INTRODUCTION

Today, rapid analysis for wine can be done with simple-to-use analytical instruments and sample adjustments. The latest applications in the industry contemplate on-line analysis of wines obtained from other fruits than grapes, using instruments like the OenoFoss wine analyzer. When using this technology for new product development, the construction of local calibrations is necessary.

A first stage in the wine routine analysis includes the measurement of the main components of taste in a fruit juice. These are: sugars (expressed as TSS, glucose, fructose and sucrose) and acids (expressed as total acids, malic acid, and pH) that are two of the main quality parameters that determine taste in a fruit juice. A juice is perceived as sweet or sour depending on how much its TSS/total acids proportion varies. The challenge is then to find those fruit cultivars or juice blends that can achieve the perfect balance between sugars, acids and aroma. The Danish cold-climate apple juices are particularly interesting to the wine industry, because of their moderate sugar values and high acid levels dominated by malic acid. Combined with a broad range of local cultivar specific aroma profiles, wines can be produced with high complexity and well balanced taste.

Previous studies have looked at sugars and acidity predictions of single cultivar sets and prediction of firmness. In the present paper, we study the relationships between a sample set made up with juices of 27 local apple cultivars, their main quality components with focus on sugars (total soluble solids (TSS), sucrose, fructose, glucose, malic acid and total acids (TA), as well as their ability to produce calibration models in the MID-NIR region from 4000 to 7330 cm⁻¹ and in the MID-IR region 1235 to 3997 cm⁻¹.
MATERIALS AND METHODS

27 mid to late season Danish apple cultivars were harvested at commercial maturity in September 2014, at the research facility, Pometum, Copenhagen University. They were stored at $4^\circ C$ in cold rooms for approximately one month. Before juicing, the ripen cultivars were taken out to room temperature ($20^\circ C$) for a few hours. The fruits were milled and juiced in a 20 L hydro press (Speidel, Germany). Immediately, 50 ml samples of each cultivar were collected and frozen at $-18^\circ C$ for 1-2 months.

Before laboratory analysis, the samples were thawed slowly in a fridge at $10^\circ C$ for 24 hours. After centrifugation for 5 minutes at 4000 rpm, the clear supernatant was used for analysis. Total soluble solids (TSS) were measured with a portable calibrated brix meter (Metler Toledo ‘Quick brix 60’). Total titratable acidity (TA) was measured by a Titrino (719 S Titrino Metrohm) titrating with 0.1 N NaOH to pH 8.1. Results were expressed in %Brix and grams of malic acid (most abundant acid in apple juices) per liter of apple juice (g/l).

Fructose, sucrose, glucose and malic acid (g/l) were determined by Ion-Chromatography according to Liu et al. 2015. 1 ml of sample was filtered through a 45µm filter and diluted 50 times with demineralized water before IC analysis. All the samples and standards were injected four times.

The mid-near infrared range (4000 - 7330 cm$^{-1}$) and Mid infrared MID-IR (1235 to 3997 cm$^{-1}$) of each sample was measured on a OenoFoss™ (FOSS, Hillerød, Denmark). 0.6ml of clear juice sample was used. The spectral resolution was 4 cm$^{-1}$ and the data was recorded as absorbance. The total number of data points per spectrum was 864. All samples were analyzed in duplicates and the models were obtained from their averaged values (one spectrum per juice cultivar).

Mean center, (MSC) and Autoscale pre-processing techniques, in the X and Y matrix respectively, were performed prior the calculation of PCA Principal Components Analysis and PLS (Principal least square) models. The reference data for sugars, acids in the juice samples were inspected using PCA. The spectrum was then included and modelled using PLS regression. Full Cross validation was used for the calculation of all the PLS calibration models. The models were calculated in Latentix™ software (Version 2.12. Copenhagen, Denmark). Descriptive statistics of the quality data were calculated in Excel 2010.

RESULTS AND DISCUSSION

Quality Data

The mean values of the 27 samples were: for TSS 11% Brix, Glucose= 9.21 g/l, Fructose=58.0 g/l, Sucrose = 32.2 g/l, TA=7.4g/l and Malic Acid=9.60g/l. These values are together with the ranges given in Table 1. Cultivars such as ‘Fynsk udvalg VI’, ‘Bedstefars æble’ or ‘Dronning Louise’ had over 12% Brix and TA between 6.8 and 7.2 g/l, while ‘Pinova’ (Germany) and ‘Golden Delicious’ (Italy), are reported with 9-15 and 13.3% Brix and TA of only 5.2 and 7.5 g/l respectively$^{9,10}$.

Most of the cultivars showed malic acid levels over 8 g/l, which are in the high end as compared with other apple juices from southern European regions$^7$. However, in a study from Campo et al. 2004$^{11}$, Basque Country, Spain, a wide range from 2.8 to 17 g/l was reported.

In addition, the content of individual sugars showed pronounced variation. For example ’Ørdings æble’ had the lowest fructose concentration, with only 23 g/l and ‘Blankholm’ had the highest 83.3 g/l. These proportions are similar to the literature $^{9,7}$.

On the other hand, none of the parameters showed a perfect normal distribution. The effects of the data structure on the multivariate models development are presented in the following sections.

Spectral data

Figure 1 illustrates the MID-IR and NIR spectra from 1235 to 7330 cm$^{-1}$ for all the juice samples. Moderated to strong absorbance peaks are observed at 4035, 4545, 4819 and 6840 cm$^{-1}$. Also a noisy region is visible between 4120 and 4772 cm$^{-1}$. A previous study from Rambla et al. 1997$^{12}$, detected strongest differences between the individual organic sugars in the spectral regions between 4877 and 4348 cm$^{-1}$ (2050 and 2300nm). The
structural similarity of sucrose to its counterparts fructose and glucose, makes their spectral response separation complicated. In addition, absorption bands around $6840 \, \text{cm}^{-1}$ have been attributed to water absorption\(^8\) and sometimes they are not included in juice calibration models. We included them in our study.

Juice Samples and spectral responses

The PCA plot in figure 2 shows the variability among the different apple juices. PC1 and PC2 described 63.9% of the total variation in the samples. The loading and score plots show that the amount of individual organic sugars and acids is cultivar dependent. For example, the cultivars ‘Pilehave’ and ‘Tagesminde æble’ (21, 24) were high in TA, whereas cultivar 20, ‘Nørregårds æble’, the lowest acidity. PC2 displays the relative quality of single cultivars related mostly to glucose and fructose. This is the case for cultivars 27 and 18, which were low in glucose and fructose, and cultivars 4,5,8 and 11 high. From a juice making point of view, the PCA plot in Figure 2 can also be used for selection of apple cultivars with desired sugars and acid profiles.

Figure 1. MID-IR and NIR spectra obtained from N=27 Danish apple juices

Figure 2. PCA Score (apple cultivars) and Loading plot (reference quality parameters) for the 27 apple juices.
PLS calibration model results

As discussed before, the apple juice set provided reference parameters with different skewness in their distribution histograms and generally, did not follow a normal distribution (data not shown). The performance of each parameter in two PLS calibration model with NIR and MID-IR spectra is shown in Table 1. Better model performances for all parameters were obtained using the full MID-IR region (1235-3997 cm⁻¹) than the full NIR region (4000 to 7330 cm⁻¹). Additionally, it appears that a high range on a reference parameter or normal distributed values do not necessarily result in highly robust calibration models. But the relative spread of data points within a parameter (SD) seems to be a reasonable indicator of model performance, when combined with proper bands.

Muresan et al. 2015¹³ showed evidences of improved calibration models from less cultivars (N=3), and higher sample number (N=36), which had a narrower spread of data than our set (Fructose SD=4.09/our=14.44; Glucose SD= 1.09/our=3.17; Sucrose SD= 1.18/our=10.34), and using band regions between 900 to 1500 cm⁻¹ and 650 to 1200 cm⁻¹, for the PLS analysis. They also used a more elaborated pre-treatment and band selection, for each parameter such as: elimination of noisy bands, selection of the appropriate pre-treatment for spectra, raw data and its combinations.

The results for TA and TSS, which SD were 1.38 and 1.06 respectively, showed the highest model performance in the MID-IR region, but for the NIR region TSS had the lowest, together with malic acid in terms of \( R^2 \) and RMSECV (see table 1). Glucose as compared with TA followed a similar pattern on regards to NIR but lower performance than in the MID-IR region. In contrast with most samples, fructose, that followed a normal-like distribution of data, performed worse than glucose and better than sucrose in the MID-IR region. Moreover, the omission of the ‘noisy’ part of the spectra did not improved the performance of the model, on the contrary, the higher performance results obtained by using the whole spectra, suggested that important information is also stored in the irregular portion of the spectra.

Optimal results were obtained selecting fewer bands in both regions (FTIR calibration software FOSS, version 3.0.1, 2014, data not shown). This suggest that local calibration models can be obtained in small apple juice sets (less than 28 Samples) as long as the within-sample distribution is similar as the one we report and the juices have similar quality characteristics as the data set presented.

The results in table 1 provide only a hint of the validation models in the future. The number of samples to be used in such models requires further investigation. Since the modelling for malic acid, sucrose and fructose was difficult, additional band sampling, other modelling strategies and more modelling time are required in order to obtain satisfactory results, as different studies have reported.¹⁴-¹⁶ Apart from this, the accuracy of the reference method used might also influence the overall results of the calibration models. The TA for example, uses data coming from a simple analytical method (end point titration), which generally provides superior data, which in apple juice is also quite specific as malic acid is strongly dominant (>98%). In contrast TSS measurements are also a technically simple and robust measurement, however usually highly unspecific as it accounts for everything that is soluble in the juice.

Table 1. PLS calibration model results of a set of 27 apple juices using the NIR and MID-IR region of the spectra. The numbers in parenthesis () represent the difference between high and low ranges.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TSS</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Total Acids</th>
<th>Malic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Range</td>
<td>8.8-13.5(4.7)</td>
<td>4.1-17.8(13.7)</td>
<td>22.9-83.3(60.4)</td>
<td>0.7-53.4(52.7)</td>
<td>5-10.7 (5.7)</td>
<td>6.3-14.4 (8.1)</td>
</tr>
<tr>
<td>SD*</td>
<td>1.06</td>
<td>3.18</td>
<td>14.44</td>
<td>10.34</td>
<td>1.38</td>
<td>1.91</td>
</tr>
<tr>
<td>#PCs</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>RMSECV</td>
<td>1.05</td>
<td>2.76</td>
<td>11.20</td>
<td>13.39</td>
<td>1.03</td>
<td>2.39</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.04</td>
<td>0.26</td>
<td>0.38</td>
<td>0.11</td>
<td>0.42</td>
<td>0.03</td>
</tr>
<tr>
<td>NIR Region (4000 to 7330 cm⁻¹)</td>
<td>( R^2 )</td>
<td>0.70</td>
<td>0.75</td>
<td>0.62</td>
<td>0.54</td>
<td>0.84</td>
</tr>
<tr>
<td>MID-IR Region (1235 to 3997 cm⁻¹)</td>
<td>( R^2 )</td>
<td>0.58</td>
<td>1.59</td>
<td>9.20</td>
<td>6.92</td>
<td>0.55</td>
</tr>
</tbody>
</table>

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CONCLUSION

It was possible to construct NIR calibration models for organic sugars and acids, from a small apple juice set, based on the full near infrared region available at an Oenofoss winescan. Calibration models in this region had lower quality than those in the MID-IR region. The relevant information was better described at the MID-IR spectral region, from 1235 to 3997 cm⁻¹. Taking into account that a small set of only 27 samples was used, the results presented are quite satisfactory for some of the parameters.

It is important to look not only at distribution histograms, but also at the spread of data points (SD), the accuracy of the reference method and the quality of the spectra, to have an initial idea of the data quality for multivariate calibration models. The multivariate statistics presented, however, provide only a hint of the performance of prediction models in the validation stage, where, using different number of samples, the full performance of the model prediction performance can be evaluated.

Additional samples covering a broader range of sugars and acids concentrations, with similar data spread as the best models presented in this study, and the combination of NIR plus MID-IR regions, may greatly improve the overall calibrations performance and provide robust prediction models. This is being further investigated. The models presented must be used as a first attempt only. The differences in chemistry of the individual juice sample not included in this study (e.g. ammonia, amino N, potassium, density, colors, etc.) may have also influenced the performance of the NIR calibration models. More research is required in this aspect.

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References

1. Jongen W. Fruit and Vegetable Processing. 2002. 120-123