally qualitative and nonspecific for the type of caramel color. More recently, size exclusion chromatography has been used for the detection of caramel color in food ingredients (Frischenschlager et al., 1982; Hellwig et al., 1981) and breads (Magrian et al., 1985; Rabe et al., 1988), and pyrolysis GC−MS has been used for the detection of caramel color in vinegar, brandy, and soft drinks (Dross et al., 1987). These latter methods all involved prior separation of the high molecular weight portion of caramel color from the matrix and allowed the identification of the type of caramel color in some instances. Finally, the caramel markers 4-methylimidazole (4-MeI) and 2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole (THI) have been detected in breads (Magrian et al., 1985; Rabe et al., 1988) or beer (Lawrence and Charbonneau, 1987).

The goal of this work was to determine if caramel color was added to four batches of adulterated acerola juice product. The potential for caramelization of the authentic acerola juice concentrate had to be addressed because it is a sugar-containing vacuum-dried product. Samples of the suspect caramel color raw material and authentic acerola juice concentrate were available for

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analysis. A two-step approach was taken: (1) classification of the suspect caramel color raw material via detection of caramel markers and HPLC−UV profiling and (2) detection of added caramel color in the adulterated acerola juice product via HPLC−UV profiling. The reliability of this approach was evaluated by analyzing 10 commercial caramel colors in the same manner. This is the first report of an adulterated acerola juice product and also the first report in which added caramel color has been matched to its commercial source.

MATERIALS AND METHODS

Reference Materials. 5-(Hydroxymethyl)-2-furaldehyde (5-HMF, 99% purity) was obtained from Sigma (St. Louis, MO). 4-Methylimidazole (4-MeI, 98% purity) was obtained from Aldrich (Milwaukee, WI). 2-Acetyl-4(5)-tetrahydroxybutylimidazole (THI) was donated by the International Technical Caramel Association (Washington, D.C). Authentic acerola juice concentrate (origin Brazil) was donated by Weinstein Nutritional Corporation (Costa Mesa, CA). Samples of 10 different commercial caramel colors (liquids or powders) were obtained by request from two U.S. caramel color manufacturers.

Sample Preparation. *Caramel Raw Material.* Liquid caramel colors were mixed thoroughly before sampling. For determination of 5-HMF, THI, and 4-MeI, 0.15-0.30 g of whole liquid caramel color or 0.10−0.15 g of whole powder caramel color was used. A volume of water was added and thoroughly mixed with the sample: 1.0 mL for liquid caramel color, 2.0 mL for powder caramel color. The samples were then filtered through nylon cartridge filters (0.20 μm for liquid caramels, 0.45 μm for powder caramels). For some caramel colors, further dilution with water was necessary to bring the analytical peak within the calibration range. For HPLC−UV profiling, 0.15−0.30 g of whole liquid caramel color or 0.10−0.15 g of whole powder caramel color was used. After 10.0 mL of water was added, the sample was sonicated for 10 min and filtered through a 0.20 μm nylon filter. The filtrate was diluted 1:3 with water prior to analysis.

Finished Products. The sample size was 1.5 g. After 3.0 mL of water was added, the sample was sonicated for 30 min and filtered through a 0.45 μm nylon filter.

HPLC Analysis. All HPLC experiments were conducted with a Hewlett-Packard series II 1090 liquid chromatograph with UV and online diode array detection. The diode array feature was used to obtain online spectra (190−600 nm) for peaks of interest. For all experiments, a 4.6 mm × 15 cm Zorbax Rx C18 (5 μm) column was used, and the buffer was 0.05 M potassium dihydrogen phosphate, 0.005 M sodium octane sulfonate, adjusted to a pH of 3.0. For determination of 4-MeI in caramel color raw material and for HPLC−UV profiling of caramel colors or finished products, the mobile phase was 85:15 buffer:methanol at 1.00 mL/min and detection was at 215 nm. For determination of 5-HMF in caramel color raw material, the mobile phase was 92.5:7.5 buffer:methanol at 1.00 mL/min and detection was at 285 nm.

RESULTS AND DISCUSSION

Analysis of Suspect Caramel Color Raw Material and Commercial Caramel Colors for Caramel Markers. The Food and Nutrition Board of the National Academy of Sciences in the U.S. has recommended dividing caramel colors into four classes (Food and Nutrition Board, 1994) according to the presence or absence of reactants which are used during manufacture, specifically ammonium and sulfite compounds. Class I caramel colors are manufactured in the absence of either ammonium or sulfite compounds. Class II caramel colors are manufactured in the presence of sulfite compounds but without ammonium compounds. Class III caramel colors are manufactured in the presence of ammonium compounds but without sulfite compounds. Class IV caramel colors are manufactured in the presence of both ammonium and sulfite compounds. Other acids or alkalies may or may not be present during manufacture of all four classes. The class designation for caramel colors does not apply outside the U.S.; other terminology such as "ammonia caramels" or "sulphite−ammonia caramels" is used.

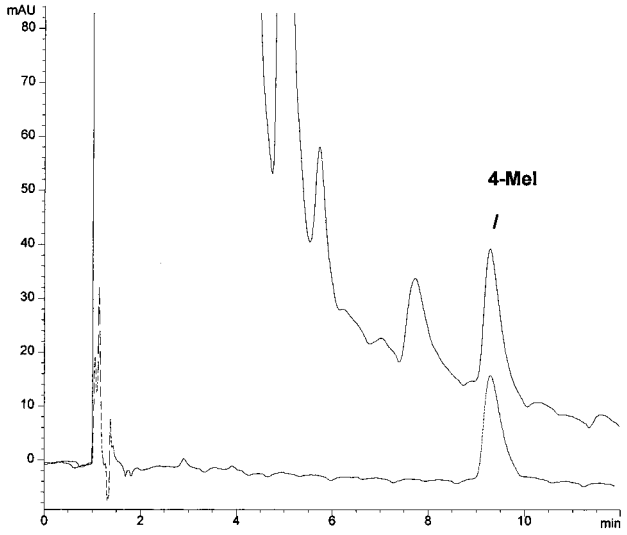

Figure 1. Chromatogram of class IV manufacturer B liquid 2 caramel color (upper trace) vs 4-MeI standard chromatogram (lower trace). Detection is at 215 nm.

Various low molecular weight compounds have been detected in the four classes of caramel color and are considered markers of caramel color. The compound 5-(hydroxymethyl)-2-furaldehyde (5-HMF) has been detected in all four classes of caramel color (Licht et al., 1992a,b). The compound 4-methylimidazole (4-MeI) has been detected in class III and class IV caramel colors, but not in class I or class II caramel colors (Licht et al., 1992a−c). The compound 2-acetyl-4(5)-tetrahydroxybutylimidazole (THI) has been detected only in class III caramel colors (Licht et al., 1992b,c).

To analyze for the three marker compounds in the present work, two similar but separate HPLC assays were conducted. 4-MeI was determined in the first assay; 5-HMF and THI were determined in the second assay (see Materials and Methods). To ascertain the reliability of the analytical methods applied, 10 commercial caramel colors from two U.S. manufacturers which represented the four classes of caramel were also analyzed for the marker compounds. One of the class IV caramel colors (manufacturer B liquid 1) had the same trade name as the suspect caramel color raw material. Representative chromatograms for a class IV caramel color overlaid with the chromatogram of the appropriate standard or standard mix are given in Figure 1 (first assay for 4-MeI) and Figure 2 (assay for 5-HMF and THI).

The analytical results for all three marker compounds in the 10 commercial caramel colors and the suspect caramel color are given in Table 1. 4-MeI was detected in all of the class III and class IV caramel colors and was not detected in any of the class I or class II caramel colors. 4-MeI was also detected in the suspect caramel color.

Table 1. Determination of Marker Compounds in Commercial Caramel Colors

carmel class	caramel identification	4-MeI content[a] (μg/g)	5-HMF content[a] (μg/g)	THI content[a] (μg/g)
class I	manufacturer A (liquid)	not detected[b]	460	not detected[b]
class I	manufacturer B (liquid)	not detected[b]	8200	not detected[b]
class I	manufacturer B (powder)	not detected[b]	510	not detected[b]
class II	manufacturer A (liquid)	not detected[b]	18000	not detected[b]
class III	manufacturer A (liquid)	14	not determined[c]	not determined[c]
class III	manufacturer B (liquid)	24	not determined[c]	not determined[c]
class III	manufacturer B (powder)	50	not determined[c]	not determined[c]
class IV	manufacturer A (liquid)	140	not determined[c]	not determined[c]
class IV	manufacturer B (liquid1)	160	420	not detected[b]
class IV	manufacturer B (liquid 2)	300	60	not detected[b]
class IV	manufacturer B (powder)	480	230	not detected[b]
class IV[d]	suspect (liquid)	130	56	not detected[b]

[a] All results represent the average of two trials and are reported to two significant figures. Values are not normalized for color units. [b] Detection limits based on standard injections were 0.5 μg/g for 5-HMF and 1 μg/g for both 4-MeI and THI. The actual detection limits in the sample matrices will vary depending on specific interferences and were not determined. [c]Determination not possible due to major coeluting interferences. [d] Classification of suspect caramel color is based on manufacturer's labeling and is consistent with analysis.

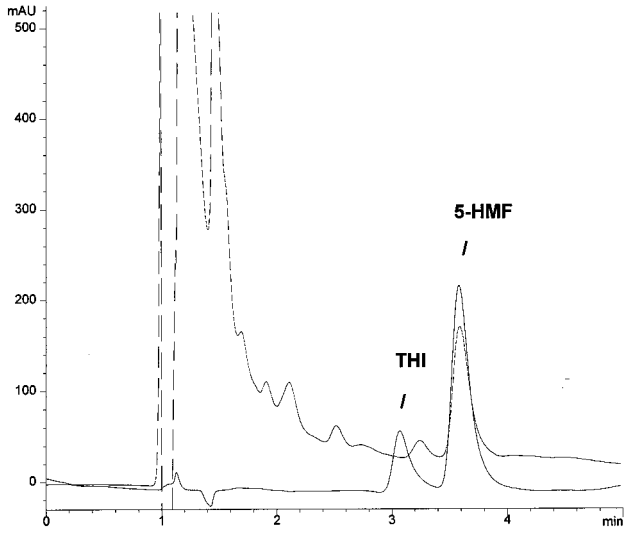

Figure 2. Chromatogram of class IV manufacturer B liquid 1 caramel color (dotted line) vs THI and 5-HMF mixed standard chromatogram (solid line). Detection is at 285 nm.

Major coeluting interferences completely precluded the analysis for 5-HMF and THI in all of the class III and one of the class IV caramels. However, 5-HMF was detected in all of the remaining caramel colors tested including the class I, class II, and class IV caramel colors and the suspect caramel color; THI was not detected in any of these caramel colors. Detection of all compounds was based on the observation of peaks with both retention times and online UV−vis spectra that matched those of the corresponding standards. Note that THI has been previously found only in class III caramel colors for which the present approach was unsuccessful.

We observed varying degrees of interference for the determination of the three marker compounds in the caramel colors tested. For the determination of 4-MeI, we observed the least interference from the class IV caramel colors, both because of higher levels of 4-MeI and less coeluting material. For the determination of 5-HMF and THI, major interferences were observed for all of the class III and one of the class IV caramel colors as noted above. Interferences were minimal for all other caramel colors tested including the supect caramel color. Despite interferences, the results for duplicate trials were reproducible to within 3−10% for all of the caramel colors tested (% reproducibility calculated as follows: % reproducibility = [range/average] × 100%).

The range of 4-MeI contents observed in this work for the class III caramel colors (14−50 mg/g) is several times lower than the range observed for the class IV caramel colors (130−480 mg/g). These results are consistent with results from a comprehensive caramel color analytical study (Licht et al., 1992a,b) in which the 4-MeI contents ranged from <10 to 43 mg/g for class III caramel colors (Licht et al., 1992b) and from 112 to 1276 mg/g for class IV caramel colors (Licht et al., 1992a).

Table 2. Spike/Recovery Experiments for Marker Compounds in a Class IV Caramel Color Matrix

	percent recovery (spiking level, μg/g)	
compound	lower level spike[a]	higher level spike[a]
4-methylimidazole (4-MeI)	97 (44)	92 (130)
5-(hydroxymethyl)-2-furaldehyde (5-HMF)	165 (22)	105 (210)
2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)-imidazole (THI)	60 (33)	60 (96)

[a] Each result is the average of two trials.

The range of 5-HMF contents observed in this work was 60−420 mg/g for class IV caramel colors and 460−18000 mg/g for class I and class II caramel colors. The previous study (Licht et al., 1992a) reported a higher 5-HMF range for class IV caramel colors (500−26000 mg/g) than was observed in this work; the detection of 5-HMF was reported in all class I and class II caramel colors, but no values were reported (Licht et al., 1992b). The lower range of 5-HMF contents found for class IV caramel colors in the present study may be due to instability of 5-HMF in the matrix: the 5-HMF content of class IV manufacturer B liquid 1 was redetermined approximately 5 weeks after the analysis reported in Table 1 and was found to have decreased by nearly 50% to 220 mg/g.

Spike/recovery experiments for all three marker compounds were conducted using class IV manufacturer B liquid 1 as the caramel color matrix. Each compound was spiked into the caramel color matrix at two different levels for a total of six spiking experiments; unspiked caramel color was analyzed at the same time. Good recovery of 4-MeI (>90%) was obtained at both the lower and higher spiking levels. Recovery of 5-HMF was quantitative (105%) at the higher spiking level but was high (165%) at the lower spiking level. Recovery of THI was consistent at 60% at both spiking levels. Results are summarized in Table 2. On the basis of these spike/recovery experiments, the values reported for 4-MeI in Table 1 are considered to be accurate for class IV

manufacturer B caramel colors which all have analytical profiles similar to one another. However, due to the obvious matrix differences for the other caramel colors, all other values reported for 4-MeI and 5-HMF content are considered to be estimates in the absence of further spike/recovery experiments.

On the basis of the manufacturer's labeling, the suspect caramel color raw material was class IV and was expected to contain 5-HMF and 4-MeI, but not THI. As shown in Table 1, both 4-MeI and 5-HMF were detected in the suspect caramel color, but THI was not detected. The 4-MeI content of the suspect caramel color was similar to the 4-MeI contents of both class IV manufacturer A liquid and class IV manufacturer B liquid 1. Recall that the suspect caramel color had the same trade name as class IV manufacturer B liquid 1. The 5-HMF content was similar to that of class IV manufacturer B liquid 2. However, the apparent instability of 5-HMF makes it an unreliable parameter for comparing caramel colors. No reliable match could be made between the suspect caramel color and any of the commercial caramel colors based solely on the levels of 4-MeI and 5-HMF.

HPLC−UV Profiling of Suspect Caramel Color Raw Material, Commercial Caramel Colors, and Finished Products. The results from the previous section are consistent with the classification of the suspect caramel color raw material as class IV. The remaining questions to be answered were as follows: Could the suspect caramel color be matched with one of the commercial caramel colors? Was a caramel color added to the adulterated acerola juice product? If so, could it be shown that it was the same caramel color as the suspect caramel color raw material?

One obvious approach was to analyze the adulterated acerola juice finished products for the caramel marker compounds 5-HMF and 4-MeI. This was attempted using a simple aqueous extraction of the adulterated acerola juice products and the same HPLC conditions as used for the commercial caramel colors. 5-HMF was detected in one batch of the adulterated product for which the caramel color content was rather high, later estimated to be 2.4 wt % caramel color. 4-MeI was not detected in this batch of adulterated product. Neither 5-HMF nor 4-MeI were detected in the other batches of adulterated product for which caramel color contents were later estimated to be in the range 0.2−0.4 wt %. On the basis of the anticipated levels of these marker compounds in the finished products, extensive method development would have been required to make this approach successful, including the selective extraction/isolation of the marker compounds from the finished product matrix and a more sensitive assay.

Profile Components. We were able to take a much simpler approach based on the very distinctive four-peak sequence which was observed in the HPLC chromatogram of the suspect caramel color raw material obtained under the HPLC conditions used for the determination of 4-MeI (Figure 3). This HPLC-UV profile was ultimately used to match the suspect caramel color with one of the commercial caramel colors and also to detect and estimate the suspect caramel color raw material content in the adulterated acerola juice finished products.

The specific chemical identities of the four components in the profile were not determined, but some structural information was obtained from their relative

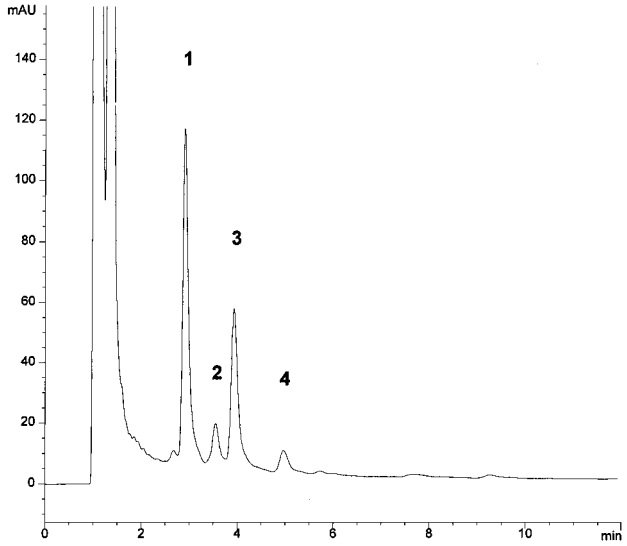

Figure 3. Chromatogram of suspect caramel color raw material including distinctive four peak sequence with retention times of (1) 2.9 min; (2) 3.6 min; (3) 4.0 min; and (4) 5.1 min. Detection is at 215 nm.

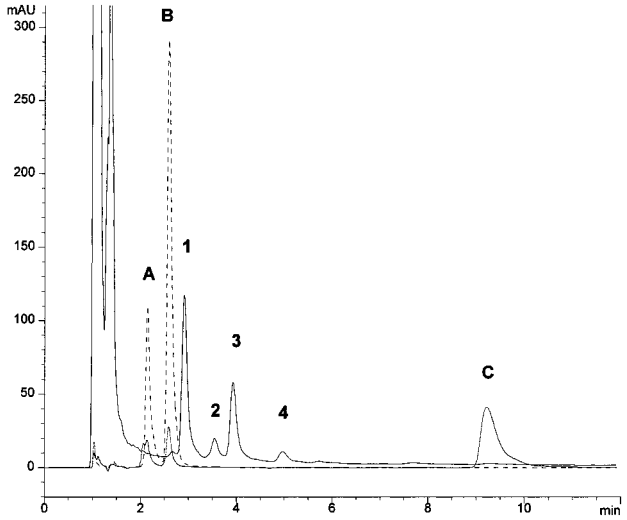

Figure 4. Chromatogram of THI, 5-HMF, and 4-MeI mixed standard with detection at 215 nm (semidotted line) or 285 nm (dotted line) vs suspect caramel color raw material chromatogram at 215 nm (solid line). Peak identification for standards: (A) THI; (B) 5-HMF; (C) 4-MeI. Peak identification for suspect caramel color is as for Figure 3.

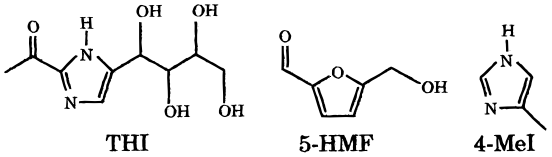

Figure 5. Chemical structures of 4-MeI, 5-HMF, and THI.

retention times and online UV−vis spectra and by comparison with the known caramel markers. A standard mixture of THI, 5-HMF, and 4-MeI provided points of reference for retention times, from which the relative component polarities could be inferred (Figure 4). The elution order of the three standard compounds was THI (2.3 min), 5-HMF (2.6 min), and 4-MeI (9.0 min). The much earlier elution of 5-HMF and THI vs 4-MeI is understood by examining their structures (Figure 5); both 5-HMF and THI have multiple polar functional groups.

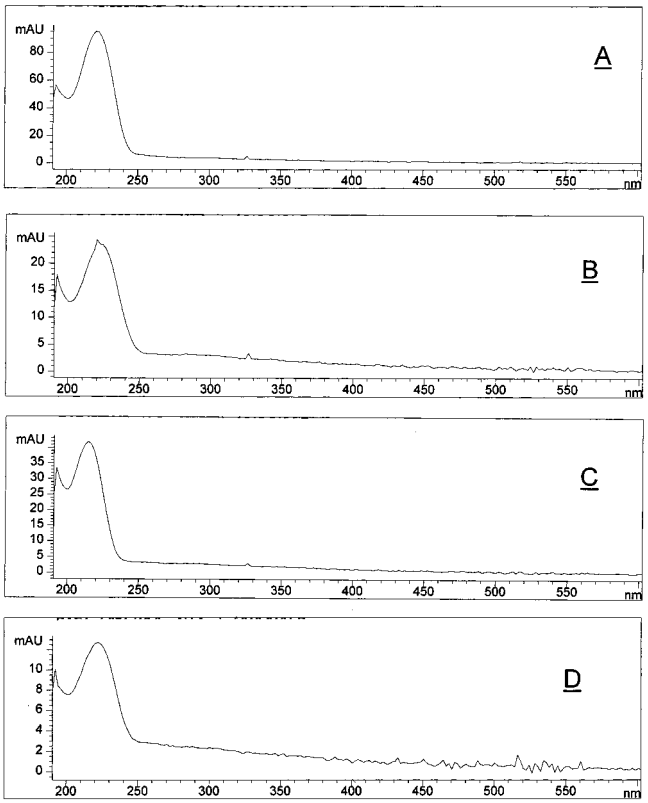

Figure 6. Online UV−vis spectra of the four components in the suspect caramel color profile corresponding to retention times and λ_{max} values of (A) 2.9 min, 222 nm; (B) 3.6 min, 223 nm; (C) 4.0 min, 216 nm; (D) 5.1 min, 222 nm.

The four profile components eluted in the retention window between 5-HMF and 4-MeI with retention times of 2.9, 3.6, 4.0, and 5.1 min, respectively. The online UV−vis spectra of all four components were rather simple comprising one major peak with a λ_{max} in the 214−222 nm range (Figure 6). The UV−vis spectrum of 4-MeI is also simple, comprising one major peak with a λ_{max} of 216 nm (Figure 7A). The presence of only a single peak in the UV−vis spectra indicates that there is only one chromophore in the structures of the unknown components and that each peak in the chromatogram probably represents a single compound. These simple spectra are very similar to the spectrum of 4-MeI which possesses a single chromophore, the imidazole ring. The UV−vis spectra of 5-HMF and THI, which each possess a second chromophore, are more complex (Figure 7B,C), and the major band is shifted to above 280 nm due to conjugation between the chromophores.

The spectral information for the four components in the profile, coupled with their relative retention times, could indicate that these compounds are conjugated dienes or heterodienes with one or more polar functional groups. The spectrum of the third component in the profile is virtually identical to the spectrum of 4-MeI and could represent a more polar imidazole compound. The spectra of the other three components are virtually identical to one another and could represent a series of structures with the same chromophore but varying amounts of polar functional groups. Another reason to suspect that these components may be nitrogen-containing compounds is that they were only detected in the class III and class IV caramel colors (see next section). Although we conducted no further work to

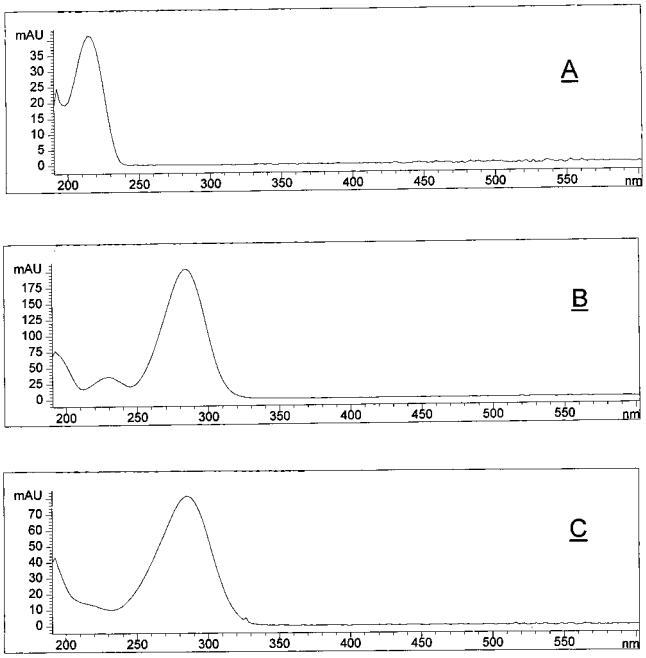

Figure 7. Online UV−vis spectra of three caramel color marker standards with λ_{max} values of (A) 4-MeI, 216 nm; (B) 5-HMF, 285 and 230 nm; (C) THI, 286 nm and broad band absorbance near 220 nm.

elucidate the structure of these components, it may be possible to do so using LC−MS.

The levels of the four components in the suspect caramel color were estimated on the basis their peak areas and using the calibration curve for 4-MeI. The four components were estimated at 0.5%, 0.08%, 0.3%, and 0.02 wt % of the suspect caramel color, respectively, in order of elution time. 4-MeI was determined at ca. 0.006 wt % in the suspect caramel color. Thus, two of the profile components (**1** and **3**) were present at 50−80 times the level of 4-MeI. The higher levels of these profile components, coupled with their structural similarity to 4-MeI and their detection in class III or IV caramel colors only, may warrant further investigation of their suitability as class-specific caramel color markers.

Profile Results for Commercial Caramel Colors. The HPLC−UV profiles of the 10 commercial caramel colors were obtained and compared to the HPLC−UV profile of the suspect caramel color. The distinctive four-peak sequence of the suspect caramel color was observed in all of the class IV caramel colors from manufacturer B (Figure 8) but not in any of the other commercial caramel colors. A given component in the commercial caramel color profiles was matched to one of four suspect caramel color components if both the retention time and UV−vis spectrum were the same.

To further compare the three class IV caramels from manufacturer B to the suspect caramel color, the area percentages of the individual peaks in the profile were calculated taking the total area of the four peaks as 100%. This treatment (Table 3) shows that the relative areas of the four peaks are similar for the suspect caramel color, manufacturer B liquid 1, and manufacturer B powder, but differ for manufacturer B liquid 2. The similar peak area percentages for manufacturer B liquid 1 and manufacturer B powder suggest that the powder was derived from the liquid; this fact was later confirmed by the manufacturer. Finally, the levels of

Table 3. Relative Peak Area Percentages of Profile Components in Class IV Caramel Colors[a]

	relative peak area (%)			
caramel color id	component 1 (2.9 min)	component 2 (3.6 min)	component 3 (4.0 min)	component 4 (5.1 min)
suspect	54	8.6	31	6.0
manufacturer B, liquid 1	56	8.0	30	5.5
manufacturer B, liquid 2	41	14	33	12
manufacturer B, powder	52	9.2	33	5.9

[a] Each result is the average of two trials.

Table 4. Estimated Percent by Weight Profile Components in Class IV Caramel Colors[a]

	percent by weight			
caramel color id	component 1 (2.9 min)	component 2 (3.6 min)	component 3 (4.0 min)	component 4 (5.1 min)
suspect	0.53	0.08	0.30	0.06
manufacturer B, liquid 1	0.65	0.09	0.36	0.06
manufacturer B, liquid 2	0.64	0.22	0.52	0.18
manufacturer B, powder	1.2	0.22	0.78	0.14

[a] Each result is the average of two trials.

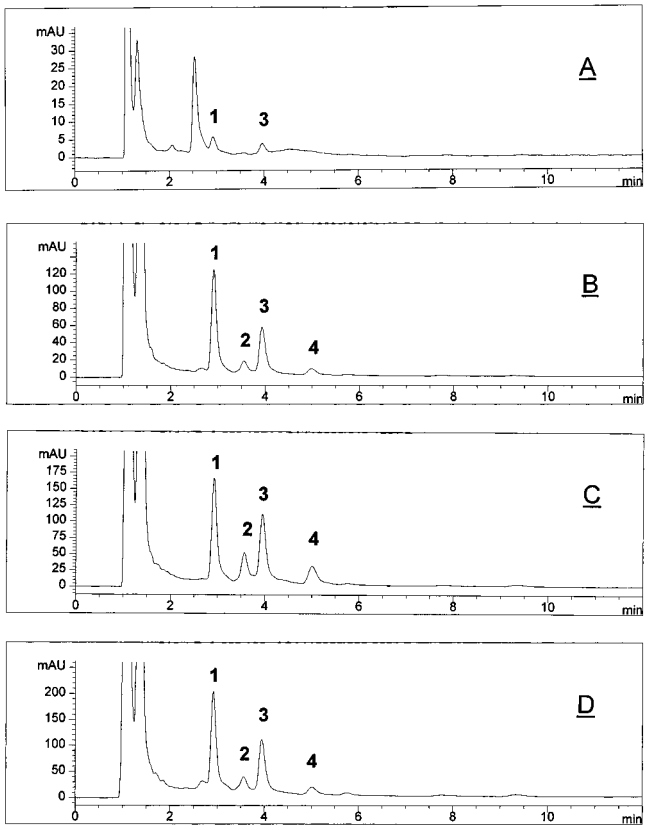

Figure 8. 215 nm HPLC profiles of class IV commercial caramel colors: (A) manufacturer A liquid; (B) manufacturer B liquid 1; (C) manufacturer B liquid 2; (D) manufacturer B powder.

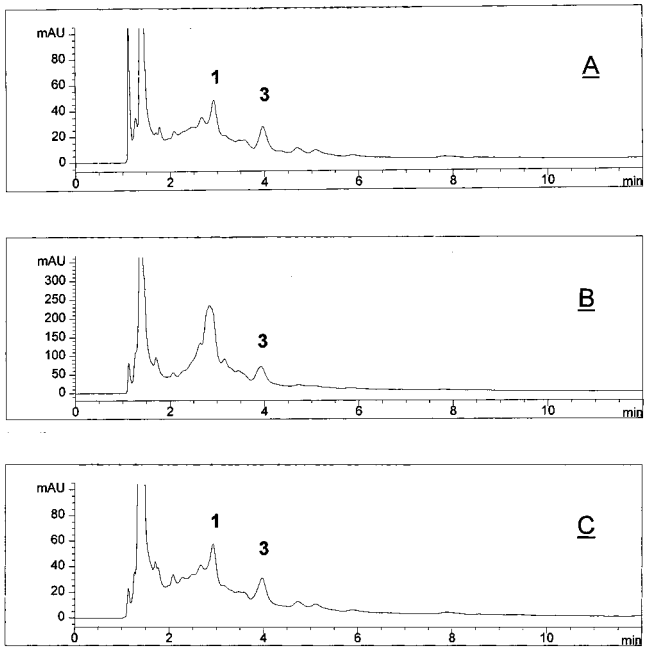

Figure 9. 215 nm HPLC profiles of class III commercial caramel colors: (A) manufacturer A liquid; (B) manufacturer B liquid; (C) manufacturer B powder.

the four components in the caramel colors were estimated as before on the basis of their peak areas relative to 4-MeI and the assayed levels of 4-MeI. The results are given in Table 4. The suspect caramel color closely matches manufacturer B liquid caramel 1. All four components are at a higher level in the powder proportional to the concentration effect of drying, about a factor of 2.

Peaks with retention times matching those of the two major peaks in the suspect caramel color profile (peaks 1 and 3, 2.9 and 4.0 min) were observed in the profile of class IV manufacturer A liquid (Figure 8A) as well as in two of the three class III caramel colors: manufacturer A liquid and manufacturer B powder (Figure 9A,C). Only one of the peaks (retention time 4.0 min) was observed in the profile of class III manufacturer B liquid; however, the 2.9 min peak could have been obscured by a major peak which eluted just prior to the expected retention time (Figure 9B). The two peaks for class IV manufacturer A liquid had online UV−vis spectra matching to the corresponding peaks in the suspect caramel color. The online UV−vis spectra of the class III caramel colors peaks could not be obtained because of coeluting components.

None of the four components from the suspect caramel color profile were detected in any of the class I and class II commercial caramel colors tested. The absence of the four components in class I and class II caramel colors and the presence of two of the components in class III and all four of the components in class IV caramel colors could be related to the manufacturing chemistry of the four classes of caramel color. Both class I and class II caramel colors are produced in the absence of am-

Table 5. Relative Peak Area Percentages of Profile Components in Adulterated Acerola Juice Products[a]

	relative peak area (%)			
sample id	component 1 (2.9 min)	component 2 (3.6 min)	component 3 (4.0 min)	component 4 (5.1 min)
suspect caramel color	54	8.6	31	6.0
adulterated batch 1	46	18	22	14
adulterated batch 2	54	7.4	33	6.3
adulterated batch 3	47	6.4	28	18
adulterated batch 4	51	8.4	32	8.8

[a] Each result is the average of two trials.

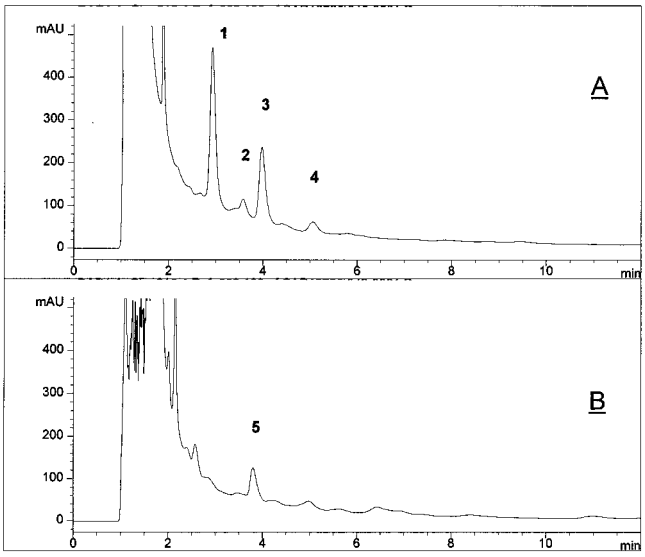

Figure 10. 215 nm HPLC profiles of (A) adulterated acerola juice product and (B) authentic acerola juice. Component 5 has different retention time and online UV−vis spectrum versus components 1−4.

monium compounds whereas both class III and class IV caramel colors are produced in the presence of ammonium compounds. Thus, the profile components could be nitrogen-containing compounds; more work would be required to address this possibility.

Profile Results for Acerola Juice Finished Products. The adulterated acerola juice batches claimed to be dry acerola juice concentrates with varying vitamin C contents. Separate information and analyses showed that the adulteration was not very sophisticated and that the finished products probably did not contain any authentic acerola juice. The formulations did contain a large amount of lactose and much smaller amounts of glucose and fructose. The relative amounts of vitamin C and the fruit sugars, as well as the ratio of glucose to fructose, were inconsistent with authentic acerola. The coloring of the adulterated product was similar to caramel coloring.

The distinctive four-peak sequence from the suspect caramel color was observed in the profiles of all four batches of adulterated acerola juice product but not in the profile of an authentic acerola juice concentrate (Figure 10). The peak area percentages for the four peaks in the adulterated acerola juice profiles were calculated and compared to the peak area percentages for the suspect caramel color (Table 5). The results for batches 2 and 4 are similar to the suspect caramel color. The peak area percentages for batches 1 and 3 are less consistent with the suspect caramel color but still indicate addition of caramel color, probably from manufacturer B. The percent by weight caramel color in the four batches of adulterated acerola juice product was

Table 6. Estimated Percent by Weight Caramel Color in Adulterated Acerola Juice Products

	percent by weight[a]		
batch number	based on component 1 (2.9 min)	based on component 3 (4.0 min)	overall average (%)
1	0.42	0.34	0.38
2	2.3	2.4	2.4
3	0.21	0.21	0.21
4	0.22	0.24	0.23

[a] Each result is the average of two trials.

calculated using the suspect caramel color as the standard. Independent calculations were carried using each of the two major peaks (2.9 and 4.0 min) and gave consistent results for the percentage of added caramel color for each batch (Table 6). Of the four batches tested, the lowest caramel color level found was 0.2 wt %. On the basis of the minimum measurable peak area for the two major peaks, the current method can detect 0.02 wt % added caramel color.

The presence of all four peaks in the adulterated acerola juice product profiles is strong evidence that caramel color was added and argues against the possibility that the four components were formed due to in-process caramelization. The absence of all four components in the authentic vacuum-dried acerola juice concentrate further supports this conclusion. The consistency between quantitative results based on the two major peaks indicates that the two compounds originate from the same source, i.e., the added caramel color.

CONCLUSION

The complex nature and limited knowledge of the chemical composition of caramel color makes its determination in finished products a particular challenge for the analytical chemist. Analysis for the caramel marker compounds THI, 5-HMF, and 4-MeI is useful for the characterization of caramel color raw materials but is much less practical for finished products in which these components are often too dilute. When questions arise as to whether caramel color was added to specific product matrixes, new analytical approaches must be devised.

HPLC−UV profiling was successfully used for the determination of added caramel color in adulterated acerola juice products. This approach allowed matching of the added caramel color to its commercial source via matching retention times, online UV−vis spectra, and area percentages of specific peaks in the raw material caramel color and finished product HPLC profiles. Quantitation was based on peak areas using a commercial caramel color as the standard; 0.2% of added caramel color was readily determined. The detection limit was 0.02% added caramel color.

ABBREVIATIONS USED

5-HMF, 5-(hydroxymethyl)-2-furaldehyde; 4-MeI, 4-methylimidazole; THI, 2-acetyl-4(5)-tetrahydroxybutylimidazole.

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