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Viability of IR spectroscopy for the accurate measurement of Yeast Assimilable Nitrogen content of grape juice

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ABSTRACT: Up to date, there have been only a few reports on the measurement of YAN and/or its components using IR spectroscopy, suffering from various limitations (number of samples, validation strategies, *etc.*). In this work, three IR spectral instruments measuring in different modes and ranges of the IR spectrum (FT-IR, FT-NIR, and ATR-MIR), were compared and evaluated for their accuracy to measure both total YAN as well as the components, FAN and ammonia, separately, using over 900 grape juice samples from 28 cultivars over three seasons. The global and vintage-based models were evaluated using $R^2_{CAL/VAL}$, RMSEC/P, and $RPD_{CAL/VAL}$. Randomization tests were used for pair-wise comparison of models. FT-IR and FT-NIR instruments gave the best results, while ATR-MIR can be used for screening purposes. Considering the accuracy, robustness, high throughput, and cost-effective nature, the models produced by both FT-IR and FT-NIR spectroscopy can provide winemakers with the opportunity to make timelier and more informed nutrient supplementation decisions, facilitating the achievement of their desired wine style and quality.

Keywords: InfraRed (IR) spectroscopy; Yeast Assimilable Nitrogen (YAN); Grape juice; Chemometrics; Randomization tests; Calibration

Declarations of interest: none

1. Introduction

IR spectroscopy provides the possibility of "fingerprinting" samples and, therefore, can provide an in-depth understanding of the chemical properties of various food and beverage products [1]. However, the potential of spectroscopic techniques would not have been realised had it not been for major developments in the field of chemometrics. Chemometric techniques such as partial least squares (PLS) regression and principal component regression (PCR) allow the simultaneous consideration of multiple variables and are also able to handle highly correlated and noisy data, addressing the inherent issues related to dealing with spectroscopic data [2]. This is due to the fact that these techniques extract latent variables from the original spectral data, thereby reducing the number of X-variables (spectral data points) to a set of non-correlated variables. This set of non-correlated

variables can then be used to explain the variation in the data [3,4] and subsequently, provide the possibility of building suitable and robust calibration models.

Due to the complexity of the winemaking process and the increasing consumer demand for high quality wines, monitoring grape and wine composition has become a necessity [1]. However, timely and cost-effective analysis is not always possible using conventional methods. This is owed to the fact that often these methods cannot be carried out on-site as they require trained personnel and the use of potentially hazardous chemicals [5]. Thus, the possibility of providing simple, rapid and cost-effective methods which are non-destructive and environmentally friendly would be an indispensable asset to the modern wine industry. These properties are all characteristic of IR spectroscopy, and although there has been widespread adoption of this technology in the food industry, the use of IR spectroscopy in the wine industry is still in its infancy [6–8].

The possible reasons for this have been highlighted in a recent publication [9], the most pertinent being the lack of understanding of the technology. Robust calibrations should be capable of providing accurate results for samples which are: (i) exposed to different environmental conditions, (ii) from different varieties and (iii) from different vintages [10]. These are essential factors to consider for the successful integration of this technology into the wine industry, especially due to the notoriously complex nature of the grape juice matrix [8]. As a result, obtaining a representative calibration set becomes a particularly challenging task [11]. Furthermore, the bulk of publications currently available on spectroscopic modelling in grape and wine research generally use a limited sample set and thus, chances are that the large degree of the variation naturally present in the population is neglected [9,12] (Dambergs et al., 2015; Skoutelas, Ricardo-da-Silva, & Laureano, 2011). Moreover, more often than not, these publications do not test their models using independent validation sets but rather report values for cross-validation which are in most cases, overoptimistic [13,14]. Cross-validation (CV) entails splitting the sample set into a predetermined number of subsets. Calibrations are then obtained by removing a different subset from the calibration data until each subset has been left out once. Thus, CV may lead to overoptimistic results as the samples used to validate the model have also been used to calibrate the model [15].

Yeast assimilable nitrogen (YAN) can be defined as nitrogen sources present in the grape juice matrix that can be taken up by yeast during fermentation. These sources include free amino nitrogen (FAN) and ammonia [16]. YAN is an essential component of grape juice as it plays a major role in fermentation efficiency by providing the necessary nutrients required for the growth and proliferation of yeast, thereby reducing the chances of stuck or sluggish fermentations [17]. Furthermore, YAN has been highlighted as a driver of quality by influencing the organoleptic qualities of wine [18]. This is primarily owed to the free amino nitrogen (FAN) portion of YAN, as certain amino acids (branched-chain and aromatic) have been identified as precursor molecules for the production of particular aroma compounds [19]. Thus, it is important to not only measure total YAN before the start of fermentation, but also to have knowledge of its composition. Consequently, this information will ensure

more informed decision-making regarding nutrient supplementation strategies and assist in avoiding unnecessary prophylactic nutrient additions.

In order to assess whether the models produced in this study are accurate enough for industrial use, it is important to understand the parameters, YAN, FAN and ammonia, in the context of the winemaking environment. Yeast assimilable nitrogen is an essential nutrient required by yeast during fermentation. In the absence of sufficient concentrations, yeast will not be able to produce the required amounts of biomass that is necessary to carry a fermentation through to dryness, and therefore, fermentations may become stuck or sluggish [17,20]. In addition to the large amounts of residual sugar that will be present in the wine, stuck or sluggish fermentations are normally accompanied by the formation of off-flavors, such as H_2S [21]. Furthermore, insufficient concentrations of the FAN component of YAN have been reported to lead to a very neutral wine devoid of desirable fruity and floral aromas. This is because the branched-chain and aromatic amino acids (which form part of the FAN component of YAN) have been identified as the precursor molecules for the formation of these favorable aromas [19,22].

The exact amount of YAN, FAN and ammonia which is optimal for the yeast during fermentation is highly strain dependent, however, a 140 mg N/L of total YAN has been benchmarked in literature as the minimum amount required to complete fermentation [23]. The range of YAN, FAN and ammonia concentrations found in various surveys across different wine regions were reviewed in a recent publication [24]. Studies done to investigate the impact of varying concentrations of YAN, FAN and ammonia on the fermentation efficiency and organoleptic qualities of the final wine have found that, at above a certain threshold, the amount of YAN becomes redundant. For example, the production of fruity and floral esters has been observed to plateau when total YAN concentrations reach more than 250-300 mg N/L, and have even been found to decrease when YAN concentrations reach approximately 500 mg N/L [25]. Furthermore, very high total YAN concentrations (>450-500 mg N/L) may result in the production of unwanted compounds such as biogenic amines, carcinogens and protein haze, as well as leading to microbial instability [16]. Therefore, having excessive concentrations of YAN will decrease the quality of the final product.

These margins of concern are, however, over approximately a 50 mg N/L (total YAN) range, depending on the nitrogen demand of the particular yeast strain used. Therefore, the use of ATR-MIR may be plausible from a screening point of view but will not allow for precise decision-making regarding nitrogen supplementation. It is important to note that the RMSEP reported is an *average* of the errors and that, in some cases, this error may be a lot larger than the value reported as the RMSEP. Therefore, there is a chance that winemakers may be completely misguided by the prediction value given by ATR-MIR.

Up until now, there have been only a few reports on the measurement of YAN and/or its components using IR spectroscopy. The first study attempted to calibrate an FT-NIR instrument for the measurement of FAN using 97 settled grape juice samples from various white varieties [26]. They were, however, unsuccessful, obtaining a

large standard error of prediction (SEP) of 272.1 mg N/L. Thus, instead, a Soft Independent Modelling of Class Analogy (SIMCA) was used to classify the samples as having either high, medium or low concentrations of FAN. In a comparison done by Dambergs *et al.* [27], MIR was shown to outperform NIR for the measurement of YAN, FAN, and ammonia, as higher ratio of standard error of performance to standard deviation (RPD) and lower standard error of cross-validation (SECV) values were observed using MIR. On the other hand, Shah *et al.* [13], investigated the viability of using ATR-MIR to measure various grape juice parameters including YAN, FAN, and ammonia. SEP values of 42.4 mg N/L, 36.7 mg N/L and 17.2 mg N/L were obtained for YAN, FAN, and ammonia, respectively. Furthermore, a RPD of approximately 2 was obtained for each of these parameters, indicating a qualitative rather than quantitative determination of these grape juice parameters. In another study, 71 grape juice samples from the Lisbon region in Portugal were used to build a calibration for YAN using FT-MIR spectroscopy. An R^2 of 0.993, SEP of 5.9 mg N/L and an RPD of 7.8 was obtained [12]. These results may, however, be overoptimistic due to the limited number of samples included in the model in combination with the use of a cross-validation strategy rather than external validation.

Thus, IR spectroscopy shows potential for the measurement of YAN concentration and composition. However, for this technology to become a feasible option for industry, a few key issues need to be addressed. These include building calibrations with larger data sets including different varieties, origins, and vintages, as well as independent validation to adequately test the accuracy and robustness of these models. Therefore, the aim of this study is to fully investigate the viability of various infrared spectroscopic instruments for the accurate quantification of YAN, FAN, and ammonia concentrations by incorporating independent and robust validation strategies.

2. Materials and methods

2.1. Sample collection

A total of 905 grape juice samples were collected over three vintages (2016 – 2018) directly from commercial wineries at a ripeness level suitable for commercial winemaking. Red grape juice samples were collected after crushing and white after settling. An unsupervised strategy was employed [24]. This meant that no specific cultivars or origin was targeted. Consequently, samples were collected from 28 different cultivars, 12 white and 16 red. Furthermore, these samples were collected from 14 different grape-growing districts situated in the Western Cape of South Africa, classified according to the demarcation set by the Wine of Origin System of South Africa (SAWIS, 2017). Samples were coded immediately upon collection and stored at -20°C until analysis.

2.2. Analytical methods

2.2.1. Reference method

The components of YAN: FAN and ammonia, were measured separately by enzymatic assay using the Megazyme™ K-PANOPA (Ireland) for FAN and Enzytec™ Fluid Ammonia (R-Biopharm, Germany) for ammonia. This was performed on the Arena 20XT (Thermo Fisher Scientific, Waltham, MA) which provides automated spectrophotometric readings. The individual values for FAN and ammonia were then summed to determine the total amount of YAN available and were expressed as mg N/L.

2.2.2. Infrared spectroscopy scanning

The samples were thawed at room temperature on the day of analysis and were centrifuged at 5000 rpm for 5 min in a 7366 Hermle centrifuge (Wehingen, Germany) prior to analysis. Spectra were collected from three bench-top infrared instruments, namely: a multi-purpose analyser (MPA) FT-NIR instrument (Bruker Optics, Ettlingen, Germany), Alpha-P ATR FT-MIR spectrometer (Bruker Optics, Ettlingen, Germany), and WineScan™ FT120 (FOSS Electric, Denmark).

FT-NIR spectra (12500-4000 cm^{-1}) were collected by the MPA in transmission mode in a 1 mm cuvette. The absorbance spectrum obtained for each sample was acquired at a resolution of 2 cm^{-1} and at a scanning velocity of 10 kHz, averaged over 32 scans. Air was used as background and an air spectrum was taken periodically during the scanning of the samples and was automatically subtracted from each individual sample spectrum.

Spectra in the mid-infrared range (4000-600 cm^{-1}) were collected by the Alpha-P ATR FT-MIR spectrometer. Each sample was scanned at a resolution of 4 cm^{-1} and at a scanning velocity of 7.5 kHz, averaged over 64 scans to give a final reading. Instrumental control of the MPA FT-NIR and the Alpha-P ATR FT-MIR were carried out using OPUS software (OPUS v. 7.0 for Microsoft, Bruker Optics, Ettlingen, Germany).

The WineScan™ FT120 measures primarily in the mid-infrared region (4000-929 cm^{-1}), however, a small section of the near-infrared region is also included (5011-4000 cm^{-1}). This instrument recorded spectra at a resolution of 4 cm^{-1} in transmission mode which was then converted into a linearized absorbance spectrum. Each measurement was averaged over 20 readings to give a final measurement. Prior to analysis of the grape juice samples, the background absorbance in the grape juice sample is accounted for using the FOSS Zero Liquid S-6060 (WineScan™ manual).

2.3. Data analysis and strategy

Calibration models and model accuracy were evaluated using OPUS software (OPUS v. 7.2 for Microsoft, Bruker Optics, Ettlingen, Germany). This software correlates the reference values to the spectra through the use of the partial least-squares regression (PLS) algorithm. The accuracy and reliability of the models were assessed

based on a set of performance evaluation indices which included the correlation coefficient of calibration and validation ($R^2_{CAL}$and R^2_{VAL}), the root-mean square error of calibration (RMSEC) and validation (RMSEP) as well as the RPD in calibration and validation (RPD_{CAL} and RPD_{VAL}).

The optimum number of latent variables (*i.e.* rank) to avoid overfitting of the model was algorithmically determined [28], Rank was, however, not used as a criteria to compare the reliability of the models in this study. Instead, a provision was made which allowed for a maximum of 20 latent variables to be considered during model optimization. This number was considered to be low enough to avoid overfitting of the models as YAN is a minor component, producing a rather weak signal in a highly complex matrix. Moreover, the chances of overfitting were further decreased by external validation strategies in addition to the large number of samples that were gathered from a variety of different cultivars, vintages and origins – ensuring that both calibration and validation sets would be representative of the population.

An untargeted-type strategy, modeling the entire spectral fingerprint followed by variable selection based in the statistical results as opposed to a variable selection based on the chemistry of the targeted compounds, was employed during the modelling process. For each instrument, the spectra from all samples were uploaded to the OPUS software with their corresponding reference values for either YAN, FAN, or ammonia. The sample set was divided into a 66/34 calibration to validation set using the Kennard-Stone algorithm by selecting the "automatic selection of test samples" feature. Thus, an external validation set was used. The models were then let to run using the "general B" option incorporated in the software package. This option automatically divides the spectra into ten sub-regions into an interval PLS strategy. The regions used for the top five models were further investigated for optimization of the calibration model. These regions were then manually selected using the "user defined optimization regions" function which allows a manual selection of ten sub-regions of any size using the "general B" option. Furthermore, pre-processing techniques such as smoothing, standardization, transformation, and normalization were used for model optimization.

Once the optimum regions were identified for a specific instrument and sample parameter, a subsequent model was built using these settings, but the sample set was divided into a 50/50 ratio of calibration/test. The models including samples from all the different varieties, origins and vintages will from hereon be referred to as "global models" and differentiated based on their calibration to validation ratio (66/34 or 50/50).

During the optimization of each model, outliers were removed and the pre-processing method which resulted in the lowest RMSEP and highest RPD was selected. Outliers were detected by the Mahalanobis distance for each calibration spectrum from which a threshold is calculated. This threshold determines whether the spectra of an unknown sample can be reliably predicted or not.

To assess the robustness of the models, it was tested to see whether the YAN, FAN, and ammonia concentrations from samples from a new vintage (2018) could be accurately predicted by a calibration model built based on samples from the previous two vintages (2016 and 2017). In other words, 2016 and 2017 grape

juice samples were used as the calibration set to train the model, while 2018 was used as an independent test set. These calibration models included samples from all three vintages, origins, and the respective red or white varieties.

RPD values and RMSEC/V are often used to compare the accuracy of calibrations. However, this approach is simply based on a direct comparison between the reported error values and an accurate statistical evaluation of model performance is therefore not provided. A randomization test reported by Olivieri [29] has been proposed to evaluate the statistical significance of the prediction performance of two calibrations. This test may be used to compare the prediction accuracy of two instruments *i.e.* the performance of different spectroscopic instruments. This may also include calibrations based on different infrared regions, measuring principle (transmission or reflectance), or even different sample formats. The differences between the square errors and the mean differences are calculated from a set of predicted samples from two different calibrations. Significant differences between two calibrations are given by p-values < 0.05 as the null hypothesis (RMSE1=RMSE2) is rejected. The test was evaluated using a MATLAb code (MATLAB R2016b version, Mathworks Inc., Natik, MA) provided elsewhere [29].

3. Results and discussion

3.1. Tasks and rationale

The rationale of the 66/34 global model was to test the viability and subsequently, the robustness of IR spectroscopy in an industrial context – where samples originate from different varieties, growing regions and vintages. A calibration model is considered robust when the model could accurately predict the tested variable, irrespective of unknown changes occurring in the external environment [10]. Due to the innate complexity of fruits and vegetables, samples belonging to different 'batches' (*i.e.* different varieties, origins and vintages) are considered as the most important factor influencing model robustness in the application of IR spectroscopy to agricultural systems [10,30]. This is an important factor to consider in the field of spectroscopy as an inherent feature of this technology is to look at the matrix in its entirety, and subsequently the interactions occurring in the given matrix [2]. Furthermore, robustness was ensured by assessing models with an independent validation set which avoids potentially overoptimistic results that could be obtained by using a cross-validation strategy.

A subsequent model was built with the calibration and independent validation set adjusted to a ratio of 50/50. This was done to further assess the robustness of the models built by a particular instrument as less samples are included in the training set, as well as increasing the number of independent samples the model is required to predict.

Furthermore, due to the number of environmental factors that influence the grapevine during the growing season, a specific vine may result in a substantially different grape juice matrix from one year to the next, known as the vintage effect. Practically speaking, a calibration model would be built using samples from previous

vintages and then used to predict the concentration values of samples from a new vintage. Therefore, the next task assigned to each instrument included building a calibration model from two vintages (2016 and 2017) and using it to independently predict the samples from a new vintage (2018). Again, to ensure a realistic situation and increase the robustness, the samples from all the vintages (including both calibration and validation sets) included samples from an array of different cultivars and growing conditions.

3.2. Nitrogen status of samples

The 905 samples had reference concentrations which spanned over a range of 44.88-483.67 mg N/L, 29.83-365 mg N/L and 1.16-344.97 mg N/L for YAN, FAN and ammonia, respectively (Supplementary Table 1). These concentrations are comparable to what has previously been published for various YAN surveys in other wine regions of the world [31–33]. Thus, another concern of spectroscopic calibration was addressed by ensuring that a large number of samples were collected over a realistic range of concentration values. This dataset is therefore regarded as representative and thus most likely capable of robust calibration of IR spectroscopic instruments for the accurate prediction of the nitrogen status of the grape juice matrix.

The dataset used to test the ability of predicting the nitrogen status of a sample from a new vintage had 799 samples included in the calibration set (2016 and 2017) and 106 in the validation set (2018) (Supplementary Table 2).

3.3. Assessment of IR spectroscopy for the purpose of nitrogen status quantification

3.3.1. Fourier-transform infrared (FT-IR) spectroscopy prediction models

Strong water absorption peaks (1552-1755 cm^{-1}; 3552-3042 cm^{-1}) can be observed in the FT-IR spectra of grape juice. This characteristic of FT-IR spectroscopy has been reported to impede its use in quantification of various compositional parameters in the grape juice matrix [34]. For example, the peak ranging between 1552-1755 cm^{-1} coincides with the absorption of the amino acid side-chains which absorbs between 1480-1800 cm^{-1} [35]. Furthermore, sugar and water absorbing at 3552-3042 cm^{-1} overlap with the 1° N-H_2 groups present in YAN. Regions related to N absorption features were commonly included in the calibrations (Supplementary Table 3). These regions (1164-1601 cm-1) are related to primary and secondary amine bending, aromatic amino acids, and oxynitrogen compounds (aliphatic and aromatic nitro compounds and organic nitrates). Other regions were also included that may correspond to absorption features of the other molecular bonds part of the nitrogen containing molecules (2993-1786 cm^{-1} and 4618-3977 cm^{-1}) [36]. All the models built using FT-IR spectroscopy in transmission mode produced models suitable for quantification as all RPD_{VAL} values were observed to be above 3.

Generally, the global models for all the parameters (YAN, FAN, and ammonia) and for both tested ratios (66/34 and 50/50) (Table 1 and 2) were found to perform better than the tasks of predicting the nitrogen status of samples from a new vintage (Table 3). Furthermore, global models employing the 66/34 ratio performed better

than the 50/50 ratio. RPD_{VAL} values of the 66/34 approach were all found to be above 4 with a RPD_{VAL} of 5.2 obtained for the prediction of total YAN – considered appropriate for quality control purposes [13]. The 66/34 ratio was found to have the lowest error in prediction for all parameters tested as a RMSEP of 13.9, 11.8 and 5.07 mg N/L was observed for YAN, FAN and ammonia, respectively. The models built based on the 50/50 ratio of calibration/validation were, however, comparable to the models employing the 66/34 ratio as RPD_{VAL} values were also generally observed to be above 4, except for FAN (RPD_{VAL} 3.89). For both ratios, the prediction of FAN was found to be a more difficult task, resulting in a lower RPD_{VAL} compared to YAN and ammonia. Interestingly, although a decrease in the RPD_{VAL} was observed for FAN for the 50/50 ratio compared to the 66/34 ratio, a slight improvement in the average prediction accuracy could be observed for the 50/50 global model (Table 1). The rank for the global models (66/34 and 50/50) were observed to range between 16 and 20 (Table 1).

A SEP of 5.9 mg N/L and an RPD of 7.8 was obtained by Skoutelas *et al.* [12] for the calibration of YAN using FT-IR. The higher RPD and lower error of prediction obtained in this study is most likely due to the model only receiving samples from a single vintage (n=71), the removal of a large number of samples considered to be outliers (n=28/71), as well as the model not undergoing any external validation.

The models built to predict a new vintage also performed accurately, with RPD_{VAL} and rank values of 4.24 and 13, 3.84 and 17, and 4.23 and 18 for YAN, FAN and ammonia, respectively (Table 3). The error in prediction obtained by this model (17.6, 11.5 and 7.32 mg N/L, for YAN, FAN and ammonia, respectively) was comparable to what was observed for both global models. Therefore, using FT-IR spectroscopy to predict the nitrogen status of grape juice samples from a new vintage has proven to be a viable possibility. Thus, by testing the robustness of the models by adding samples from a different growing season, this study has managed to successfully address one of the major concerns regarding the application of this technology in agriculture. However, it must be kept in mind that these calibrations still need to be updated and maintained in the future to ensure that the accuracy and robustness is maintained [9].

3.3.2. Fourier-transform near-infrared spectroscopy (FT-NIR)

The NIR spectra, characterized by the overtones and combination bands caused by the fundamental vibrations occurring in the mid-infrared range, was dominated by the overtones of the O-H stretch (7274-6338 cm^{-1}) and a combination band of O-H stretching and bending (5417-4495 cm^{-1}), induced by the presence of water in the grape juice matrix [37]. Despite this, NIR spectroscopy has been reported to be appropriate for quantification purposes as the band shape is often typical of a specific compound or a group of compounds [34]. A common region at 4856-4285 cm^{-1} was always used in the models which is related with N-H and C=H stretching modes (4397 cm^{-1}) and proteins (4812 cm^{-1}) (Supplementary Table 3). Additional regions were often included in the models at higher wavelengths generally excluding the water overtones (7274-6338 cm^{-1} and 5417-4495 cm^{-1}).

As with FT-IR spectroscopy, the 66/34 global model performed the best when looking at both the RPD_{VAL} and RMSEP statistics (Table 1). A better RPD_{VAL} was, however, observed for the prediction of ammonia concentrations of samples from a new vintage (RPD_{VAL} of 3.51 compared to 2.9 for the 66/34 model) although, the difference between the two models in terms of the RMSEP was considered irrelevant (8.47 vs 8.46 mg N/L for the 66/34 and 2016+2017/2018 model, respectively). Furthermore, RPD_{VAL} value for the 66/34 global model to predict total YAN was also close to 5, as was the case for FT-IR spectroscopy. In terms of the other parameters, higher RPD_{VAL} values were obtained for FAN (RPD_{VAL} 3.43 and 3.08) compared to ammonia (RPD_{VAL} 2.9 and 2.72), for both global models (66/34 and 50/50, respectively) for FT-NIR spectroscopy. This is in contrast to what was found for FT-IR, where ammonia was found to be more accurately predicted than FAN. As RPD_{VAL} values for FT-NIR were found to be more than 3 for YAN and FAN for both global model ratios, this method was found to be adequate for accurate quantification of these parameters [10]. Although decreased accuracy was obtained for the quantification of ammonia ($RPD_{VAL} < 3$), these values are still deemed satisfactory ($RPD_{VAL} > 2.5$) [10]. Furthermore, the rank of these models was observed to range between 17-20 (Table 1).

The task of predicting a new vintage (Table 3) resulted in higher RPD_{VAL} values than for the 50/50 global models (Table 2). This may be due to the larger number of samples used to train these models in addition to the reduced number of samples tested against these models. Furthermore, this model also outperformed the 50/50 global model in terms of the RMSEP for FAN and ammonia, obtaining errors of 13.7 and 8.46 mg N/L, respectively. Rank of these models ranged between 16-20. Interestingly, the prediction of total YAN of a sample from a new vintage using FT-NIR was observed to be (although marginally), better than what was found for FT-IR spectroscopy (Table 3). The results for FT-NIR spectroscopy to predict the FAN and ammonia concentrations of a new vintage were also considered to be adequate for accurate quantification ($RPD_{VAL} > 3$) [10]. Therefore, FT-NIR spectroscopy can be considered a viable technique for the prediction of samples from a new vintage and a feasible option for industrial use.

3.3.3. Attenuated total reflectance mid-infrared spectroscopy

ATR-MIR spectra of the grape juice samples were mainly characterized by a strong sharp peak at 950-1100 cm^{-1}, corresponding to water peaks, whereas peaks occurring between 1480-1800 cm^{-1} are related to C=N, C=C and C=O stretching and N-H bending, corresponding to bonds found in amino acids and their side chains [35]. The carboxylic acid O-H stretch produced peaks between 2800-2970 cm^{-1}, which can be owed to amino acids as well as organic acids present in the grape juice medium and, therefore, could lead to interferences in the spectra, hampering accurate quantification. Furthermore, the presence of sugars can also interfere with accurate quantification due to the sp^3 C-H stretch found in this region as well as the alcohol O-H stretch occurring between 3388-3600 cm^{-1}, coinciding with primary and secondary amino nitrogen groups (1°N-H; 2°N-H). The regions included in the spectra cover 3991-778 cm^{-1} which indicates that almost the totality of the spectra was included throughout the different tested calibrations (Supplementary Table 3). The reported models included

nitrogen related regions corresponding to primary, secondary, tertiary, aromatic and/or oxy nitrogen compounds, searching for predictive information during the interval selection optimization process. Additional regions were also sometimes included possibly overlapping with other compounds [38].

Overall, ATR-MIR was not found to be suitable for accurate quantification purposes as RPD_{VAL} values were never observed to be more than 2.5 [10], with many found to be less than 2 (Table 1-3). Rank values for ATR-MIR were generally lower than for other spectroscopies, ranging between 11-15. However, following the trend of the abovementioned spectroscopies, both global models were still found to be generally more accurate than what was observed for the other tasks. The highest RPD_{VAL} was obtained for the prediction of YAN in the 50/50 global (RPD_{VAL} 2.3), however, a higher RMSEP was obtained for this model (26.9 mg N/L) compared to the 66/34 model (24.8 mg N/L; RPD_{VAL} 2.07). Furthermore, as with FT-NIR spectroscopy, higher RPD_{VAL} were obtained for YAN and FAN ($RPD_{VAL} > 2$) compared to ammonia ($RPD_{VAL} < 2$). This trend was not only observed for the global models, but generally throughout the tasks of robustness assigned to the instrument.

RPD_{VAL} for the prediction of a new vintage ranged between 1.62 (ammonia) and 2.17 (FAN). Together with the lower RPD_{VAL}, higher errors in prediction (RMSEP) were observed for this task (Table 3) compared to the global models (Table 1 and 4.5) as well as compared to the other spectroscopies for the same task. Again, rank values were observed to be lower than for other spectroscopies, ranging between 7-11.

3.4. Overall trends

3.4.1. Comparison of the performance of the instruments

Overall, for each instrument, total YAN predictions were observed to be more accurate than measuring the components separately. This was shown through the higher RPD values obtained for YAN than for FAN and ammonia separately, as well as the lower error in prediction (RMSEP) found for YAN compared to the sum of the errors obtained for FAN and ammonia (Tables 1-3). Furthermore, for all tasks (global and vintage models) FT-IR was able to predict total YAN and ammonia more effectively than FAN, whereas FT-NIR and ATR-MIR was able to predict total YAN and FAN more effectively than ammonia.

FT-IR (WineScan™ FT120) outperformed both other instruments for the measurement of all three of the investigated parameters, throughout all the given tasks. This is because consistently higher RPD_{VAL} as well as lower RMSEP were observed for this instrument compared to the other spectroscopies. However, the MPA, measuring in the NIR range in transmission mode, also produced models capable of accurate quantification, although the validation statistics were slightly less optimal than what was found for FT-IR. It would, however, be advisable to rather use FT-IR for the quantification of ammonia compared to FT-NIR as FT-IR obtained $RPD_{VAL} > 4$ compared to < 3 for FT-NIR.

ATR-MIR was, however, not comparable to either FT-IR or FT-NIR spectroscopy for any of the parameters or tasks assigned. This is due to the consistently lower RPD_{VAL} and higher RMSEP obtained throughout. Thus,

this instrument is only suitable for screening purposes and not for the accurate quantification of any of the parameters tested. It was surprising that the FT-NIR spectral instrument outperformed the ATR FT-MIR instrument as MIR spectra are produced due to the fundamental stretching, bending and rotating vibrations produced by various functional groups present in the sample. On the other hand, spectral signatures in the near infrared region are only due to the complex overtones of these fundamental vibrations. Furthermore, the combination bands, such as those produced by C-O stretch and the N-H band in protein, as well as water, which is a major component of most fruits and vegetables, can result in a highly convoluted NIR spectrum, decreasing the chances of accurate quantification and interpretation [6,10]. However, the regions that were selected for the optimization of the models for the FT-IR models primarily fell within the mid-infrared range (YAN ~4200-1200 cm^{-1}; FAN ~4600-1400 cm^{-1}; ammonia ~3000-1200 cm^{-1}). Thus, it is hypothesized that the mode that the spectra was collected in (reflectance *vs*. transmission) also played a major role in the difference in performance obtained between the instruments and thus, transmission mode was found to be more suitable than reflectance for this application. This could be explained by the minimal penetration depth of the evanescent wave in the juice absorbing medium that caused weaker spectral features in the nitrogen absorbing regions and consequently lower prediction accuracies. In other words, pathlength differences between FT-NIR and FT-IR applications (scale in millimetres) and that of the ATR-MIR technique (scale in microns) might explain the results observed.

Additionally, to further evaluate model performance, a randomization test was performed. The direct comparison of the statistics (R^2, RMSE, and RPD) obtained from the process of model optimization do not indicate if the performance of the reported calibration is statistically significant. The test was therefore applied with the objective of evaluating instrument performance. This includes a comparison of different regions within the infrared range as well as two different measuring principles (transmission *vs.* attenuated total reflectance). A pairwise comparison between instruments was thus performed (Table 4). The randomization test was explored for the global models with 66/34 calibrations/validation ratio. Samples that were used in the calibration set in all three spectroscopic techniques were included.

Non-significant differences in the predictions were observed between FT-IR and FT-NIR techniques for the quantification of YAN. On the contrary, when both FT-IR and FT-NIR where compared with ATR-MIR spectroscopy, significant differences were obtained for this parameter. These results are in accordance with the lower prediction performance observed for the latter technique. Interestingly, despite very similar calibrations being reported for FAN analysis for both FT-IR and FT-NIR, significant differences were still obtained. This indicates that FT-IR is providing significantly more accurate predictions when compared to NIR. Both instruments outperformed again ATR-MIR with significant differences being observed for the comparisons. Finally, when predictions for ammonia were evaluated, significant differences were observed for the three pair-wise comparisons. FT-IR was again found to statistically outperform the other two spectroscopy instruments. These results supported the hypothesis raised earlier suggesting that spectral collection mode (transmission or

reflectance), followed by the region of choice within the infrared are playing major roles in the ability of the different spectroscopy applications to predict nitrogen components in juice samples.

4. Conclusion

To the authors' knowledge, this is the first study of its kind, incorporating such a large degree of variability for the purpose of quantifying the nitrogen status of the grape juice matrix. This variability is demonstrated by the large number of samples as well as the number of different grape varieties, origins, and vintages incorporated in both the calibration and validation sets. In addition to this, an independent validation set was used. This is a shortcoming highlighted in most other studies in this field which impedes the widespread use of this technology for routine analysis of fruits and vegetables.

The results obtained in this study show that it is indeed possible to calibrate IR spectroscopic instruments for the accurate measurement of YAN, FAN, and ammonia concentrations. Transmission FT-IR spectroscopy was, however, observed to show the most promising results; however, FT-NIR spectroscopy also produced models capable of good to excellent quantification, primarily for YAN and FAN. Furthermore, both of these instruments showed sufficient robustness against samples originating from different varieties, growing conditions, and vintages, addressing the concerns of applying this technology to the agricultural industry. Therefore, applying this rapid, cost-effective, and environmentally friendly method in an industrial setup is a plausible option, despite the inherent variability and complexity of the grape juice matrix. Moreover, the possibility of measuring the YAN status of samples from a new vintage are one of the most important findings in this study as it demonstrates the feasibility of this technology in an industrial set-up. This is because calibrations will most likely be based on samples originating from previous vintages and used for analysis of subsequent vintages.

In light of this, using FT-IR, or even FT-NIR spectroscopy would be more beneficial than ATR-MIR as there are lower RMSEP and higher RPD_{VAL} values. High RPD values are important as the RPD of a model is an indicator of how reliable the model is *i.e.* it indicates how reliable the RMSEP of the model is. Furthermore, the RMSEP reported for these two instruments are low enough in the context of the YAN status of grape must to allow for optimal and precise nitrogen supplementation.

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References

[1] M. Gishen, D. Cozzolino, R. Dambergs, The Analysis of Grapes, Wine, and Other Alcoholic Beverages by Infrared Spectroscopy, Handb. Vib. Spectrosc. (2010). doi:DOI: 10.1002/9780470027325.s8960.

[2] D. Cozzolino, W.U. Cynkar, S. N, R. G Dambergs, P.A. Smith, N. Shah, R.G. Dambergs, P.A. Smith, A brief introduction to multivariate methods in grape and wine analysis, Int. J. Wine Res. 1 (2009) 123–130. doi:10.2147/IJWR.S4585.

[3] F. Liu, Y. He, L. Wang, G. Sun, Detection of Organic Acids and pH of Fruit Vinegars Using Near-Infrared

Spectroscopy and Multivariate Calibration, Food Bioprocess Technol. 4 (2011) 1331–1340. doi:10.1007/s11947-009-0240-9.

[4] S. Wold, M. Sjöström, L. Eriksson, PLS-regression: a basic tool of chemometrics, Chemom. Intell. Lab. Syst. 58 (2001) 109–130. doi:10.1016/S0169-7439(01)00155-1.

[5] B.H. Gump, B.W. Zoecklein, K.C. Fugelsang, R.S. Whiton, Comparison of Analytical Methods for Prediction of Prefermentation Nutritional Status of Grape Juice, Am. J. Enol. Vitic. 53 (2002) 325–329. doi: http://dx.doi.org/10.1016/j.lwt.2013.05.009.

[6] D. Cozzolino, The role of visible and infrared spectroscopy combined with chemometrics to measure phenolic compounds in grape and wine samples, Molecules. 20 (2015) 726–737. doi:10.3390/molecules20010726.

[7] M.J. Martelo-Vidal, M. Vázquez, Evaluation of ultraviolet, visible, and near infrared spectroscopy for the analysis of wine compounds, Czech J. Food Sci. 32 (2014) 37–47. doi:10.17221/167/2013-CJFS.

[8] R. Bauer, H.H. Nieuwoudt, F.F. Bauer, J. Kossmann, K.R. Koch, K.H. Esbensen, FTIR spectroscopy for grape and wine analysis, Anal. Chem. 80 (2008) 1371–1379. doi:10.1021/ac086051c.

[9] R. Dambergs, M. Gishen, D. Cozzolino, A Review of the State of the Art, Limitations, and Perspectives of Infrared Spectroscopy for the Analysis of Wine Grapes, Must, and Grapevine Tissue, Appl. Spectrosc. Rev. 50 (2015) 261–278. doi:10.1080/05704928.2014.966380.

[10] B.M. Nicolaï, K. Beullens, E. Bobelyn, A. Peirs, W. Saeys, K.I. Theron, J. Lammertyn, Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review, Postharvest Biol. Technol. 46 (2007) 99–118. doi:10.1016/j.postharvbio.2007.06.024.

[11] C.D. Patz, A. Blieke, R. Ristow, H. Dietrich, Application of FT-MIR spectrometry in wine analysis, in: Anal. Chim. Acta, 2004: pp. 81–89. doi:10.1016/j.aca.2004.02.051.

[12] D. Skoutelas, J.M. Ricardo-da-Silva, O. Laureano, Validation and comparison of formol and FT-IR methods for assimilable nitrogen in vine grapes, South African J. Enol. Vitic. 32 (2011) 262–266. doi:10.21548/32-2-1386

[13] N. Shah, W. Cynkar, P. Smith, D. Cozzolino, Use of attenuated total reflectance midinfrared for rapid and real-time analysis of compositional parameters in commercial white grape juice, J. Agric. Food Chem. 58 (2010) 3279–3283. doi:10.1021/jf100420z.

[14] A. Versari, G.P. Parpinello, A.U. Mattioli, S. Galassi, Determination of grape quality at harvest using Fourier-transform mid-infrared spectroscopy and multivariate analysis, Am. J. Enol. Vitic. 59 (2008) 317–322.

[15] E. Anderssen, K. Dyrstad, F. Westad, H. Martens, Reducing over-optimism in variable selection by cross-model validation, Chemom. Intell. Lab. Syst. 84 (2006) 69–74. doi:10.1016/j.chemolab.2006.04.021.

[16] S.-J. Bell, P.A. Henschke, Implications of nitrogen nutrition for grapes, fermentation and wine, Aust. J. Grape Wine Res. 11 (2005) 242–295. doi:10.1111/j.1755-0238.2005.tb00028.x.

[17] P.A. Henschke, V. Jiranek, Yeast: Metabolism of nitrogen compounds. In: Wine Microbiology and Biotechnology, Res. Gate. (1993) 77–164. doi:10.1089/end.2014.0018.

[18] A. Kovtun, K. Torn, J. Kotta, Long-term changes in a northern baltic macrophyte community, in: Est. J. Ecol., 2009: pp. 270–285. doi:10.3176/eco.2009.4.03.

[19] A.Y. Smit, The impact of nutrients on aroma and flavour production during wine fermentation, PhD Thesis, Stellenbosch : Stellenbosch University, 2013.

[20] L.. Bisson, Stuck and Sluggish Fermentations, Am. J. Enol. Vitic. 50 (1999) 107–119.

[21] M. Gobbi, F. Comitini, G. D'Ignazi, M. Ciani, Effects of nutrient supplementation on fermentation kinetics, H2S evolution, and aroma profile in Verdicchio DOC wine production, Eur. Food Res. Technol. 236 (2013) 145–154. doi:10.1007/s00217-012-1870-0.

[22] A. Rapp, G. Versini, Influence of Nitrogen Compounds in Grapes on Aroma Compounds in Wine, Wein-Wissens. 51 (1991). doi:10.1016/S0167-4501(06)80257-8.

[23] M. Bely, J.M. Sablayrolles, P. Barre, Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in oenological conditions, J. Ferment. Bioeng. 70 (1990) 246–252. doi:10.1016/0922-338X(90)90057-4.

[24] G. Petrovic, M. Kidd, A. Buica, A statistical exploration of data to identify the role of cultivar and origin in the concentration and composition of yeast assimilable nitrogen, Food Chem. 276 (2019) 528–537. doi:10.1016/j.foodchem.2018.10.063.

[25] M. Vilanova, M. Ugliano, C. Varela, T. Siebert, I.S. Pretorius, P.A. Henschke, Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by Saccharomyces cerevisiae wine yeasts, Appl. Microbiol. Biotechnol. 77 (2007) 145–157. doi:10.1007/s00253-007-1145-z.

[26] M. Manley, A. van Zyl, E.E.H. Wolf, The Evaluation of the Applicability of Fourier Transform Near-Infrared (FT-NIR) Spectroscopy in the Measurement of Analytical Parameters in Must and Wine, South African J. Enol. Vitic. 22 (2001) 93-100. doi:10.21548/22-2-2201.

[27] R.G. Dambergs, B. Kambouris, W.U. Cynkar, L.J. Janik, D. Cozzolino, P.A. Henschke, M. Gishen, A comparison of near infrared and mid-infrared spectroscopy for thecanalysis of yeast assimilable nitrogen in grape juice., in: Australian Wine Industry Technical Conference Inc:, Melbourne, Australia, 2004: pp. 334–335.

[28] J.L. Aleixandre-Tudo, H. Nieuwoudt, J.L. Aleixandre, W. du Toit, Chemometric compositional analysis of phenolic compounds in fermenting samples and wines using different infrared spectroscopy techniques, Talanta. 176 (2018) 526–536. doi:10.1016/j.talanta.2017.08.065.

[29] A.C. Olivieri, Practical guidelines for reporting results in single- and multi-component analytical calibration : A tutorial, Anal. Chim. Acta. 868 (2015) 10–22. doi:10.1016/j.aca.2015.01.017.

[30] Y. Wang, D.J. Veltkamp, B.R. Kowalski, Multivariate instrument standardization, Anal. Chem. 63 (1991) 2750–2756. doi:10.1021/ac00023a016.

[31] C.E. Butzke, Survey of Yeast Assimilable Nitrogen Status in Musts from California, Oregon, and Washington, Am. J. Enol. Vitic. 49 (1998) 220-224.

[32] K.M. Hagen, M. Keller, C.G. Edwards, Survey of biotin, pantothenic acid, and assimilable nitrogen in winegrapes from the Pacific Northwest, Am. J. Enol. Vitic. 59 (2008) 432–436.

[33] G. Nicolini, R. Larcher, G. Versini, Status of yeast assimilable nitrogen in Italian grape musts and effects of variety, ripening and vintage, Vitis - J. Grapevine Res. 43 (2004) 89–96.

[34] A. Ricci, G.P. Parpinello, L. Laghi, M. Lambri, A. Versari, Application of infrared spectroscopy to grape and wine analysis, in Infrared Spectroscopy: Theory, Developments and Applications. pp. 17-41, Nova Science Publishers, Inc, 2014.

[35] A. Barth, The infrared absorption of amino acid side chains, Prog. Biophys. Mol. Biol. 74 (2000) 141–173. doi:10.1016/S0079-6107(00)00021-3.

[36] A. Veselá, A.S. Barros, A. Synytsya, I. Delgadillo, J. Čopíková, M.A. Coimbra, Infrared spectroscopy and outer product analysis for quantification of fat, nitrogen, and moisture of cocoa powder, Anal. Chim. Acta. 601 (2007) 77–86. doi:10.1016/j.aca.2007.08.039.

[37] H. Büning-Pfaue, Analysis of water in food by near infrared spectroscopy, in: Food Chem., 2003: pp. 107–115. doi:10.1016/S0308-8146(02)00583-6.

[38] J. Coates, Interpretation of Infrared Spectra, A Practical Approach, in: Encycl. Anal. Chem., 2006. doi:10.1002/9780470027318.a5606.

Table 1. Summary statistics of the global models with calibration/validation ratio of 66/34.

Global Model: Calibration/Validation: 66/34													
		N	**Range (mg N/L)**	**Pre-processing**	**Rank**	**R^2_{CAL}**	**RMSEC**	**RPD_{CAL}**	**R^2_{VAL}**	**RMSEP**	**RPD_{VAL}**	**Slope**	**Bias**
FT-IR	YAN	893	53.27-470.5	None	20	94.56	14.5	4.29	96.25	13.9	5.2	0.953	1.56
	FAN	882	32.28-342.9	First Derivative	16	92.67	11.9	3.69	94.03	11.8	4.09	0.906	0.145
	Ammonia	886	6.63-167.1	First Derivative	20	95.79	4.95	4.87	95.32	5.07	4.63	0.953	0.269
FT-NIR	YAN	889	53.27-470.5	None	18	95.06	14	4.5	95.77	14.5	4.87	0.954	0.907
	FAN	887	32.28-342.9	None	18	91.01	12.7	3.33	91.47	14.5	3.43	0.918	-0.755
	Ammonia	887	8.64-127.6	Constant Offset Elimination	20	90.18	7.62	3.19	87.94	8.47	2.9	0.935	-0.984
ATR-MIR	YAN	885	63.08-438.1	None	15	87.19	22.4	2.79	82.22	24.8	2.07	0.831	2.07
	FAN	879	32.28-267.1	Constant Offset Elimination	11	79.41	19	2.2	76.23	22.7	2.05	0.754	-0.325
	Ammonia	871	6.09-127.6	Constant Offset Elimination	14	74.54	10.7	1.98	71.71	13.2	1.88	0.675	0.873

Table 2. Summary statistics of the global models with calibration/validation ratio of 50/50.

Global Model: Calibration/Validation: 50/50													
		N	**Range (mg N/L)**	**Pre-processing**	**Rank**	**R^2_{CAL}**	**RMSEC**	**RPD_{CAL}**	**R^2_{VAL}**	**RMSEP**	**RPD_{VAL}**	**Slope**	**Bias**
FT-IR	YAN	886	44.8-469.4	First Derivative	18	94.25	15.6	4.17	94.3	15.4	4.19	0.942	-0.0719
	FAN	883	32.28-342.9	First Derivative	19	94.09	11.6	4.11	93.18	11.5	3.89	0.925	-0.499
	Ammonia	886	1.16-167.1	None	20	95.87	4.84	4.92	94.45	5.77	4.25	0.922	0.119
FT-NIR	YAN	891	53.27-470.5	None	17	95.63	14.1	4.78	94	15.6	4.09	0.948	0.65
	FAN	887	32.28-342.9	None	18	92.96	12.8	3.77	89.15	14.7	3.08	0.942	-2.47
	Ammonia	883	1.16-167.1	None	20	90.23	7.61	3.2	86.43	9.12	2.72	0.849	-0.42
ATR-MIR	YAN	879	53.27-438.1	Constant Offset Elimination	15	87.33	23.5	2.81	81.06	26.9	2.30	0.885	1.5
	FAN	877	32.28-267.1	Constant Offset Elimination	13	84.61	17.8	2.55	75.13	21.1	2.01	0.792	-1.67
	Ammonia	879	6.09-127.6	None	13	75.2	11.8	2.01	66.55	13.1	1.73	0.72	-0.938

Table 3. Summary statistics of the models built to predict the nitrogen status of a new vintage.

Vintage Model: Calibration/Validation: 2016+2017/2018													
		N	**Range (mg N/L)**	**Pre-processing**	**Rank**	**R^2_{CAL}**	**RMSEC**	**RPD_{CAL}**	**R^2_{VAL}**	**RMSEP**	**RPD_{VAL}**	**Slope**	**Bias**
FT-IR	YAN	893	59.09-388	MSC	13	91.75	18.5	3.48	94.36	17.6	4.24	0.971	-1.86
	FAN	882	44.31-267.9	None	17	91.74	12.7	3.48	93.11	11.5	3.84	0.904	-1.42
	Ammonia	886	8.68-147.6	MSC	18	93.83	5.77	4.03	94.4	7.32	4.23	0.936	0.249
FT-NIR	YAN	892	59.09-388	None	16	93.8	16	4.02	94.36	17.5	4.26	0.966	-2.75
	FAN	888	44.31-269.3	Constant Offset Elimination	17	89.64	14.3	3.11	91.49	13.7	3.43	0.94	0.268
	Ammonia	882	8.68-135.6	Constant Offset Elimination	20	89.33	7.66	3.06	91.83	8.46	3.51	0.867	0.498
ATR-MIR	YAN	892	59.09-388	SNV	8	70.23	32.9	1.83	75.83	34.1	2.05	0.681	-4.67
	FAN	883	44.31-267.9	Min-Max Normalization	11	75.26	21.2	2.01	77.46	21.3	2.17	0.782	-5.2
	Ammonia	875	8.68-120.2	First Derivative + SNV	7	63.86	13.2	1.66	61.62	16.7	1.62	0.592	-1.22

Table 4. Calibration statistics summary for the three studied spectroscopy techniques and the three nitrogen measurements evaluated. Pairwise comparison of the different spectroscopy techniques included in the study for the three nitrogen parameters evaluated. P-values lower than 0.05 indicate significant differences in the predictions delivered by the two techniques.

	FT-IR		FT-NIR		ATR-MIR		N	FT-IR vs. FT-NIR	FT-IR vs. ATR-MIR	FT-NIR vs. ATR-MIR
	RMSEC	RPD	RMSEC	RPD	RMSEC	RPD				
YAN	14.5	4.29	14	4.5	22.4	2.79	415	0.342	<0.001	<0.001
FAN	11.9	3.69	12.7	3.33	19	2.2	566	0.025	<0.001	<0.001
Ammonia	4.95	4.87	7.62	3.19	10.7	1.98	195	<0.001	<0.001	<0.001