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McGinnis, T. P., Water and Core Technologies Research, Nalco Company, 1601 W. Diehl Road, Naperville, IL, 60563-1198. **Analytical Characterization of Conditioned Diffusion Juices and Related Process Samples.**

ABSTRACT

A conditioning system developed and applied by Nalco has been shown to result in improved synthetic thin juice quality. Various analytical techniques have been employed to characterize the composition of juices subjected to conditioning in a number of beet sugar factories. In addition to diffusion and thin juices, samples of condensed vapors were analyzed in order to characterize the types of volatiles liberated from the juices. The analytical results give insight into the improved purity and decreased color of thin juices examined in field studies during the 2003-2004 slicing campaign.

INTRODUCTION

Nalco has become involved in the development and application of a patented juice conditioning technology. The fundamental concepts of this technology have been discussed in previous presentations[1,2]. Studies have shown that thin juices prepared from conditioned raw juices show increases in purity and decreases in color, versus thin juices prepared from unconditioned raw juices. With respect to juice purity, it was theorized that there were no sucrose losses occurring in the raw juice during the conditioning. However, the absolute levels of sucrose in raw juices before and after treatment needed to be determined in order to assess the validity of the theory. As air stripping and vacuum are applied as part of the conditioning process, it was theorized that volatile organic components were being removed. The removal of these materials was thought to potentially affect both purity and color. However, the types and amounts of volatiles removed needed to be characterized to better understand the processes taking place.

Analytical techniques were developed to characterize key chemical components, in order to better understand changes in the composition of conditioned juices. High Performance Liquid Chromatography (HPLC) was used to quantitate the absolute amounts of sucrose present in raw juices prior to and after conditioning. For volatiles, components were analyzed by first liberating them from solution via gas purging and then concentrating them with cold trapping. The trapped materials were then thermally desorbed, followed by separation and identification using gas chromatography with mass spectrometric detection (Purge and Trap Gas Chromatography with Mass Spectrometry, or PTGCMS).

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[1] **The Development and Application of a Juice Conditioning System for Increased Sugar Quality and Recover.** Sanders, D., Nalco Company, 1601 W. Diehl Road, Naperville, IL, 60563-1198

[2] **Improved Beet Sugar Purification from "Conditioned" Diffusion Juice: Field Studies During the 2003-2004 Campaign.** Saye, D. J., and Dang, X., Separations and Commercial Processes Research, Nalco Company, 1601 W. Diehl Road, Naperville, IL, 60563-1198

PROCEDURES

Reagents

All water used for dilutions and solution preparation was prefiltered and deionized using a Milli-Q gradient water purification system (Millipore, Billerica, MA).
Sucrose (Ultrapure Bioreagent grade, 99.9+%) was obtained from J. T. Baker (Phillipsburg, NJ).
Ethyl acetate (A.C.S. grade, 99+%) was obtained from J. T. Baker.
d_5-Ethyl-d_3-acetate (99 atom % D) was obtained from Sigma-Aldrich (Milwaukee, WI).
All reagents were used as supplied without further purification.

HPLC

All raw juices analyzed had been frozen immediately after collection, prior to and after the conditioning unit, and were maintained in a frozen state at all times prior to sample preparation. Raw juices were thawed just prior to sample preparation and analysis. An aliquot of the thawed raw juice was centrifuged at 9000 rpm for 5 minutes. A 1 g aliquot (weighed to the nearest 0.1 mg) of the supernatant was diluted to volume in a 25 mL volumetric flask with deionized water and well mixed. A portion of this dilution was filtered with a 0.45 micron Millipore Millex-HV filter prior to introduction into an autosampler vial.

All samples were analyzed versus multiple point external calibrations prepared using deionized water and A.C.S. grade sucrose. Calibration solutions ranged from approximately 100 – 10,000 ug sucrose per mL. All calibrations were linear, with R^2 values in excess of 0.999. Instrumental components and general conditions are listed below.

Column:	Aminex HPX-87H (Bio-Rad Laboratories, Hercules, CA) 300 mm x 7.8 mm, with guard
Mobile Phase:	Water, no pH adjustment
Flow Rate:	0.6 mL/min.
Temperature:	85 C with column oven (Eppendorf, Hamburg, Germany)
Injection Volume:	20 uL, via autoinjector module
Detection:	Refractive Index, 50 C Cell Temp. (Erma, Tokyo, Japan)
Data Analysis:	TotalChrom Client Server Software version 6.1 from Perkin Elmer (Boston, MA)

PTGCMS

Qualitative screening of raw juices was performed by placing approximately 1 gram of thawed juice or process sample in the purge tube connected to the purge and trap concentrator, and analyzing the sample per the conditions described below. Ionization in the mass spectrometer was achieved with standard conditions of 70eV. Identification of the volatile components was achieved via matching of the obtained mass spectra with those included in the NIST 2002 mass spectral library, along with the appropriate evaluations and judgment of the analyst.

Quantitative analysis of a representative volatile component, ethyl acetate, was performed using perdeuterated ethyl acetate as an internal standard. This allowed for the comparison of compounds that behave very similar to one another chromatographically, and elute at nearly the

same time into the spectrometer. The two compounds differ enough by mass, however, such that characteristic ions of the separate compounds can be independently monitored for quantitative comparison. Using isotopically labeled compounds as internal standards in quantitative mass spectrometry is preferred over truly external calibrations. Changes occurring in the ion source of mass spectrometers over time can result in varying degrees of ionization between analyses. However, the ratios of signature ions in compounds eluting at the same time into the mass spectrometer are much less prone to variation. As a result, the ratioing of labeled and unlabeled fragments provides a stable quantitative entity.

The perdeuterated component was spiked into samples at a constant, known amount for each analysis. The mass spectrometer was operated in selected ion monitoring (SIM) mode, with ions only collected at m/z values of 61, 70, and 88 (for ethyl acetate), and at 66, 76, and 96 (for d_5-ethyl-d_3-acetate). The three ions chosen for each compound are not produced during the ionization of the other compound. Therefore, the detection of these ions at the proper elution time denote the presence of the compounds. For calibration and quantitation purposes, only ion abundances at m/z 61 and 66 were measured and used in calculations, as they were not seen to be significantly present in any of the "non ethyl acetate" compounds noted to be eluting in the same time frame under full scan mass spectral analyses.

Calibration was performed with a series of solutions consisting of varying amounts of ethyl acetate and a known amount of the perdeuterated analogue. These solutions were all prepared in 14% w/v sucrose in water, in order to mimic the bulk matrix of raw diffusion juice. Volatile organics, including ethyl acetate, were removed to undetectable limits in the sucrose solution with thorough vacuum degassing. For each analyzed calibration sample, the ratio of ion abundances at m/z 61 to m/z 66 was determined, and plotted vs. the micrograms of ethyl acetate present. The plot of ion ratios vs. ug of ethyl acetate gave a linear response with a correlation coefficient of > 0.99.

For the quantitation of ethyl acetate in a raw juice sample, a known weight of juice was spiked with the known amount of perdeuterated compound. The m/z 61/66 ratio was determined for the two compounds at the time they coeluted. This ratio was combined with the calibration line, and the weight of sample used, to calculate the amount of ethyl acetate present in the juice. Instrumental components and general conditions for the PTGCMS are listed below.

P & T Concentrator:	Tekmar 3000, with Cryofocusing Module (Tekmar, Mason, OH) Purge gas; Helium Purge Time and Temperature; 10 min. @ 90 C Initial Trap; Tenax A Trap (Supelco, Bellefontaine, PA), maintained @ -20 C via liquid CO_2 throughout the sample purging period Trap Desorption Time and Temperature; 10 min. @ 225 C Cryofocusing Unit; Mounted at column head and maintained @ -120 C via liquid N_2 throughout the duration of the trap desorption period Cryoinjection; Directly to GC via rapid desorption @ 225 C
GC:	Hewlett-Packard 6890 (Agilent, Wilmington, DE)
Inlet:	Direct from cryofocusing unit to column
Column:	J&W DB-5ms (Agilent), 30 m x 0.25 mm,

	0.25 um film thickness
Carrier:	He @ 0.5 cc/min.
Oven Ramp:	0 C (5 min. hold) – 200 C (no hold) @ 10 C/min.
Mass Spectrometer:	Hewlett-Packard 5973 MSD (Agilent)
Ionization:	Electron Impact @ 70 eV (except for SIM mode described above)
Data Analysis:	HP Chemstation (Agilent), with NIST Spectral Searching Program/Database (ChemSW, Fairfield, CA)

RESULTS AND DISCUSSIONS

Sucrose Concentration Across Conditioning Unit

Of primary importance was the need to determine if absolute sucrose levels changed as raw juice passed through the unit. The HPLC procedure was employed to address this issue. Figure 1 shows a representative chromatogram of a diffusion juice sample. A large and well defined peak is seen for sucrose. Also noted in the expanded insert are well defined peaks for glucose and fructose. Precision data for sucrose concentration was determined on two separate juices. One sample was obtained prior to the inlet of the unit, while another was sampled after the juiced had passed through the unit (these samlples are not paired, i.e. they are not inlet/outlet pairs representing the exact same juice stream). In each case, nine separate sample preparations were analyzed, with the following results;

Sample # 1 - Juice Collected Prior to Unit
n = 9
Average Wt. % = 14.02
$\pm$ 3 sigma = 0.15
% RSD = 0.37

Sample # 2 - Juice Collected After Unit
(Collected on Different Day than Sample # 1)
n = 9
Ave. Wt. % = 14.62
$\pm$ 3 sigma = 0.12
% RSD = 0.28

The low relative standard deviations of the analyses indicate that juices collected before or after the unit can be characterized to a high level of precision. Table 1 shows typical data for sucrose levels in inlet/outlet raw juice pairs. It can be seen that absolute changes are minimal across the unit, and are well within the precision of the method.

Absolute quantitations of glucose and fructose were not determined via external calibration. However, a measurement of relative area percentages of glucose and fructose peaks vs. sucrose peaks can be made. Relative area percentages of glucose and fructose vs. sucrose are shown in Table 2. The changes are small across the unit for both components. This is further indication that the sucrose is not degraded in the unit, as such degradation would likely manifest itself in the formation of invert sugars.

Figure 1

HPLC Profile of Raw Juice

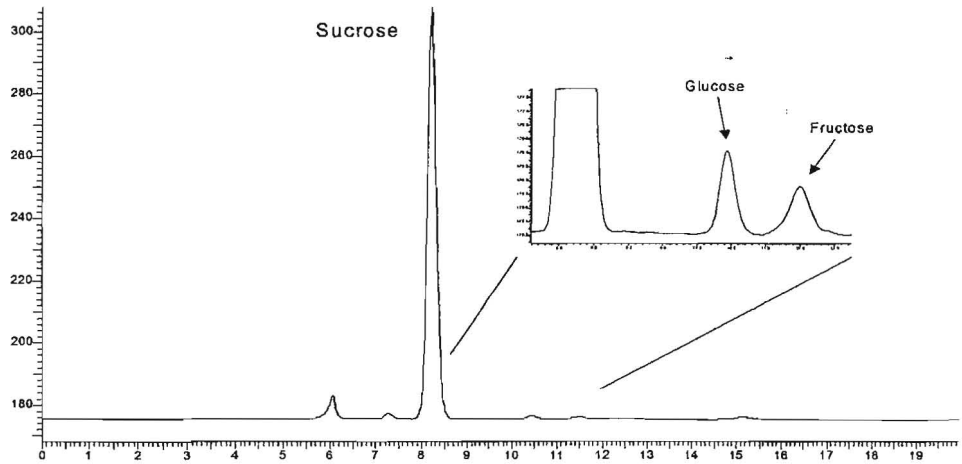


Table 1

Absolute Sucrose Concentration in Inlet and Outlet Raw Juice Samples

Sample Pair #	% Sucrose @ Inlet	% Sucrose @ Outlet	Delta Across Unit
1	14.67	14.70	0.03
2	14.52	14.53	0.01
3	14.26	14.27	0.01
4	13.87	13.87	0.00
5	14.61	14.65	0.04
6	14.43	14.42	-0.01
7	14.18	14.20	0.02
8	13.61	13.61	0.00
9	13.83	13.76	-0.07
10	15.03	15.07	0.04
11	14.86	14.85	-0.01
12	14.85	14.92	0.07

Table 2

Area Percentages of Glucose and Fructose, Relative to Sucrose

Sample Pair #	Relative % Glucose @ Inlet	Relative % Glucose @ Outlet	Delta Across Unit	Relative % Fructose @ Inlet	Relative % Fructose @ Outlet	Delta Across Unit
1	1.62	1.62	0.00	1.05	1.04	-0.01
2	1.55	1.61	0.06	0.95	1.03	0.08
3	1.67	1.71	0.04	1.08	1.11	0.03
4	1.61	1.62	0.01	1.20	1.25	0.05
5	2.12	2.12	0.00	1.54	1.54	0.00
6	1.64	1.60	-0.04	1.07	1.06	-0.01
7	1.62	1.65	0.03	1.23	1.24	0.01

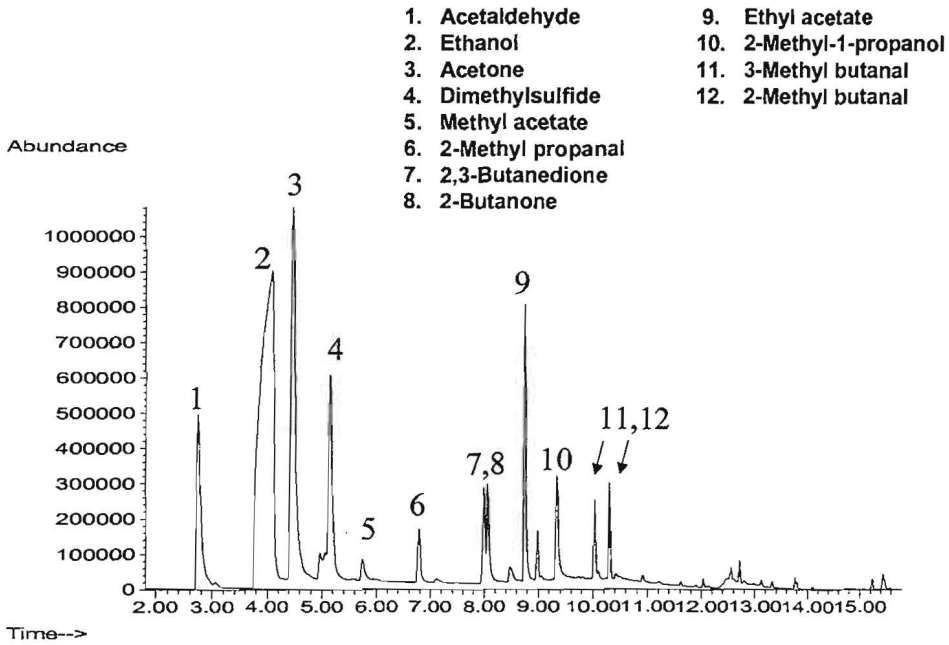
Based on the results shown in Tables 1 and 2, it is concluded that no negative effects are imparted to sucrose as it passes through the conditioning unit.

Volatiles Present In Raw Juice

Qualitative analysis of the raw juice by PTGCMS revealed that a variety of low molecular weight organic components can be purged from raw juices under relatively mild conditions. Figure 2 shows the total ion chromatogram resulting from the analysis of a typical raw juice.

Figure 2

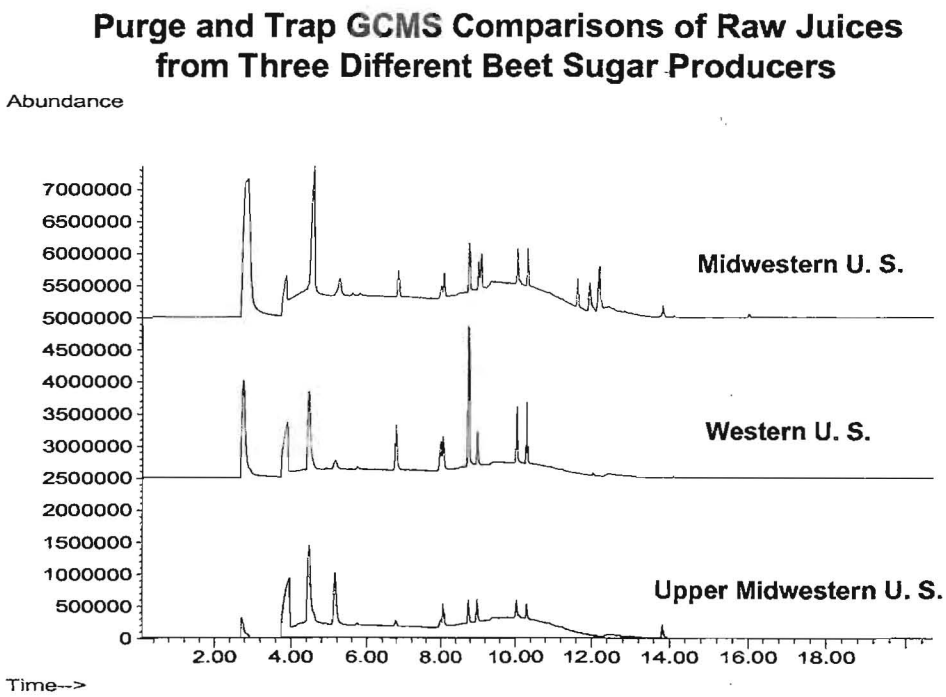
Purge and Trap GCMS Profile of Raw Juice



The identified components are primarily C_2 to C_5 carbonyl containing compounds. These are present along with ethanol, which exhibits a large tailing peak due to overload conditions on the analytical column. Relative or absolute amounts of components are difficult to determine from this type of GCMS analysis. The different types of compounds are likely to have differing degrees of partitioning from the juice matrix during the purging process. Also, the degree of ionization in the mass spectrometer can be significantly different from one compound to the next, even if the number of carbon atoms and functional groups are similar. In general, since purge and trap techniques have the ability to isolate and concentrate analytes away from a bulk matrix prior to analysis, small quantities can be detected with ease. Based on the analysis above, it was initially estimated that volatiles removed by the purge and trap analysis were on the order of parts per million in the juice.

Juices from various parts of the United States were analyzed in the same fashion. It was seen that the basic volatile components were present in all. This is shown in Figure 3.

Figure 3



In all cases, there is a similar volatiles pattern, with signature compounds present along with ethanol. There is some variation in the types and relative amounts of some components, and this may be related to the quality of the beets from which the juice was produced. It has been noted that, in general, juices prepared from fresh, frozen beets have less overall volatiles compared to juices originating from beets that have degraded due to thawing, microbiological activity, etc.

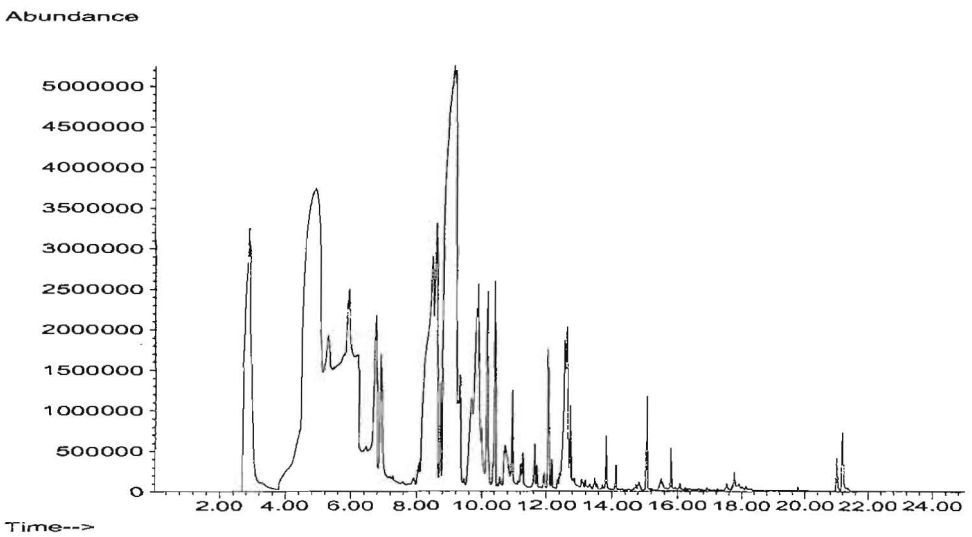
Analysis of juices collected before and after the conditioning unit suggested that some of the volatiles were being removed. In general, a profile of a treated juice would show a lower abundance of volatiles than an untreated juice. However, this was difficult to quantitate for reasons stated earlier. Also, the mechanism by which volatiles were isolated from the juice by PTGCMS is not the same physically as those occurring in the conditioning unit.

Some evidence that volatile components were being removed during the actual treatment was obtained by analyzing vapors from the unit. It was possible to tap condensation lines into the air stripper and vacuum chamber portions of the conditioning unit, and collect condensed vapors with chilled loops. These condensed liquids were then analyzed by PTGCMS. An example of a condensate is shown in Figure 4. Approximately 70 components were tentatively identified in this chromatogram via spectral matching. The majority of these are carbonyl containing compounds (aldehydes, ketones, esters), but also included are a number of ethers, sulfides, and pyrazines. This showed conclusively that there were organic components residing in the vapor phase in the conditioning unit. While some of these may have had the opportunity to partition back into the juice during the conditioning process, it was thought that a good portion

of them were being eliminated as exhaust. As sampled, the volatiles were highly concentrated in the resulting solution, owing to some of the broad and overloaded peaks in the figure.

Figure 4

Purge and Trap GCMS Profile of Condensed Vapor from Conditioning Unit Vacuum Chamber



Quantitation Of Volatiles In Raw Juices

As mentioned earlier, the quantitation of components by GCMS can be problematic due to changes occurring in the instrument's ion source with time. Particularly difficult can be the separate quantitation of numerous components simultaneously in the same sample. To better understand the extent of the removal of volatiles during the conditioning process, it was decided to focus on a single model compound. Ethyl acetate was found to be present in all of the juices examined, as well as being highly abundant in the condensates. The method described earlier using a perdeuterated analogue as an internal standard, was employed to see if ethyl acetate was being eliminated during conditioning. Table 3 shows the levels of ethyl acetate determined in a number of inlet/outlet juice pairs collected over a two day period. It is seen that for this data set the ethyl acetate levels vary from about 100 to 300 ppb in the inlet samples. This variation is likely due to changes in beet quality during the time elapsed. In most cases there is a distinct decrease in the amount of ethyl acetate occurring across the unit, with the average removal being approximately 20%. Ethyl acetate is simply a marker compound in this study, and the other volatile components may exhibit different behavior in terms of overall volatility and the ability to partition out of the juice matrix. However, it is reasonable to assume that many of the other volatile components also are removed during the conditioning process along with ethyl acetate.

Table 3
Ethyl Acetate Levels in Raw Juices Collected Before and After the Conditioning Unit

Sample Pair #	ppb EA Inlet	ppb EA Outlet	% Reduction Across Unit
1	320	264	18
2	244	191	22
3	229	192	16
4	340	261	23
5	123	101	18
6	229	189	17
7	113	94	17
8	100	98	2
9	118	92	22
10	111	94	15
11	118	82	30

Impact Of Volatiles On Thin Juice Purity

The levels of ethyl acetate in the juices was seen to be in the parts per billion range. The amount of volatile organics expected to be removed by the conditioning process would therefore be estimated to likely be on the order of ppm. Removal values of this magnitude do not represent a sufficient mass of non-sugars to explain the purity increases described in the previous Nalco presentations. Therefore it is thought that the removal of volatile organics by the conditioning equipment does not directly impact enhanced purity of juices.

Impact Of Volatiles on Thin Juice Color

While volatiles removal is not a likely source of purity enhancement, it could very well be involved in reduction of color. The earlier Nalco presentations described reductions in thin juice color when the conditioning unit was in operation. Many of the components which have been identified as volatiles in the raw juice, and which are likely removed from them during the conditioning process, are known to be involved in a number of complex reaction pathways which lead to the production of color bodies in solution. Examples would be Maillard-type reactions of carbonyl compounds with amines, etc. Although the intial amount of volatile components in raw juice may be relatively small, these are still responsible in part for color formation in thin juices. Removal of some of these volatiles would be expected to decrease the amount of components available for color producing reactions, and lead to a decrease in resulting thin juice color.

CONCLUSIONS

Analytical methods were developed to characterize key chemical components present in raw juices treated with Nalco's juice conditioning technology. It was found that sucrose is not degraded when juice is treated, and accordingly, invert sugars are not generated. A number of volatile components were identified as present in raw beet diffusion juices. It was shown that the Nalco conditioning treatment appears to reduce the levels of some of the volatile components.

The absolute levels of volatile organics in the raw juices is small, and hence their removal is likely not directly involved in the purity enhancements seen in resulting thin juices. The volatiles that are removed may, however, be directly related to the decreases in thin juice color that have been noted. The compounds that are likely removed are largely carbonyl containing low molecular weight materials, which are known to participate in reactions which form color bodies in solution.

REFERENCES

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