

Wine Metabolomics: Objective Measures of Sensory Properties of Semillon from GC-MS Profiles

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S Supporting Information

ABSTRACT: The contribution of volatile aroma compounds to the overall composition and sensory perception of wine is well recognized. The classical targeted measurement of volatile compounds in wine using GC-MS is laborious and only a limited number of compounds can be quantified at any time. Application of an automated multivariate curve resolution technique to nontargeted GC-MS analysis of wine makes it possible to detect several hundred compounds within a single analytical run. Hunter Valley Semillon (HVS) is recognized as a world class wine with a range of styles. Subtle characters reliant upon the development of bottle maturation characteristics are a feature of highly esteemed HVS. In this investigation a metabolomic approach to wine analysis, using multivariate curve resolution techniques applied to GC-MS profiles coupled with full descriptive sensory analysis, was used to determine the objective composition of various styles of HVS. Over 250 GC-MS peaks were extracted from the wine profiles. Sensory scores were analyzed using PARAFAC prior to development of predictive models of sensory features from the extracted GC-MS peak table using PLS regression. Good predictive models of the sensorial attributes honey, toast, orange marmalade, and sweetness, the defining traits for HVS, could be determined from the extracted peak tables. Compound identification for these rated attributes indicated the importance of a range of ethyl esters, aliphatic alcohols and acids, ketones, aldehydes, furanic derivatives, and norisoprenoids in the development of HVS and styles. The development of automated metabolomic data analysis of GC-MS profiles of wines will assist in the development of wine styles for specific consumer segments and enhance understanding of production processes on the ultimate sensory profiles of the product.

KEYWORDS: *multivariate curve resolution alternative least-squares (MCR-ALS), Semillon wine, sensory, partial least-squares (PLS), volatile compounds, parallel factor analysis (PARAFAC)*

■ INTRODUCTION

Flavor and aroma chemists have attempted to ascertain the most important chemical constituents contributing to the aroma perception of specific products, and mapping the concentration of these compounds to consumer preference remains the driver of research in consumer product aroma quantification. Flavor is an individual consumer's perception of the complex interactions of tactile and aroma compounds present in a food, and mapping these interactions to compositional data is an enormously challenging task.

In terms of aroma, compounds are typically described in relation to their concentration, chemical class, perceived odor description, odor thresholds in a matrix of similar composition to the food of interest, and the odor activity value (OAV). The OAV is calculated as the ratio of the quantified value of the compound in the food to the odor threshold, with values greater than one considered to impart contribution to the perceived odor of the food.¹ Significant limitations in the interpretation of OAVs arise. First, the determination of aroma thresholds is subjective and prone to considerable imprecision depending upon assessor experience and the matrix used to present the compound to assessors.¹ Also, compounds with an OAV less than one are considered to have little or no immediate impact on the aroma of a food or beverage; however, the notion of odor families² and the synergistic or suppressive interactions of compounds with similar functional

groups to food and beverage aroma is not accounted for solely by the use of OAV.³

Significantly some compounds in low concentrations and with OAV greater than one may contribute positively to product aroma, but when present at high concentrations, their odor contribution to the overall product may become dominant and negatively correlated with product acceptance. Such observations have been reported for specific marker compounds associated with minty, eucalyptus aromas,⁴ or yeast spoilage characters⁵ in wine. Alternatives and adjuncts to OAV such as aroma extract dilution analysis,⁶ CHARM,⁷ and other methods that employ olfactory detection during gas chromatography are important contributors to the understanding of aroma compound concentrations and sensory perception.

Each of the above approaches requires considerable investment of time and personnel for the determination of discrete analytical values for compounds of interest in a sample. The application of multivariate curve resolution (MCR) techniques makes it possible to decompose complex and information-rich data sets to matrices corresponding to concentration and spectral profiles.^{8–11} This approach circumvents the requirement for a priori information regarding the presence of specific

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analytes in the sample and expedites data analysis. Extracted concentration profiles can be used in multivariate data analysis pertaining to metabolomic composition, and if descriptive sensory data are available predictive models of the sensory attributes could be constructed. Such models will then enable the identification of compounds with important sensory features for the product, assist in the elucidation of manufacturing processes which influence their concentration, and shed light on the process associated with product development and manufacture.

In this investigation we report a novel and comprehensive semi-automated data analysis approach using MCR applied to a GC-MS data set of Hunter Valley Semillon (HVS) wines and map these results to sensory features obtained by descriptive sensory analysis. This is the first report of the use of multivariate curve resolution techniques applied to the analysis of GC-MS data with subsequent analysis to create predictive models of the sensory features of wines. This approach enables us to report for the first time the identification of important compositional aspects of the wines and establish objective measures of some sensory properties.

EXPERIMENTAL SECTION

Chemicals. All chemicals were of analytical grade and purchased from either Sigma-Aldrich Australia or Fluka Australia. Deionized water ($18 \text{ M}\Omega\text{cm}^{-1}$) was prepared using a Milli-Q filtration system. Ultra-high-purity helium was obtained from BOC gases, Australia.

Sensory Descriptive and Exploratory Data Analysis of Wines. The wines used in the present study (Table 1) were part of

Table 1. Wine Sample Details and Allocation in Calibration and Independent Test Sets Used for Predictive Modeling

wine code	vintage	wine style ^a	number of sample replicates	
			calibration set	independent test set
A	2001	4	2	1
B	2005	2	2	1
C	2002	4	2	1
D	2006	2	2	1
E	2006	2	2	1
F	2006	3	1	2
G	2006	2	2	1
H	2003	3	2	1
I	2002	4	1	2
J	2006	2	2	1
K	1996	4	2	1
L	2004	4	2	1
M	2006	1	2	1
N	2005	2	2	1
O	2002	3	2	1
P	1998	4	1	2

^aWine style is indicated according to Blackman and Saliba.¹² Style 1 characterized by residual sugar, styles 2 and 3 characterized by fruit flavor spectrum, and style 4 characterized by bottle age.

a larger investigation which first characterized HVS and subsequently examined the impact of bottle age on the wine's sensory characteristics.^{12,13} This particular sensory data set was collected during a repeated appraisal of the wines after an additional three years of cellar maturation. A total of 16 different wines were examined using sensory descriptive analysis. Full details of the sensory aspects of these wines, panel training, and data collection are described elsewhere¹² and will be described here only briefly. Sixteen appropriately trained panelists rated the wines sensory features in triplicate in twelve tasting sessions

held over a two-week period. The Compusense program generated random three-digit identification numbers for each wine at each session. The order of presentation of the wines was determined using a randomized complete block design. Sensory data were modeled using Parallel Factor Analysis (PARAFAC)^{14,15} as the assessors responses can be considered a four-dimensional structure of dimensions 16 (samples) \times 15 (sensory attributes) \times 48 (16 \times 3; sensory assessor \times replicate). The data were mean centered across mode 1, and no scaling was applied as it can be reasonably expected that sensory data will have similar variances as the same scale has been used during evaluations.¹⁵ PARAFAC models extracting decreasing numbers of components, commencing with 4 factors, were fitted to the sensory data until the core consistence results had stabilized.^{14,16} Factor loadings from the model were plotted to visualize the relationship between samples and sensory attributes. To assess the quality of the loadings, the residual sum of squares for each mode (samples, attributes, and assessors) were used and variables exceeding the 99% confidence interval (CI) were flagged as those that were poorly fitted. Assessors whose responses exceeded the 99% CI of the model were removed and the PARAFAC model was recomputed with the remaining data. A sample replicate mean sensory score was computed from the remaining assessor sensory scores for each sensory attribute and this was used for regression modeling.

Wine Sample Extraction and GC-MS Analysis. Wines were assessed, extracted, and analyzed in random order on the same day of sensory panel appraisal to minimize compositional variations between sensory and analytical replicates. Prior to extraction, 50 mL of wine was spiked with 50 μL of internal standard (IS) comprising 3-*tert*-butyl-4-hydroxyanisole to give a final concentration of 4000 $\mu\text{g L}^{-1}$. Samples were extracted with LiChrolute-EN cartridges (Merck) as described previously.¹⁷ Briefly, solid-phase cartridges were conditioned with 4 mL of each of the following solvents: dichloromethane, methanol, and 12% ethanolic aqueous solutions. The wines were passed through each cartridge at approximately 2 mL min^{-1} , followed by a rinse with 2 mL of 12% ethanol aqueous solution and dried using low pressure (approximately -0.3 bar for 15 min). The retained components were eluted with 1.5 mL of dichloromethane and spiked with 25 μL of IS mix comprising 2-octanol and 4-hydroxy-4-methyl-2-pentanone to give final concentrations of 6670 and 6713 $\mu\text{g L}^{-1}$, respectively, transferred to sample vials, and capped for storage at 4 $^{\circ}\text{C}$ until analysis was conducted.

For GC-MS analysis, 1 μL of extract was injected into an Agilent 7890 gas chromatograph fitted with a 60 m \times 0.25 mm internal diameter fused-silica capillary column with a 0.25- μm wax (DB-WAXetr) stationary phase (J&W Scientific, Folsom, CA) using a Gerstel MPX autosampler with a Peltier tray cooler at 4 $^{\circ}\text{C}$. The injector temperature was 240 $^{\circ}\text{C}$; septum purge flow was 3 mL min^{-1} , and the split ratio was 10:1. The helium flow rate through the column was 1.5 mL min^{-1} with an average velocity of 31 cm sec^{-1} ; the column temperature was held at 40 $^{\circ}\text{C}$ for 5 min, then increased at 2 $^{\circ}\text{C min}^{-1}$ to 210 $^{\circ}\text{C}$ and held for 20 min. Mass spectra were collected at a scan rate of 4.4 s^{-1} using an Agilent 5975C mass detector operating in electron ionization mode, scanning from 35 to 350 m/z with a detection threshold of 100. The detector was switched off between 6.60 and 8.65 min during efflux of solvent. Transfer line temperature was set to 210 $^{\circ}\text{C}$, source temperature was 230 $^{\circ}\text{C}$, and quadrupole temperature was 150 $^{\circ}\text{C}$. Total elution time during which mass spectra were collected for each sample was 110 min.

Multivariate Curve Resolution Alternating Least Squares (MCR-ALS) Analysis of GC-MS data. All GC-MS files were exported in three-dimensional csv format from MSD Chemstation version E.02.00.493 for processing in MATLAB version R2011b (Mathworks, Natick, MA) where all data treatments procedures were performed. The total ion chromatogram (TIC) for each sample was overlaid without spectral alignment to enable identification of appropriate time windows for automated processing. Although this process required inspection of all samples, it is the only manual component of the peak area integration and spectral extraction process and takes less than 30 min to inspect the entire data set. Each time window was selected on the basis of a stable baseline and that incorporated peaks of a similar

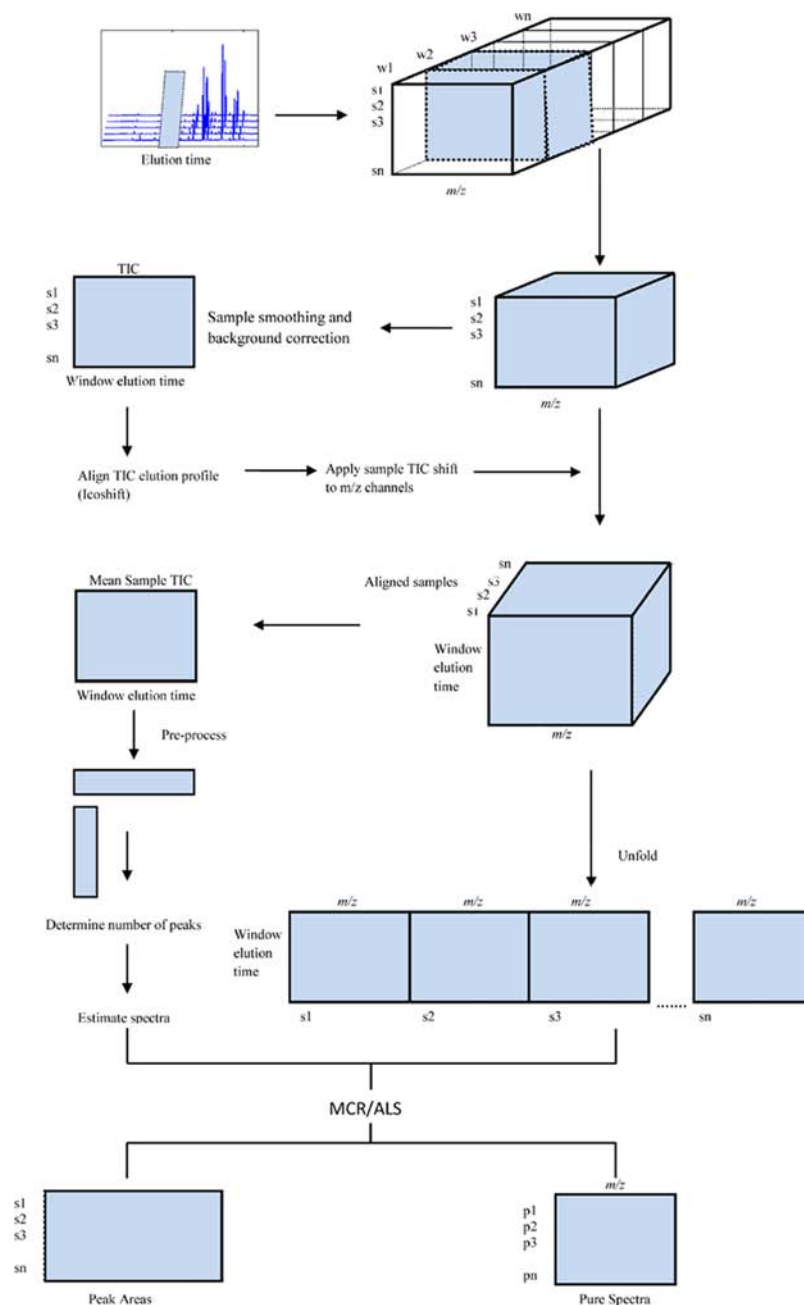


Figure 1. TIC elution profiles are examined to identify time windows based upon peak profiles. Each time window is then automatically processed separately by smoothing, background correction, and TIC peak alignment. The TIC shift is then applied to each sample m/z channel to create a 3D matrix of aligned spectra for the time window. PCA of the mean TIC is used to determine the number of peaks in the time window along with an initial estimate of spectra. The unfolded aligned spectral matrix is then processed by MCR/ALS to extract pure spectra and peak areas for each sample. This process is repeated for each time window. Spectra are exported to NIST for preliminary compound identification, and sample peak areas are collated for further data analysis.

profile height, across an average elution time of approximately 1.5 min. Files were processed using a modified approach to published procedures.¹⁸ Each m/z channel was smoothed by subtracting the minimum value and filtered by convolution using a vector of size 7. Convolution is advantageous in that the signal is linearly filtered and shift invariant thereby retaining elution time profiles. A smoothed and corrected TIC for each sample was then determined from the sum of each m/z channel in the time window, and this profile was used for alignment of elution profiles using the maximum cross-correlation approach with the mean sample as the target vector.¹⁹ The sample shift from the alignment of the TIC was then applied on a sample-by-sample basis to each m/z channel to align all sample elution profiles within the time window thereby creating an aligned three-dimensional

matrix (elution time* m/z channel*sample). A two-dimensional matrix representing the mean m/z elution profile was then used to determine the number of interesting features (peaks) within the time window. The number of features was determined by principal component analysis (PCA) of the transformed matrix; an offset of 1 was added to all m/z channels which were then down weighted by log10 transform, mean centered, and variance scaling using the Pareto equation prior to PCA. This approach accommodates the number of peaks observed within the time window without overestimating features of interest.^{11,20} To enable the iterative MCR-ALS procedure, an initial estimate of either peak intensity or spectra is required. Spectra associated with each peak of interest were estimated with the SIMPLISMA approach²¹ using the PURE algorithm. Estimated spectra

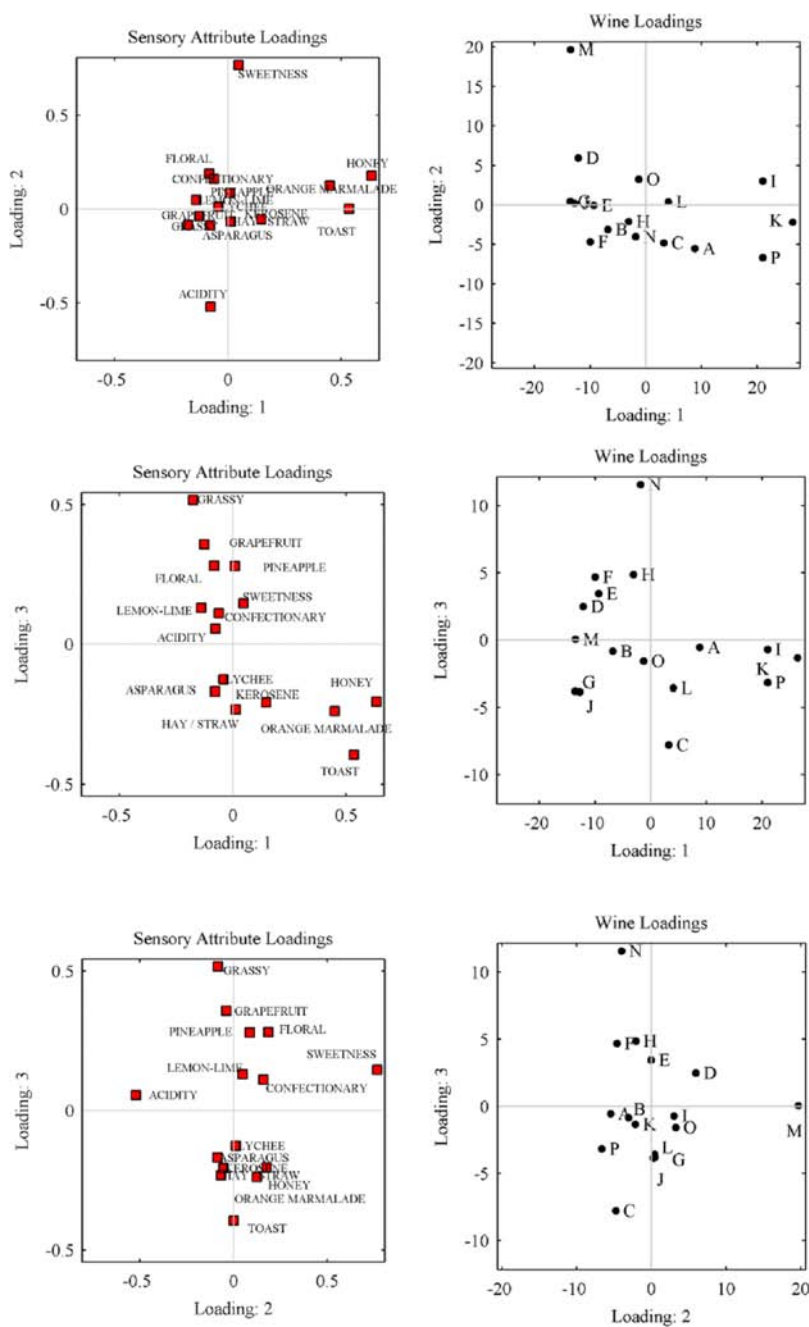


Figure 2. PARAFAC loadings for wines and sensory attributes extracted from sensory descriptive analysis. Wines are coded by letter as identified in Table 1

were then passed along with the column augmented elution profile of all samples (elution time*(samples*m/z)) in the time window to the MCR-ALS algorithm.¹⁰ MCR-ALS iteratively determines the matrix concentration (C) and spectral (S) profiles of interesting features by solving the classic least-squares matrix equation

$$\mathbf{X} = \mathbf{CS}^T + \mathbf{E} \quad (1)$$

where X is a matrix of the unfolded elution time window of dimensions N (elution time) by J (samples (K)*m/z scans (L)); C is a matrix of concentrations of dimensions (N*K) by R (number of features or peaks to be determined), i.e., the chromatographic profile; S are the spectral profiles of dimensions R by L, and E are the residual errors (N by J).

To solve this problem, the MCR-ALS approach iteratively solves for C and S using the following equations:

$$\mathbf{C} = \mathbf{XS}(\mathbf{S}^T\mathbf{S})^{-1} \quad (2)$$

$$\mathbf{S} = \mathbf{X}^T\mathbf{C}(\mathbf{C}^T\mathbf{C})^{-1} \quad (3)$$

where superscript T and -1 represent the transposed and pseudoinverse matrices, respectively. Thus for each iteration an updated estimate for both C and S can be obtained until the solution converges and the residual matrix remains unchanged. For each iteration of the ALS process constraints must be applied to the concentrations and spectra such that C must always be positive, all negative values replaced by zero; C is unimodal for all samples; elution time profiles for samples must not vary by more than 2.5%; S must always be positive and of equal length. The approach for peak table and spectral data extraction from the GC-MS files is summarized in Figure 1.

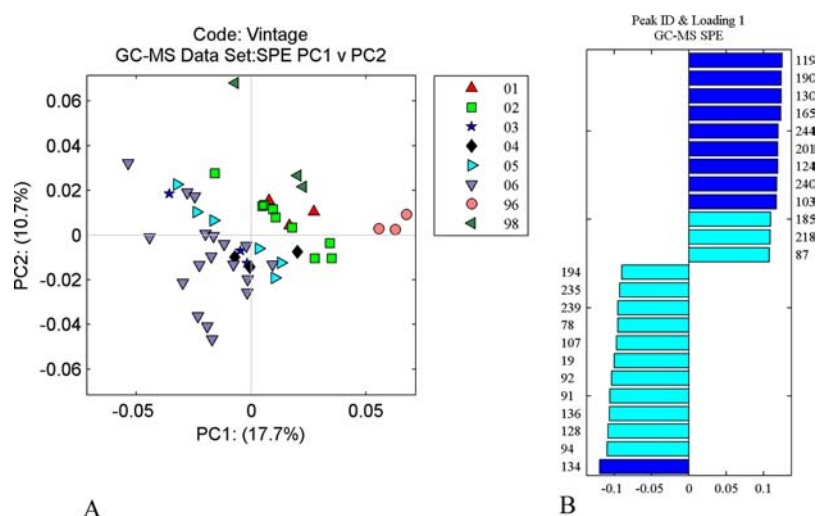


Figure 3. (A) PCA scores of peak areas extracted by MCR-ALS with wines coded for vintage. (B) Loadings from the PCA and peak number indicated on the vertical axis. Darker loadings indicate absolute peak loadings exceeding the 90th percentile.

Spectral libraries of peaks derived from MCR-ALS were exported from Matlab in a format compatible with the National Institute of Standards and Technology (NIST) Mass Spectral Search Program (version 2.0) using purposely written scripts. Identification of target compounds of interest and internal standards was conducted by matching the extracted spectra with the NIST-08 Library, and confirmed using published retention time indices and/or pure compounds run in identical GC-MS conditions. Retention indices were determined by analyzing an alkane standard mix (C8–C32 *n*-alkane) in hexane using the same oven ramp and gas flow conditions.

Analysis of GC-MS Peak Areas to Identify Interesting Sample Groups. Extracted peak areas were preprocessed by normalizing all peaks to the peak area associated with the injection IS (4-hydroxy-4-methyl-2-pentanone) to eliminate minor injection discrepancies between samples and then to the peak area for the IS for extraction (3-tert-butyl-4-hydroxyanisole) to eliminate discrepancies associated with extraction efficiency. Preprocessing of peak areas was done as described above prior to PCA. Peaks associated with the IS were excluded from the PCA. Interesting sample groups were identified in scores plots, and a nominal cutoff of 0.9 times the highest absolute loading value for a specific principal component (PC) was used to establish important peaks associated with these groups.

To facilitate interpretation of PCA models, factor rotation algorithms were employed. Typically PCA models have loadings rotated to maximize simplicity and assist interpretation, however, it is also possible to rotate scores to simplicity when specific groupings of samples are expected²² such as the present investigation. Factor rotations may be either oblique or orthogonal and for ease of interpretation orthogonal rotation was used as preservation of the angular dependence of loadings and scores was maintained. Varimax and quartimax rotations were investigated by conducting PCA on the preprocessed peak table and then rotating the PCA model in accordance with published methods.²²

Identification of Peaks Associated with Wine Sensory Features. The peak data table was regressed across the replicate mean sensory values for each wine calculated following the removal of the poorly performing sensory assessors' results. Prior to regression, sample replicates were assigned to either a calibration or independent test data set to determine the predictive ability of the regression models such that both data sets contained at least one independent sample replicate for each different wine (Table 1) and approximately 60% of the samples were placed in the calibration set. Peak selection within the calibration set was conducted using the forward interval (iPLS) regression for each analyte and model combination.²³ Predictive models of the sensory attributes from the peak areas were constructed using either PLS1 or PLS2 regression analysis using the SIMPLS algorithm with cross validation using random sample subsets

with six data splits. The number of latent variables chosen for each model was determined from eigenvalues and inspection of the local minima of the root-mean-square errors of calibration, cross validation, and prediction such that each metric did not substantially deviate from the others. Sample outlier and influence plots (data not shown) were constructed to identify samples with *Q* residuals or Hotelling T^2 values exceeding the 95% confidence interval and these samples were excluded and the regression model was recomputed.²⁴ For PLS2 models multiple predictands were selected based upon the PARAFAC modeling of sensory data such that attributes closely modeled by the same loading or those with similar loading values (Figure 2) were modeled together. For PLS models with good predictive ability, the variables (peaks) used in the model were identified, and regression coefficients and selectivity ratios (SR)²⁵ were plotted to visualize the influence of each peak on the model. The SR enabled the identification of peaks with the most influence on the predictive models of the sensory scores and these peaks identified using NIST database searches of extracted spectra, matching corresponding retention indices and injection of pure compounds where possible. The NIST search was conducted using a maximum *m/z* constraint of 350 and results were filtered to only include forward match and reverse match results of greater than 800. All PCA and PLS regression analysis was performed using the PLS toolbox (version 6.5, eigenvector Research Inc., Wenatchee, WA).

RESULTS AND DISCUSSION

Sensory Modeling using PARAFAC. PARAFAC modeled the sensory scores using three loadings for each mode (wine samples, sensory attributes, and panellists). Responses from one panellist were removed from the data cube prior to the final model as the sum of squares of residuals for this assessor was greater than the 99% CI (Supporting Information Figure S1). The final PARAFAC model explained 19% of sensory data variability with a core consistency of 94%. While this is a low amount of explained variability, the crucial sensory features for aged HVS are well-defined. Loading plots for modes 1 (wines) and 2 (sensory attributes) are shown in Figure 2. Loading 1 models wine sensory features associated with bottle development. The attributes of honey, toast, and orange marmalade aromas are distinctive and sought-after features of quality aged HVS wines. Wines I, K, and P have the most accentuated developed character and the highest sensory ratings for these characters. These three wines, together with the wines A, L, and C are all positively located in loading 1 and belong to "style

Table 2. Identity of Compounds Associated with Hunter Valley Semillon Bottle Age and Wine Style Identified in PCA Plots

peak ^a	compound	CAS	Kovats RI expt	Kovats RI reported ^b	identified ^c	reported aroma ^d
62	1-pentanol	71-41-0	1213	1244 ⁴³	RI, MS, cmp	balsamic
68	acetoin	513-86-0	1222	1291 ⁴⁴	RI, MS, cmp	butter, cream
73	2-hydroxy-2-methylbutyric acid	3739-30-8	1303	1361 ⁴³	RI, MS, cmp	
103	furfural	98-01-1	1412	1482 ⁴⁵	RI, MS, cmp	bread, almond
105	2-acetyl furan	1192-62-7	1502	1511 ⁴⁶	RI, MS	balsamic
119	2,3-butanediol (R,S)	513-85-9	1509	1582 ⁴³	RI, MS, cmp	fruit
124	isobutyric acid	79-31-2	1511	1584 ⁴⁴	RI, MS, cmp	rancid, butter, cheese
130	ethyl-2-furoate	614-99-3	1605	1599 ⁴³	RI, MS	
133	ethyl decanoate	110-38-3	1606	1641 ²⁸	RI, MS, cmp	grape
134	4-methyl benzaldehyde	529-20-4	1607	1644 ⁴⁷	RI, MS	
137	ethyl methyl succinate	4676-51-1	1607	1641 ⁴⁸	RI, MS	
153	diethyl glutarate	818-38-2	1801		RI, MS, cmp	
163	2-acetyl-2-methyltetrahydrofuran	32318-87-9	1806		MS	
165	benzyl alcohol	100-51-6	1900	1851 ⁴⁹	RI, MS, cmp	sweet, flower
172	NID (MW 151) ^e					
180	ethyl 3-hydroxy-3-methylbutanoate	18267-36-2	2001		MS	
190	1H-pyrrole-2-carboxaldehyde	1003-29-8	2003	2036 ⁵⁰	RI, MS	
201	1-methyl-1H-pyrrole-2-carboxyaldehyde	1192-58-1	2074	2112 ⁵⁰	RI, MS	
217	NID (C13 norisoprenoid, MW 210)		2202		MS	
230	triethyl citrate	77-93-0	2500		RI, cmp	
231	2-furoic acid	88-14-2	2501		MS	
240	vanillin	121-33-5	2504	2598 ⁵¹	RI, MS, cmp	vanilla
244	acetovanillone	498-02-2	2603	2685 ⁴⁵	RI, MS, cmp	vanilla

^aPeak numbering is assigned on chromatographic elution profile. ^bRI: published retention indices for similar stationary phase. ^cMS: NIST mass spectral database match with minimum forward and reverse matching of 800. cmp: matched compound with pure standards ^dReported aromas from Flavornet (<http://www.flavornet.org>) unless indicated. ^eNot identified, tentative molecular weight indicated.

4",¹² i.e., typical of aged HVS. This group of wines contains the oldest wines examined, ranging in vintage year from 1996 through to 2004. Loading 2 models the mouth feel sensations of acidity and sweetness with each of these attributes at opposing dimensions for this loading. The position of each of these sensory features within loading 2 is to be expected, as the presence of residual sugar moderates the perception of acidity.²⁶ One wine, designated M, is modeled by this dimension and is the only wine for style 1 in the study. Loading 3 models the remaining sensory attributes with prominent primary fruit-derived features in the positive segment and the typical aged sensory features possessing negative loading values. No distinctive separation of wines according to wine style is obvious within this dimension.

MCR-ALS Extraction of Peaks and Peak Table. A peak area table consisting of a total of 253 peaks, including the internal standards, was obtained from the chromatographic profiles and this was subjected to data analysis. The extracted peak areas ranged in several orders of magnitude and thus logarithmic transformation and Pareto scaling was considered appropriate to moderate the influence of compounds with large MSD responses relative to concentration and is frequently employed for metabolomic studies.²⁷ PCA of the peak area table, excluding the internal standards, shows that wines are positioned along PC1 (Figure 3A) according to vintage and hence bottle age. Good clustering for each wine sample triplicate indicates that the extraction, GC-MS analysis, and MCR-ALS analysis procedures are robust and free from spurious results. The corresponding loading bar chart for PC1 identifies peaks with the most significant influence upon the PCA. A nominal value of the 90th percentile of peaks based upon the absolute value of the largest loading from the PCA was used to identify the most significant peaks areas (Figure

3B). The identity of the compounds associated with these peaks was confirmed with a NIST database search of the extracted spectra, matching Kovats retention time indices (Table 2) and pure compounds when available. Of interest are the positive loadings for vanillin and acetovanillone which are usually associated with extraction from oak wood during wine conservation. HVS however, is typically a nonoaked wine and the presence of vanillin and acetovanillin in aged HVS suggests other mechanisms are responsible for their formation, and low concentrations of these compounds have been reported in Semillon juice.³⁵ Ferulic acid, a grape-derived hydroxycinnamic acid, is reported to form vanillin in ethanolic solutions³⁶ and this may be an important mechanism in the development of HVS bottle age character.

The majority of the important compounds have positive loadings (Figure 3) and it can therefore be inferred that these compounds increase in concentration during bottle age. This group of compounds is characterized by a range of ethyl esters of organic acids, furan based compounds, and acetoin. These results are generally consistent with reported increases in ethyl esters of both branched and diprotic organic acids for bottle-aged wines,³¹ wines aged at different temperatures and or duration,^{30,32} and measures of volatile compounds during accelerated aging conditions of Chardonnay.³³ The concentration of furanic derivative compounds is also reported to increase in wines with residual carbohydrate during aging.^{32,34} As some of the HVS wines in this study have considerable cellar age it is therefore expected that the formation of these compounds would occur during the maturation process.

HVS wine styles are generally characterized by the presence of residual sugar (style 1), the spectrum of fruit flavor (styles 2 and 3), and bottle-aged character (style 4).¹² Rotated scores coded for wine style show a general separation of samples

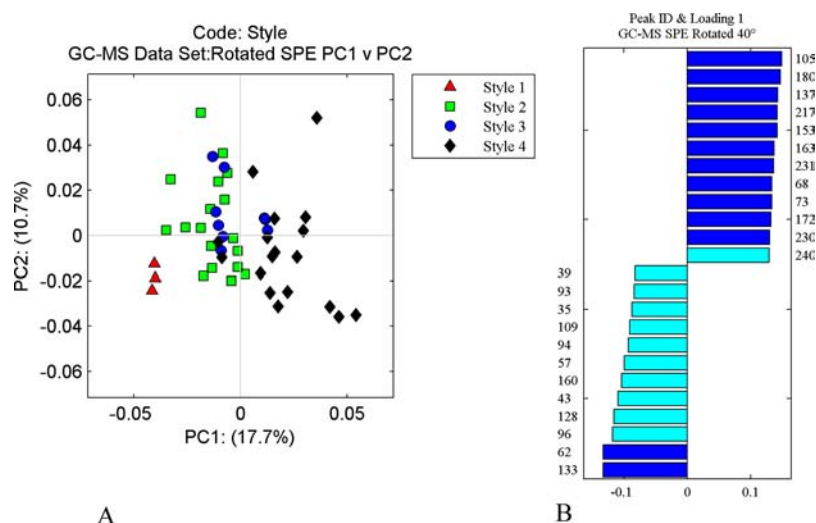


Figure 4. (A) Quartimax rotation of PCA scores with wines coded for style. (B) Rotated loadings from the PCA and peak number indicated on the vertical axis. Darker loadings indicate absolute peak loadings exceeding the 90th percentile.

according to style (1 and 4) along PC1 (Figure 4) and the variable loadings (Figure 4), which illustrate the most important compounds based upon the relative loading values, are generally in agreement with previously reported sensory description of aged HVS wines arising from the formation of compounds during wine aging reactions.¹² The loading directions infer that two compounds, 1-pentanol and ethyl-decanoate, diminish in concentration within the wines during aging (loadings are negative) which is in agreement with previously reported measures of these volatile compounds in young and aged HVS,²⁸ bottle-aged Verdejo,²⁹ and bottle-aged Sauvignon blanc.³⁰

Prediction of Sensory Scores from GC-MS Peaks. PLS regression of the extracted peak areas across the sensory scores was conducted using both PLS1 for single, and PLS2 algorithms for multiple, sensory attributes based upon the sensory PARAFAC modeling. PLS2 models did not fit the data more clearly and regression coefficients were more difficult to interpret than PLS1 models for single attributes and therefore only PLS1 models are presented. Sensory data are inherently noisy (variable) and thus a conservative minimum value for R^2 of 0.70, and a ratio of the range of sensory attribute scores to the RMSEP of 5 or greater, analogous to the range error ratio,³⁷ was used as a measure of the predictive value for each model (Table 3). Sensory attributes for which good models were established were honey, toast, orange marmalade, and sweetness, and these sensory attributes were also those with the largest range of panellist scores and which broadly help define some of the most important sensory features of HVS styles. The range of sensory scores for all attributes is relatively low reflecting the subtle sensory profile of HVS, and attributes with the largest range of sensory ratings tend to be those associated with bottle age. The low range of sensory scores in this investigation highlights the requirement for a well trained panel that can reliably and consistently rate specific sensory attributes so objective models of the sensory data can be derived from GC-MS profiles. Identification of the peaks selected for PLS regression models (Table 4) and examination of the regression coefficients (Figure 5) reveals the relative change in peak area and thus concentration of each compound in the PLS model. For most compounds the regression coefficients are positive

Table 3. Sensory Scores Mean, Range and PLS Regression Model Performance for Prediction of Sensory Attributes Using the Extracted Peak Table from MCR-ALS GC-MS Profiles

sensory attribute	sensory scores			PLS model performance			
	min	max	mean	R^2	RMSEP	RER ^a	LV ^b
lemon–lime	2.21	4.67	3.58	0.580	0.447	0.18	7
floral	0.83	2.97	1.63	0.256	0.482	0.23	2
grapefruit	1.21	2.83	2.15	0.397	0.469	0.30	6
pineapple	0.87	2.27	1.61	0.560	0.317	0.23	5
confectionery	0.52	2.35	1.11	0.321	0.396	0.32	3
hay/straw	1.49	3.63	2.63	0.346	0.511	0.24	5
grassy	0.52	2.40	1.37	0.573	0.418	0.22	4
asparagus	0.50	1.63	0.98	0.439	0.283	0.25	4
lychee	0.67	1.96	1.22	0.332	0.431	0.33	10
orange marmalade	1.07	4.77	2.20	0.876	0.394	0.11	6
honey	2.03	6.87	3.65	0.824	0.580	0.12	4
toast	1.61	5.83	3.44	0.837	0.605	0.14	2
kerosene	1.48	3.43	2.49	0.480	0.526	0.27	5
acidity	4.03	6.10	5.06	0.689	0.422	0.48	6
sweetness	2.60	5.70	3.57	0.719	0.552	0.18	5

^aRange error ratio (response range: RMSEP). ^bNumber of latent variables used in regression modeling.

indicating an increase in concentration of the compounds as the sensory ratings increase and development during aging.

Although inspection of the regression coefficients offers insight to the overall predictive power, it offers no insight to the explanatory value of specific variables within the model. The iPLS selection technique continuously adds variables until convergence within the model. Consequently, peaks are selected but may contribute only small improvements to the predictive acuity once the most influential peaks have been selected. The SR indicates the relative importance of the variables in the predictive models based upon the explained variance and has better discrimination for metabolomic studies than variable importance in projection scores.³⁸ A higher SR means that the specific variable explains a greater proportion of the variation for the predicted attribute. Based upon the SR, the

Table 4. Identity of Compounds with High Efficacy for Predictive Models of Sensory Attributes for HVS

predicted sensory attribute	peak	compound	CAS	Kovats RI	Kovats RI reported ^a	identified ^b	reported aroma ^c
honey	130	ethyl-2-furoate	614-99-3	1605	1599 ⁴³	RI, MS	sweet ⁴¹
honey	233	5-hydroxymethyl furfural	67-47-0	2501	2410 ⁴³	RI, MS, cmp	cardboard; blackberry ⁵²
honey & toast	240	vanillin	121-33-5	2504	2555 ⁵¹	RI, MS, cmp	vanilla
toast	117	2,3-butanediol (R,R)	6982-25-8	1507	1523 ⁵³	RI, MS, cmp	butter, cream
toast	153	diethyl-glutarate	818-38-2	1800		MS, cmp	
toast	211	4-vinyl guaiacol	7786-61-0	2201	2192 ⁴⁵	RI, MS, cmp	clove, curry
toast	245	3-oxo- α -ionol	34318-21-3	2603	2623 ⁵⁴	RI, MS	spice
orange marmalade	51	3-penten-2-one	625-33-2	1118	1120 ⁵³	RI, MS, cmp	fruity ⁵⁵
orange marmalade	116	propanoic acid	79-09-4	1507	1523 ⁵³	RI, MS, cmp	pungent, rancid, soy
orange marmalade	126	NID (MW 105) ^d		1601		MS	
orange marmalade	128	3,7-dimethyl-1,5,7-octatrien-3-ol	29957-43-5	1603	1586 ⁵⁶	RI, MS	citrus, floral ⁵⁷
orange marmalade	165	benzyl alcohol	100-51-6	1900	1883 ⁵⁸	RI, MS, cmp	sweet, flower
orange marmalade	237	ethyl succinate	1070-34-4	2404	2440 ⁵⁹	RI, MS	
orange marmalade	243	pyroglutamic acid, ethyl ester	7149-65-7	2602		MS	
sweetness	58	ethyl hexanoate	123-66-0	1210	1239 ⁵¹	RI, MS, cmp	apple peel, fruit
sweetness	106	2-ethyl hexanol	104-76-7	1500	1446 ⁴³	RI, MS, cmp	rose, green
sweetness	124	isobutyric acid	79-31-2	1511	1584 ⁶⁰	RI, MS, cmp	rancid butter, cheese
sweetness	143	NID (MW 248)		1704			
sweetness	175	2-phenyl ethanol	60-12-8	1902	1940 ⁴⁵	RI, MS, cmp	honey, spice, rose
sweetness	180	ethyl-3-hydroxy-3-methylbutanoate [†]	18267-36-2	2001		MS	
sweetness	181	2,6-dimethyl-7-ocene-2,6-diol	29210-77-3	2001	1949 ⁵⁵	RI, MS	smoked, fruity ⁵⁴
sweetness	217	NID (C13 norisoprenoid, MW 210)		2202		MS	
sweetness	258	tyrosol	501-94-0	2750	3012 ⁴³	RI, MS	

^aRI: published retention indices for similar stationary phase. ^bMS: NIST mass spectral database match with minimum forward and reverse matching of 800. cmp: matched compound with pure standards. ^cReported aromas from Flavornet (<http://www.flavornet.org>) unless indicated. ^dNot identified, tentative molecular weight indicated.

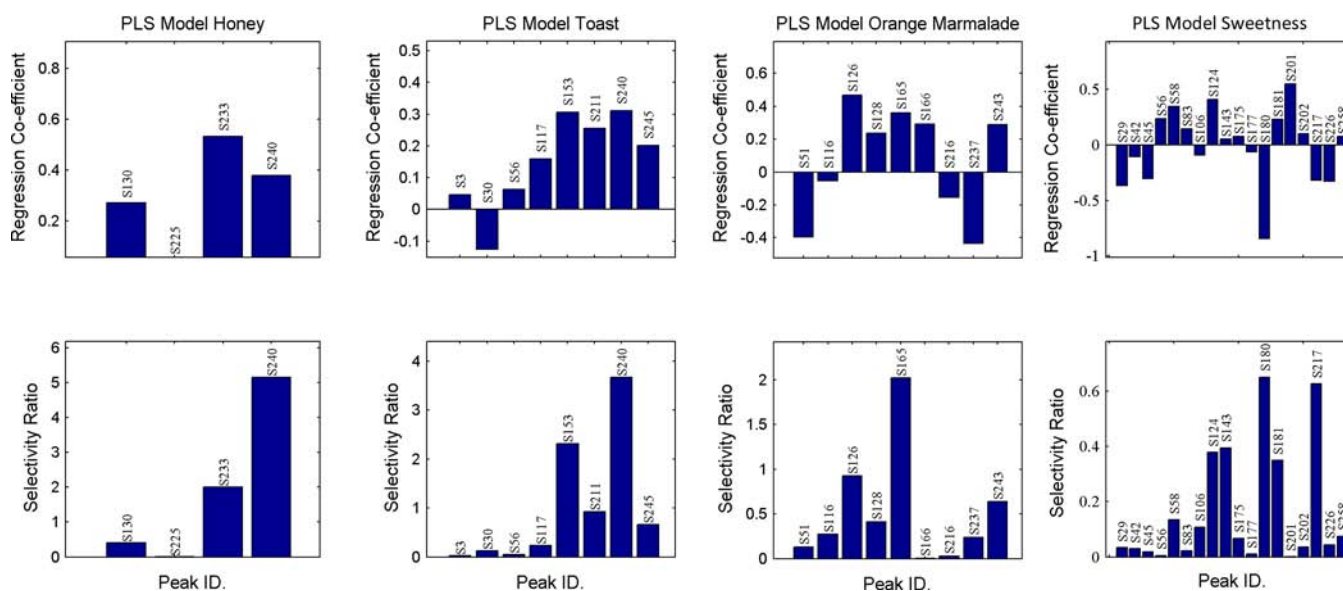


Figure 5. Regression coefficients and selectivity ratios for peaks areas used for predictive models of sensory attributes. Peak identification numbers appear above each bar.

most influential peaks have been identified for the predictive models of sensory attributes with the largest range of sensory scores.

The important compounds identified for predictive models and their reported aromas are listed in Table 4. Most of these compounds could be readily identified using matching data from reported Kovats retention indices, NIST searches, and, where available, analogous compounds run under the identical instrumental conditions. The sensory acuity of honey was

modeled with the fewest compounds, the most important being vanillin and 5-hydroxymethylfurfural (SHMF) and of lesser importance ethyl-2-furoate. One compound associated with honey aroma could not be identified and the SR for this compound was low reflecting an overall low level of influence for this compound. As mentioned previously, the presence of vanillin in HVS is likely a consequence of bottle aging. Furanic based compounds are important aroma constituents with their formation arising from Maillard reactions and nonenzymatic

carbohydrate degradation. At low pH, furfural and 5-HMF form from pentose and hexose condensation and rearrangement reactions, respectively, and subsequent reactions result in the formation of a variety of aroma compounds.³⁹ The long-term storage of foods in heat or in acidic environments enhances their production and it is expected that this class of compounds would arise in aged HVS. Furfural is also a nonoxidative degradation product of ascorbic acid⁴⁰ and may accumulate during the bottle aging of wines. The contribution of ethyl furoate is of less importance to the perception of honey aroma based upon the SR. This compound has also been reported to be associated with the perception of sweet aromas in sherry wines along with increases in concentration of SHMF during aging.⁴¹ Furoic acid can emanate from the oxidative degradation of ascorbic acid⁴² and hence may form the ethyl ester over time or with increased temperatures as reported previously.^{30,32} The perception of toast is influenced chiefly by five compounds which, in descending order of the SR, are vanillin, diethyl glutarate, 4-vinyl guaiacol, 3-oxo- α -ionol, and 2,3-butandiol. With the exception of diethyl glutarate these compounds have reported aromas of vanilla, clove/curry, spice, and cream/butter, respectively. All of these compounds have positive regression coefficients indicating they contribute positively to increased toast aroma perception in the wines as their concentration increases. The predictive model for orange marmalade consists of a range of compounds with diverse chemical class including alcohols, aliphatic acids, carbonyls, norisoprenoids, and ethyl esters. Compounds modeled with positive regression coefficients and large SR have reported aromas of sweet, citrus, and floral (Table 4) with the most important of these being benzyl alcohol. Of interest is the inclusion of 3-penten-2-ol and ethyl succinate with negative regression coefficients indicating the contribution of these compounds diminishes as orange marmalade aroma intensifies during bottle development. The SR for these compounds is relatively small indicating a minor contribution to the overall sensory rating for this attribute. One compound (peak 126) has a moderate influence on the model, and could not be identified based upon the stringent criteria used in this investigation, and olfactory detection may be required to assist in the authentication of this peak.

Of interest is the good model for sweetness which is typically a mouthfeel attribute in wine and could reasonably be expected to not model well with GC-MS data. The PLS model for sweetness is the most complex of the models in this investigation, consisting of 18 compounds with 2 of these not identified. The diversity of the identified compounds, regression coefficients, and SR for the compounds in this model indicates a complex array of aromas associated with sweetness. This is in agreement with the reported association of the perception of sweetness in HVS with both floral and honey attributes.¹² The predictive models thus obtained from the GC-MS profiles using MCR-ALS in this study enable the elucidation of the chemical attributes associated with all important sensory dimensions for the reported styles of HVS.

Identification of important marker compounds associated with style and bottle age character provides clues to the chemical development of HVS wine bouquet during aging. Full descriptive sensory analysis of the wines conducted simultaneously with the GC-MS profiling has enabled good predictive models of the sensory attributes that define HVS styles. While the sensory attributes of honey, toast, and orange marmalade appear to develop along a similar dimension during bottle aging

of HVS, it is clear from the PLS models that each sensory character is composed of distinguishing compounds. With the exception of vanillin, which is important for both honey and toast, no other compound is replicated within the predictive models. The use of SR for ranking the relative merit of compounds associated with the prediction of sensory features from the GC-MS data provides objective insight into the importance of specific marker compounds associated with wine sensory features. Future applications of rapid and automated GC-MS data analysis will enable consumer preference ratings to be more rapidly linked to objective instrumental measures and ascertain the impact of wine production techniques upon the composition of wine.

■ ASSOCIATED CONTENT

📄 Supporting Information

Figure S1: Residual sum of squares for each mode of the sensory data cube from PARAFAC modeling. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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■ ABBREVIATIONS USED

HVS, Hunter Valley Semillon; GC-MS, gas chromatography mass spectroscopy; OAV, odor activity value; CI, confidence interval; IS, internal standard; TIC, total ion chromatogram; MCR-ALS, multivariate curve resolution alternating least-squares; PCA, principal component analysis; PC, principal component; PLS, partial least-squares; PARAFAC, parallel factor analysis; SR, selectivity ratio; NIST, National Institute of Standards and Technology; SHMF, 5-hydroxymethylfurfural

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