Process control in flavour generation: NIR-MVA as a tool to monitor the formation of key odorants

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Abstract

Sensors that are suitable to monitor chemical reactions leading to the formation of potent odorants in foods, and consequently, enabling process control, are of increasing demand. In the present work, real time kinetic analyses were made by developing new methodologies combining near infrared spectroscopy (NIR) with rich information detectors such as GC and LC-MS. These were applied to study the dynamics in phenylacetaldehyde formation through a number of reactions, namely (i) glucose and phenylalanine, (ii) gallic acid and phenylalanine and iii) gallic acid, phenylalanine and glucose. Phenylacetaldehyde as well as other reaction intermediaries were monitored during 60 min with a frequency for data acquisition of 3 spectra/min. Samples were collected in 10 minutes reaction intervals, and target analysis was performed using mass spectroscopy (GC-ITMS and LC-ESI-QqTOF-HRMS). For comparison, the spectral data were analysed in a conventional way fitting kinetics for specific wavelengths. Multivariate alternative least squares (MCR-ALS) method was applied to model the spectral data with the quantification of the reaction compounds, and to perform deconvolution of spectral data. Different reaction rates were observed according to the perturbation, i.e. metals addition, temperature increase and substrate class. The obtained results were in line with those obtained by LC-ESI-QqTOF-HRMS. The developed NIR spectroscopy method showed to be a good alternative for real time, high-throughput and low-cost analysis process monitoring, unlocking chemical information related to specific compounds such as, phenylacetaldehyde, benzaldehyde, quinones and dicarbonyls, and the impact on phenylacetaldehyde formation. Also, it showed that the information captured by NIR spectroscopy can accurately predict the phenylacetaldehyde concentration under real time conditions.

Introduction

Several factors affect the formation of Strecker aldehydes in wines, in particular the formation of phenylacetaldehyde. In particular compounds such as antioxidants [1], metals [2] and glucose [3] are key compounds in mechanisms responsible for aldehydes formation such as Maillard reaction and oxidation of phenolic compounds. Commonly the kinetic studies are performed by taking points at specific times during an experiment The disadvantage of this time point approach is the loss of information between the intervals.

Process analytical technology (PAT) involves the combined use of in-process monitoring, allowing a dynamic measurement of reaction kinetics in short periods of time. Monitoring is at the core of PAT and is done in situ or at-line on whole samples by multi-parametric methods. Many spectroscopic techniques are multi-parametric methods. Near-

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infrared (NIR) spectroscopy is one of the techniques to be suitable for a variety of PAT applications. Major advantages of NIR spectroscopy regard its non-destructive nature and its immediate (real-time) delivery of results. However, this technique is based on indirect measurements, resulting in convoluted and broad spectra, that are very difficult to interpret with the unaided eye. It requires calibration and the use of a reference technique, such as GC-MS or HPLC with mathematical and statistical tools (chemometrics) to extract analytical information from the corresponding spectra [4]. This represents a major limitation for its usage, nevertheless, in recent studies it has been successfully applied in different food systems such as meat, fruits, vegetables, grains, dairy products, oils and beverages mainly for quality control [5]. In the present study, NIR spectroscopy was applied to investigate its add-value, as tool to monitor key odorants formation, such as phenylacetaldehyde formation in wine related model systems, as well as to verify its potential to unlock chemical information related to specific compounds (phenylacetaldehyde, benzaldehyde, quinones and dicarbonyls) involved in the reaction mechanism of phenylacetaldehyde formation.

Experimental

Sample preparation

Three equimolar model systems (2.4 mM) comprising i) glucose and phenylalanine (M) and ii) gallic acid and phenylalanine (O) and iii) gallic acid, glucose and phenylalanine (MO) were prepared in 12% (v/v) aqueous ethanol and tartaric acid (0.03 M) buffered to pH 3.4 with NaOH. The metal ions, copper(II) and iron(II), were added to the model system at concentrations of 6.3 µM and 0.1 mM, respectively, in the form of Cu(II)sulfate·5H2O and Fe(II)sulfate·7H2O. In the solutions with no metal addition, a 50 µM EDTA solution was also added. The solutions were prepared in 20 mL vials. The vials were closed with an internal silicone septum and an external screw cap.

Offline measurements

A design of experiments was performed consisting of storing two model solutions (O & MO) at three different temperatures (T=40, 60 and 90ºC). Phenylacetaldehyde was identified and quantified by GC-MS and the reaction intermediaries such as the gallic acid, quinone and the hydroxysulphonic acid of phenylacetaldehyde (HASA) by LC-ESI-QqTOF-HRMS, respectively [3].

Online measurements - NIR acquisition and data analysis

On-line and offline acquisition of spectra was performed with the use of a portable DLP NIRscan Nano Evaluation Module (EVM) (Texas Instruments, Dallas, USA). Absorbance spectra were measured in the wavelength range from 900 to 1700 nm, at intervals of 3.51 nm. Diffuse reflectance NIR spectra were continuously collected in-line and non-invasive during the process, a spectrum has been acquired each 1 minute for 160 minutes.

Data manipulation with NIR spectra was performed using PLS Toolbox version 8,0 (Eigenvector Research, Inc., USA) for MatlabR2014a for Mac (Mathworks, USA). It is common in NIR spectroscopy to apply pre-treatments designed to avoid a mix-up between the relevant information and the spectral noise. After the acquisition either multiplicative scatter correction (MSC) or a 7- point second order Savitzky-Golay filter (second order derivative pre-processing) were performed.

Principal component analysis (PCA) was used for sample classification to obtain process trajectories/perturbations from the spectral data. Partial least squares-regression (PLS-R) was applied to develop a model to predict phenylacetaldehyde content in online measurements.

Results and discussion

The two major questions addressed in this study were: (1) how much information can NIR spectroscopy capture from Strecker aldehyde formation in wine related model systems and (2) how accurate is the predicted phenylacetaldehyde concentration.

The approach used focused not only on the multi-parametric assessment of NIR spectra but also on the ability to define process kinetics (i.e. process-signatures or fingerprints) related to several perturbations such as the addition of metals and antioxidants during the reaction time taken from consecutive spectra acquisitions.

A representative scheme of NIR spectra pre-processing is shown in Figure 1. The raw spectrum is characterized by broad and unresolved bands; pre-processing techniques (MSC + SG + Mean Centre) clearly allowed a better enhancement of spectral information.

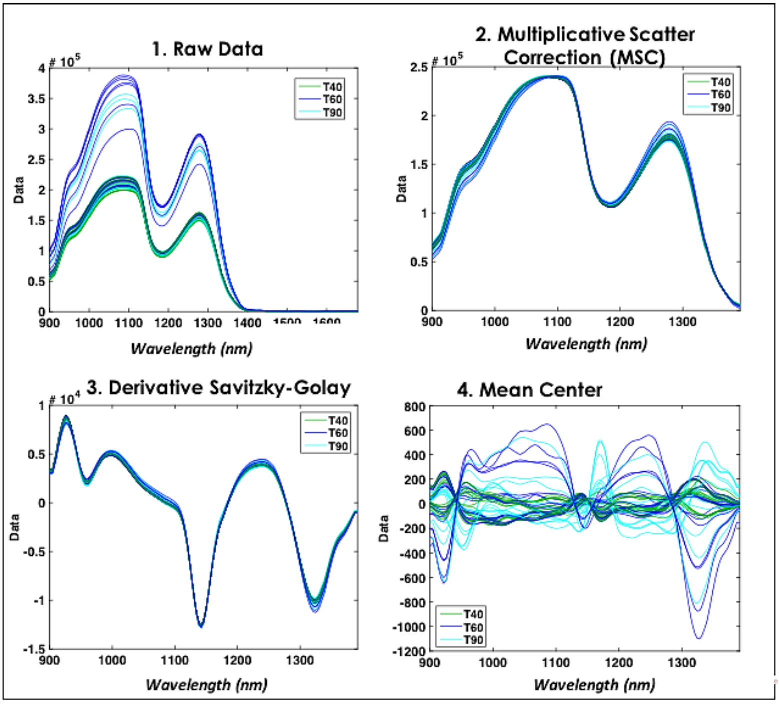

Figure 1: A) Pre-processing of spectras obtained in offline measurements (coloured according temperature) B) Scores plots from PCA analysis of PC1 vs. PC2 and PC1 vs. PC3

Offline measurements - results

The effect of temperature was well captured by the score plot of the three first principal components analysis (Figure 2). The PCA was carried out on the offline measurements to investigate the variance in the spectral data and it clearly shows that temperature 90ºC (light blue) was well separated from 60 and 40ºC along PC2. Along

PC3 a clear separation between samples with and without metals, represented in green and red respectively, was also observed. These results show that the developed NIR spectroscopy method was sensible to both temperature and metals presence which is a good indication that the sensor can be used for offline process control.

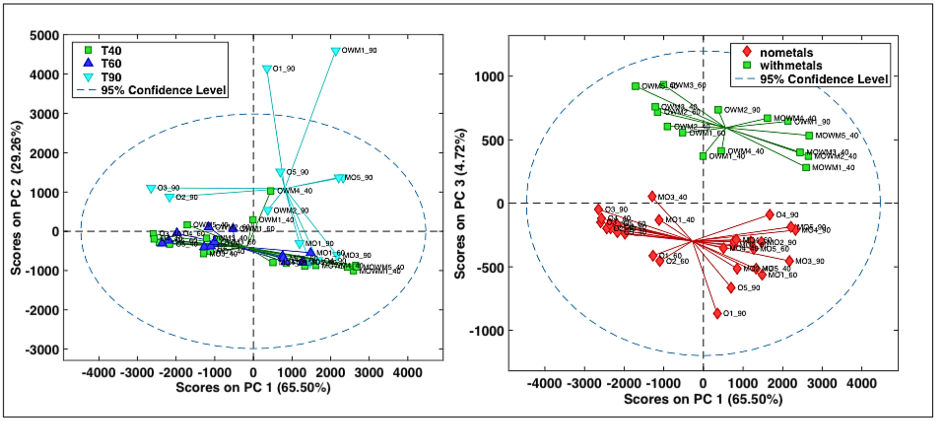

Figure 2: PCA results for the NIR data obtained in DOE offline measurements: scores plots from PCA analysis of PC1 vs. PC2 and PC1 vs. PC3

Online measurements - results

To better understand the impact of single factors in real time, sequential additions of metals and sulphur dioxide were done to the MO model system. NIR was applied to continuously collect spectra during the process, and a large amount of complex data was obtained per monitored process. Useful information from these data was extracted using suitable chemometric tools.

To further complement the obtained NIR information every ten minutes an aliquot was taken from the reactor and analysed by LC-ESI-QqTOF-HRMS. Phenylacetaldehyde content and reaction intermediates, such as: hydroxysulphonic acid (HASA) of phenylacetaldehyde were quantified.

The NIR data were again modelled using PCA and 87% of the variation was explained by the first two components (results not shown). Since this study was performed in dynamic mode as a function of time, the correlation of score plot expression can be correlated with the time of the experiment. For this purpose, the scores for the first component were plotted versus reaction time and indicated that the Strecker degradation started after 12 minutes and ended at 117 minutes (Figure 3). This endpoint also corresponded to the addition of sulphur dioxide, which was expected to stop the formation of phenylacetaldehyde due to the binding power of bisulphite to phenylacetaldehyde to form the HASA of the aldehyde. In parallel, target quantification of phenylacetaldehyde by GC-MS was done every 10 minutes during the total experiment time. Figure 3 shows that phenylacetaldehyde concentration increased during time, and the addition of SO_2 decreased its formation dramatically, confirming the results obtained with NIR Spectroscopy.

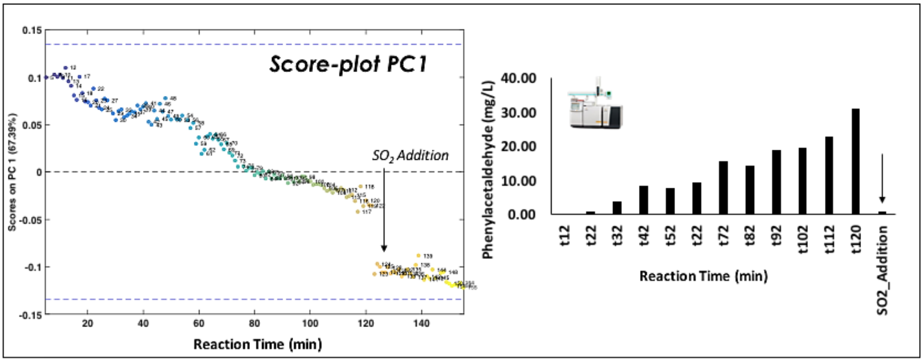

Figure 3: PCA results for the NIR data obtained during the reaction time. A) Scores of PC1 versus process time plot. B) Phenylacetaldehyde concentration measured by GC-MS during reaction time (each 10 minutes)

Furthermore, for online measurements of phenylacetaldehyde using NIR spectra a correlation coefficient of r=0,935 was obtained and a Root Mean Square Error of Calibration of 1.8 mg/L was calculated as depicted in Figure 4. An indication is given that the information captured by NIR spectroscopy can rather accurately predict phenylacetaldehyde concentration under real time conditions.

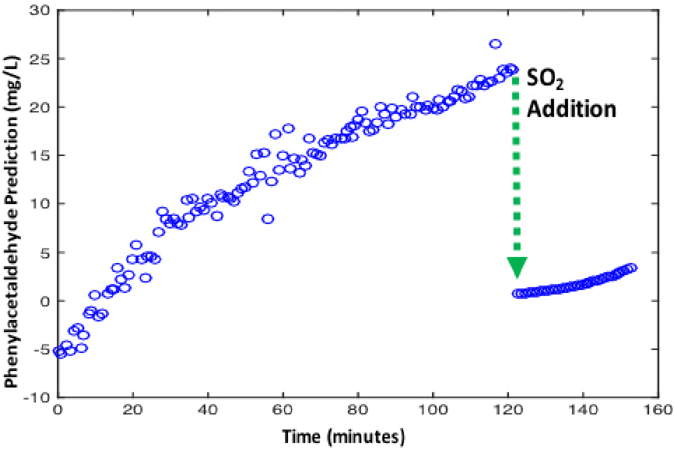

Figure 4: Phenylacetaldehyde real time prediction based on the PLS-R regression vector during the reaction time

In summary, this study evaluated the feasibility of NIR spectroscopy as a process analyser (PAT tool) for the non-invasive, on-line and real-time monitoring of Strecker aldehydes in wine related model systems. It clearly showed that it can capture the impact of the addition of metals and temperature variation on the phenylacetaldehyde formation and rather accurately predicted its concentration. The developed NIR spectroscopy method showed potential as a real time, high-throughput and low-cost analysis for process monitoring, unlocking chemical information related with specific compounds.

References

1. Nikolantonaki, M. and A.L. Waterhouse, (2012) Journal of Agricultural and Food Chemistry, 60(34): p. 8484-8491.
2. Danilewicz, J.C., (2003) American Journal of Enology and Viticulture, 54(2): p. 73-85.
3. Monforte, A.R., S. Martins, and A.C. Silva Ferreira, (2017) Journal of Agricultural and Food Chemistry
4. Porep, J.U., D.R. Kammerer, and R. Carle, (2015) Trends in Food Science & Technology, 46(2, Part A): p. 211-230.
5. Huang, H., Yu, H., Xu, H. and Ying Y., (2008) Journal of Food Engineering, 87(3): p. 303-313.