RAPID AUTHENTICATION OF CONCORD JUICE CONCENTRATION IN A GRAPE JUICE BLEND USING FOURIER-TRANSFORM INFRARED SPECTROSCOPY AND CHEMOMETRIC ANALYSIS

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ABSTRACT

Concord grape juice is associated with many health benefits, and so it can be sold at a premium price. However, there is currently no method to verify the percent composition of Concord grape juice in grape juice blends. In order to guard against potential adulteration, a rapid method for authentication is required.

Fourier Transform Infrared (FT-IR) spectroscopy was used to develop a model which predicts the percent composition of Concord grape juice. The model was based on a training set of 64 samples with Concord concentrations ranging from 50-100%. Data was collected on an external validation set with a standard error of prediction of 5.55% using 7 factors.

The results suggest the feasibility of using FT-IR coupled with chemometrics as a production-scale tool for authentication claims of Concord in grape juice blends, protecting consumers and businesses against deceptive labeling.

INTRODUCTION

Grape juice is a valuable commodity, making it susceptible to potential adulteration. According to the USDA, the U.S. grape industry has consistently been the most valuable crop among nuts, fruits and vegetables (Amanor-Boadu, Boland, Barton, Anderson, & Henehan, 2003). Grapes account for roughly one-fifth of the total fruit cash receipts (Pollack & Perez, 2008) and the consumption of grape juices increased by 13% from 1995 to 2002 (United Nations Food and Agricultural Organization, 2002). These increases reflect an increased demand for grape products that could be associated with the numerous recent findings that associate specific grape products and cultivars with a wide range of health benefits.

Specifically, Concord grape juice is often associated with many health benefits. The reported effects of Concord grape juice has included antioxidant and free radical scavenging activity, (Frankel et al., 1998; O'Byrne et al., 2002; Seeram et al., 2008; Gollucke et al., 2009) heart protective effects (Freedman et al. , 2001; Shanmuganayagam et al., 2007), and anti-aging effects on motor and cognitive functions (Shukitt-Hale, Carey, Simon, Mark, Joseph, 2006; Coles, 2007). Many of the health claims are tied to Concord grape's high phenolic content due to the presence of proanthocyanidins, anthocyanins, catechins, and flavonoids. Concord grape juice, when compared to other phenol rich beverages, ranks 3rd behind pomegranate and red wine in overall phenolic content (Seeram et al., 2008).

Given the unique health and sensory attributes of Concord grapes, the ability to identify and quantify Concord content in products is desirable. Since it has a premium value there is a risk for the potential adulteration of Concord grape juice with other grape juice varieties. Currently, chromatography is the most commonly used method for detecting adulteration and is often coupled with mass spectroscopy. However, these methods require extensive extraction and

purification steps (Nasi, Ferranti, Amato, & Chianese, 2008). In addition, chromatographic methods are time-consuming, require the use of organic solvents, and necessitate a trained technician (He, Rodriguez-Saona, & Giusti, 2007). Sensory tests require panelists to be consistently trained, and are not always accurate when used for discrimination (Martin, Mallikarjunan, & Zoeklein, 2008). Other, less common means of authentication include the use of electronic noses, which are rapid but are still not accurate enough for discrimination, and stable carbon isotope ratios, which require authenticated databases to be effective (Martin, 2008; Rossman, 2001).

The advantages of FT-IR spectroscopy as a spectroscopic technique for food analyses are well documented, including specific advantages like rapid and simple analysis, qualitative and quantitative results, no harmful solvents, non-destructive sampling, highly resolved spectra, and simple instrumentation (Baeten & Dardenne, 2002). Another advantage of FT-IR spectroscopy is that it monitors the entire chemical composition of the sample, as opposed to individual compounds, which makes monitoring subtle varietal differences feasible. Because of this, it is ideally suited for monitoring and authenticating complex food matrices. In grapes, specifically, it has been shown that differences in the composition of numerous flavan-3-ol monomers provide the best indication of varietal differences (Edelmann, Diewok, Schuster, & Lendl, 2001). FT-IR has been used to verify label claims in honey, authenticate pomegranate juice concentrate, predict the maturity and texture of cheddar cheese, discriminate red wine cultivars, and authenticate juices (Woodcock, 2009; Vardin, 2008; Fagan, 2007; He, 2007; Edelmann, 2001). Multivariate data reduction and pattern recognition techniques are used in conjunction with FT-IR spectroscopy in order to authenticate a wide range of products. Multivariate analysis has already found use in authentication and adulteration testing in such commodities as orange juice

and tomatoes (Pan & Melton, 2002; Arvanitoyannis & Vaitsi 2007). Furthermore, FT-IR spectroscopy coupled with Multivariate analysis has been been the subject of research studies evaluating its application for detecting adulteration in honey and pomegranate juice (Woodcock, 2009; Vardin, 2008).

The results of previous studies indicate the potential utility of spectroscopic techniques for authentication of food products. The objective of this study is to use FT-IR coupled with chemometrics to authenticate the concentration of Concord grape juice in a blend of several grape juice varieties.

MATERIALS AND METHODS

Grape Juice Samples

Authentic grape juice sample concentrates of 4 different grape varieties were donated by Welch's: Concord (7 different lots from 2 sources), red (3 lots from 3 sources), white (3 lots from 1 source), and Niagara (4 lots from 2 sources). All grape juice samples were shipped frozen in clear plastic or glass containers and stored at -20˚C. The entire volume of juice was thawed and mixed vigorously for 10 minutes prior to use.

Sample Preparation

Concentrates were diluted to 16^{o} Brix with distilled water using a 0-32^{o} Brix handheld refractometer (Omega's RFH Series, Stamford, CT). The samples were then semi-purified by solid phase extraction (SPE) using a Sep-Pak® Vac (6 cc, 1 g) C18 cartridge and following optimized procedures adapted from the literature (Giusti et al., 1999; George et al., 2005). Columns were pre-conditioned by washing with acidified methanol (5 ml, 0.1% HCl v/v) followed by acidified water (5 ml, 0.1% HCl v/v). The juice (5 ml) was applied onto the pre-conditioned column and phenol compounds were adsorbed onto the mini-column while the less

polar substances, essentially sugars and simple acids, were eluted by washing again with acidified water. The phenol-rich fraction was subsequently recovered with acidified methanol (5 ml) and collected.

Generation of Grape Juice Blends

To strengthen the model at high concentrations of Concord, another model was created and grape juice blends were prepared by *weight* from the phenol rich fraction obtained after SPE. This model ranged from 50-100% Concord concentration, the range where predictions in the previous model showed the most inaccuracy. Part of the error associated with the previous model was addressed by including blends of both 2 and 4 juices into this model, in addition to changing to measurements by weight. Unique blends (64) were made for the training set at regular intervals of increasing Concord concentration. The distribution of these blends is shown in Figure 5 below. Additionally, 20 more sample blends were made for the EVS.

Spectra Collection

An aliquot (15 μL) of each blend was uniformly applied to the surface of an internal amorphous material transmitting infrared radiation (AMTIR) crystal of the horizontal attenuated total reflectance (ATR) accessory (Pike Technologies, Madison, WI). The sample on the ATR accessory was vacuum dried in a vacuum desiccator to obtain a thin film, which limits the interfering absorption from moisture. The infrared spectra were recorded between 4,000 and 700 cm^{-1} on a Excalibur 3500 Fourier-Transform infrared spectrometer (Digilab, Randolph, MA) using the Resolutions Pro software (version 4.0, 2005, Varian, Inc., Palo Alto, CA). Each sample was represented by an absorbance spectrum containing 1,687 points and the entire IR range was

scanned 64 times and averaged. A 40 second delay was used to allow the sample holder time to purge environmental CO_2. For the first models (2 juice blends and original 4 juice blends prepared by volume), 3 replicate spectra were taken for each sample and concentration predictions were averaged. The similarity of each of the 3 replicates rendered this averaging exercise unnecessary and so it was eliminated from the procedure for the final model (2 and 4 juice blends prepared by weight, 50-100% Concord concentration). Since the model has potential application within industry, elimination of this tedious triple measurement step would greatly approve the rapidity of its use.

Statistical Analysis for Varietal Discrimination

The spectra were imported into the commercial multivariate statistics program Pirouette 3.11 (Infometrix Inc., Woodinville, WA). The second derivative of each spectrum was transformed using a 5-point polynomial-fit smooth function and maximum normalization function prior to multivariate analysis. By normalizing and taking the second derivative of the original spectra emphasizes the presence or absence of peaks rather than peak intensity, and therefore the presence or absence of characteristic components rather than their concentration was highlighted.

The pattern recognition method of soft independent modeling of class analogies (SIMCA) was used for classifying the high-dimensional data (infrared spectra) because it incorporates PCA for dimension reduction to summarize the most important factors (principal components) that explain nearly all of the variance as well as filter out noise. SIMCA clusters each class (grape juice variety, geography, lot, etc.) into separate probability spheres based on principal component space made up of the chosen number of principal components. The spheres were comprised of two types of points: data points that each represents complete IR spectral data of a sample and

points that represent the confidence interval of the cluster. Confidence intervals (95%) were calculated for each cluster for varietal discrimination.

Statistical Analysis for the Regression Model

The factor-based partial least squares regression (PLSR) as a calibration model used the extracted principle components to make regression models in order to predict the content of Concord grape juice in a 100% grape juice blend. A model was developed using a training set containing samples from each lot and variety of grape juice. Models were created using spectral data from unique grape juice blends of different grape juice varieties. The models generated based on these training sets were tested using a validation set of samples independent from those used in the construction of the model.

Our goal was to predict concentration of Concord grape juice with a high degree of accuracy. Predictive ability was tested with performance statistics, Standard-Error of Cross Validation (SECV) and Standard-Error of Validation (SEV).

RESULTS AND DISCUSSION

Construction of a Model for 4 Juice Blends and Validation

To improve the predictive ability of this method for higher concentration Concord blends, a model was built containing a variety of 2 and 4 juice blends ranging from 50-100% Concord grape juice concentration. In order to incorporate the complexity of blends made from multiple varieties, 4 juice blends were used and in order to incorporate as the characteristics of a blend with a large percentage of a particular variety, 2-juice blends were used. In particular, this large percentage was important to ensure accurate predictions with Concord-Niagara blends given the relative difficulty of discriminating between these two juices. Unique samples (64) made up the

training set used to build a predictive model. The model used 7 factors and had an associated error of 4.56% with an r^2 value of 0.99 as can be seen in the Y-Fit in Fig. 1. The efficacy of the model was further evaluated using an External Validation Set of 20 original samples, independent from those incorporated into the training set (Table 3). The r^2 value for the EVS was 0.94 with a standard error of prediction of 5.55%, again, using 7 factors. As opposed to the previous model, which showed more error at the extremes of its prediction range, the final model had variability equally distributed among all prediction values. Another improvement on prior attempts is both the increased number of samples in both the training set and external validation set as well as the more comprehensive distribution of the data points, when compared to data points placed at discrete intervals. In addition to the w/w measurements for blend construction and enrichment of the higher Concord concentration range, the decrease in the number of factors required likely also contributed to the decrease in associated error from 8.40% to 5.55%.

CONCLUSIONS

FT-IR analysis of grape juice samples coupled with the multivariate chemometric models, SIMCA and PLS, was found to be an appropriate method to determine the variety of an unknown, pure grape juice and the content of Concord grape juice in a grape juice blend. SIMCA was shown to provide a high level of discrimination among grape juices based on variety. Moreover, it was shown that classification of the phenolic faction depended on the presence or absence of characteristic alkene and aromatic bands which are common in phenolic compounds.

The PLS models showed a very low error of validation in comparison to a large amount of variance. The original 4 juice blend model (v/v) was able to predict Concord concentration on a

small EVS set of 6 samples with an associated error of 8.40%. To reduce this error, a much more robust PLS model containing 64 distinct blends of Concord, Niagara, red, and white in both 2 juice and 4 juice combinations was shown to have very acceptable levels for its standard error of validation. This model was focused on concentrations in the upper range of Concord concentration where the previous model was most inaccurate. It was validated against a separate set of samples and was shown to have an error of 5.55%. For application as an authentication tool within industry, this would be considered a "live model," and additional samples would continually be added to the training set to maintain and improve predictive ability.

Based on these results, FT-IR coupled with chemometrics may be a feasible method for the discrimination of pure grape juices on the basis of variety, and for the prediction of Concord grape juice in a 100% grape juice blend. It provides a method for this discrimination that is rapid, simple, and prevents the use of large quantities of hazardous solvents which has many possible applications within industry.

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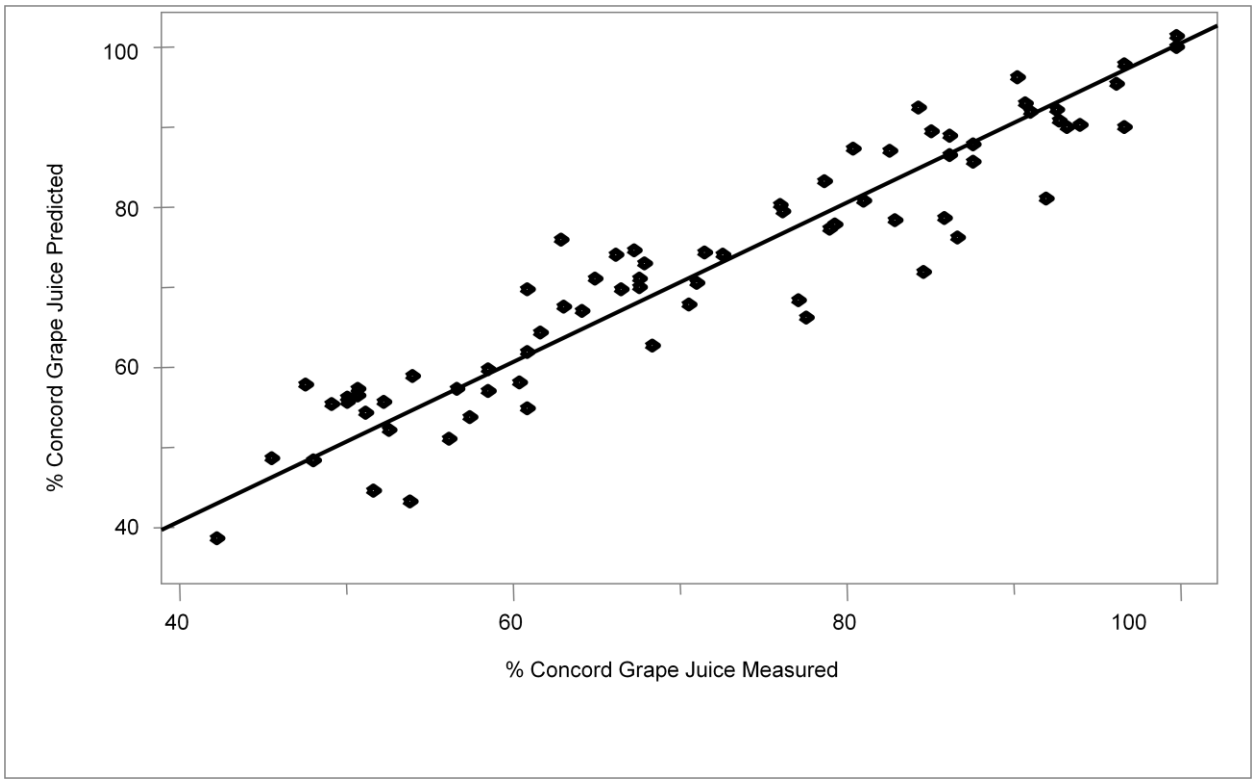
FIGURES

Figure 1: Y-Fit of PLS model of 2 and 4 juice blends made by weight
Table 1: Concord concentration predictions for the EVS for the model in Figure 10



Sample Number	Predicted % Concentration	Actual % Concentration	Difference
1	94.1	100	5.9
2	61.1	52.5	8.6
3	54.3	54.2	0.1
4	64.1	57.7	6.4
5	69.9	63.2	6.7
6	67.5	71.3	3.9
7	67.8	67.6	0.2
8	66.5	77.4	10.9
9	75.7	81.3	5.6
10	85.5	85.4	0.2
11	84.9	86.2	1.3
12	93.4	91	2.3
13	94.9	93	1.9

14	101.3	96.9	4.4
15	52.6	50.3	2.3
16	51.3	52	0.8
17	50.4	51.7	1.3
18	64.6	61.1	3.5
19	68.5	68.1	0.4
20	80.9	66.5	14.4