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Insights on the role of chemometrics and vibrational spectroscopy in fruit metabolite analysis

Y. Sultanbawa [a,b], H.E. Smyth [a], K. Truong [c], J. Chapman [c], D. Cozzolino [a,*]

[a] ARC Industrial Transformation Training Centre for Uniquely Australian Foods, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Coopers Plains, QLD 4108, Australia
[b] Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Brisbane, Queensland 4072, Australia
[c] Nanobiotechnology Laboratory, School of Science, College of Science, Engineering and Health, RMIT University, Melbourne, VIC 3001, Australia

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ABSTRACT

The last three decades have demonstrated the ability of combining data analytics (e.g. big data, machine learning) with modern analytical instrumental techniques such as vibrational spectroscopy (VIBSPEC) (e.g. NIR, Raman, MIR) and sensing technologies (e.g. electronic noses and tongues, colorimetric sensors) to analyse, measure and monitor a wide range of properties and samples. Developments in instrumentation, hardware and software have placed VIBSPEC as a useful tool to quantify several bioactive compounds and metabolites in a wide range of fruit and plant samples. With the incorporation of hand-held and portable instrumentation, these techniques have been valuable for the development of in-field and high throughput applications, opened new frontiers of analysis in fruits and plants. This review will present and discuss some of the current applications on the use of VIBSPEC techniques combined with data analytics on the measurement bioactive compounds and plant metabolites in different fruit samples.

1. Introduction

Recent developments in a wide range of disciplines associated with crops and fruit production (e.g. plant nutrition and physiology, biochemistry, chemistry and mathematics) have increased our knowledge about the compositional, nutritional characteristics and functional properties of these agricultural commodities (Cozzolino, 2009; Capozzi and Bordoni, 2013; Fardet, 2014; Zhang et al., 2010). Understanding the inherent complexity of fruit composition and chemistry, and their interrelations with consumers, nutrition, safety and physiology, demands new approaches that will allow moving to the direction of a more holistic and interdisciplinary approaches (Fardet, 2014). These new approaches have been associated with the introduction of new technologies (e.g. sensors) and advances in data analytics (e.g. machine learning) (Capozzi and Bordoni, 2013; Cozzolino 2009; 2011; Cozzolino, 2012; Granato et al., 2014; Nunes et al., 2015; Zhang et al., 2010).

For many years, researchers have been very effective to (Fig. 1) integrate and evaluate the implementation of different data analytical methods (e.g. chemometrics, machine learning) with a wide range and variety of instrumental analytical techniques to analyse the composition and functional properties of a wide range of samples, including fruits, grains, wine, etc (Capozzi and Bordoni, 2013; Cozzolino 2009; 2011; Cozzolino, 2012; Granato et al., 2014; Fardet, 2014; Nunes et al., 2015; Smyth and Cozzolino, 2011; Zhang et al., 2010).

The last three decades have also demonstrated the advantages of incorporating data analytics (e.g. machine learning, chemometrics) to modern analytical instrumental techniques (Fig. 2) such as vibrational spectroscopy (VIBSPEC) (e.g. NIR, Raman, MIR) and sensing technologies (e.g. electronic noses and tongues, optical sensors) to identify, monitor and quantify the composition, the nutritional value and functional properties in a wide range of natural products and foods (Capozzi and Bordoni, 2013; Cozzolino 2009; 2011; Cozzolino, 2012; Granato et al., 2014; Fardet, 2014; Nunes et al., 2015; Smyth and Cozzolino, 2011; Zhang et al., 2010). The growing number in this type of applications have been directly associated with the imperative to ensure that both raw ingredients and commodities meet the required minimal quality control standards required by both the market and consumers. In addition, these technologies have allowed to both identify and monitor changes in the chemical composition of the sample during transport, storage, and processing.

* Corresponding author.
E-mail address: d.cozzolino@uq.edu.au (D. Cozzolino).

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The chemical composition, nutritional value and functional properties of fruits is of importance during trade and processing, and ultimately to the consumer (Dembitsky et al., 2011; Guaadaoui et al., 2004; Kaur and Kapoor, 2001; Moure et al., 2011; Schieber et al., 2001). For example, nutrients such as sugars (SUG) and other carbohydrates (CHO), lipids (LIP), protein (PRO) and compounds containing nitrogen are very important to maintain the health and wellbeing of consumers, as well as to monitor storage conditions, or to improve agricultural practices (e.g. orchard fertilization) (Dembitsky et al., 2011; Guaadaoui et al., 2004; Kaur and Kapoor, 2001; Moure et al., 2011; Schieber et al., 2001). However, other compounds present in low concentrations like vitamins, bioactive ingredients (e.g. phytochemicals), are of great importance as they can have direct or indirect effects on human health and safety (Dembitsky et al., 2011; Guaadaoui et al., 2004; Kaur and Kapoor, 2001; Moure et al., 2011; Schieber et al., 2001). Most of these bioactive and natural compounds are well known to have a positive effect against different diseases such as cancer, cardiovascular diseases (CVD), obesity, neurodegenerative disorders, type 2 diabetes (Bazzano et al., 2003; Potter, 2005; Rabeta et al., 2013; Van Duyn and Pivonka, 2000; Wootton-Beard and Ryan, 2011). These bioactive ingredients include a wide range of phytochemicals and plant compounds such as antioxidants (e.g. phenols and flavonoids), minerals and vitamins (e.g. vitamin E, pro-vitamin A, and vitamin C) (Delgado-Vargas et al., 2000; Manganaris et al., 2018; Choe and Min, 2006; Martson and Hostettmann, 2009; Park et al., 2012).

Recent analytical developments have been improved by advances in instrumentation, hardware, software and data analytics, allowing for the growth of new applications in a wide range of fields including VIBSPEC, chromatography [e.g. gas chromatography (GC)], high performance liquid chromatography (HPLC), mass spectrometry (MS), and electrophoresis (Gorinstein et al., 2010; Sumner et al., 2003; Krüger and Schulz, 2007; McGoverin et al., 2010). Most of these techniques are very precise and specific allowing for the handling of several samples and analytes in the laboratory (e.g. R&D) as well as in an industrial setting (Gorinstein et al., 2010; Sumner et al., 2003; Krüger and Schulz, 2007; McGoverin et al., 2010). While routine analytical instrumentation and technologies are used to efficiently measure several analytes in a wide variety and types of fruits, the utilization of most of these technologies generally require several pre-processing stages prior or during analysis (e.g. isolation, filtration, extraction) (Gorinstein et al., 2010; Sumner et al., 2003; Krüger and Schulz, 2007; McGoverin et al., 2010). Nevertheless, less attention was devoted on the development and utilization of rapid instrumental methods based on VIBSPEC to analyse fruit bioactive compounds and metabolites (Gorinstein et al., 2010; Sumner et al., 2003; Krüger and Schulz, 2007; McGoverin et al., 2010; Ignat et al., 2011). Other sensing technologies (e.g. optical, colorimetric, etc.) have become highly popular due to their high sensitivity and low cost of analysis, making them also very useful for screening or high throughput applications (Oroian and Escricho, 2015; Blanco and Villaroya, 2002; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Bec and Huck, 2019; Bec et al., 2020).

A very significant advantage of the application of VIBSPEC techniques is that they can define and record the so-called *fingerprinting* of a given sample or even a single compound (Oroian and Escricho, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020). The utilization of these methods has allowed for the development and implementation of rapid and high throughput methods, avoiding the need for sample preparation or chromatographic separation. Without the need of such requirements, these technologies can be utilised and implemented in several steps during pre and post-harvest, during processing and storage, and even at the market (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020). The development of these applications has been made possible by the incorporation of data analytics, allowing for the analysis and interpretation of the data collected.

This review will present and discuss some of the current applications on the use of VIBSPEC techniques combined with data analytics to measure bioactive compounds and plant metabolites in a wide range of fruit samples.

2. Main characteristics of vibrational spectroscopy

The indistinctive advantages of VIBSPEC are associated with their non-destructive nature, where both minimal or no sample preparation is needed, in addition to chemical free requirements during the analysis of samples as reported by several authors in the field (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020). In addition to these advantages, the possibility to measure or predict several compounds or properties from a single analysis has been very attractive for in field and high throughput applications (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020).

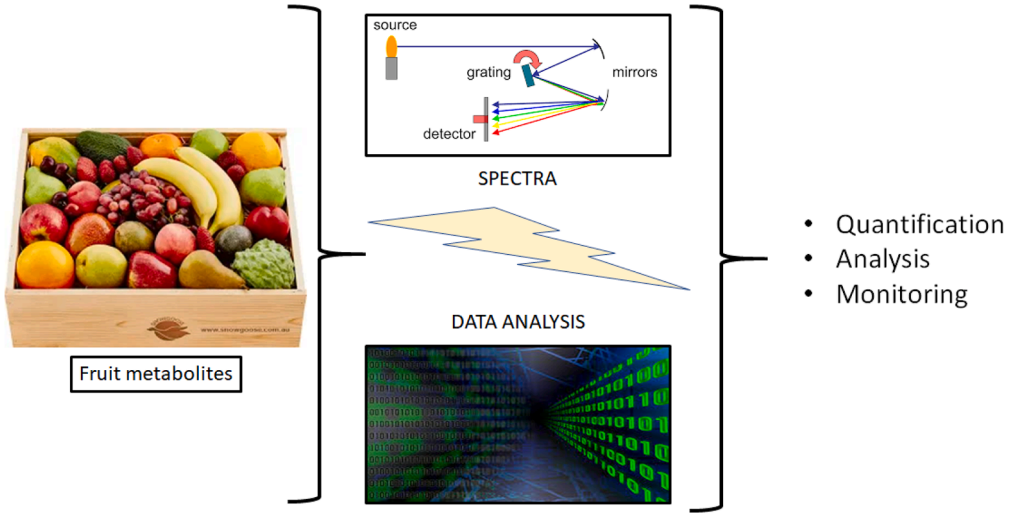

Fig. 1. Quantification and monitoring of fruit metabolites using spectroscopy and data analysis.

Vibrational spectroscopy is based on the measurement of overtones and vibrations of atoms that constitute a molecule (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020). For example, in the IR region, vibrations of carbon-hydrogen, oxygen-hydrogen and nitrogen–hydrogen as well as a diverse number of functional groups can be detected and measured in this region (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020). Biochemical, chemical compounds and metabolites of fruits and plant tissues can be measured according to the *Beer-Lambert* law where the amount of absorbed light is directly proportional to concentration (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020). During the analysis chemical bonds present in the sample will vibrate at specific frequencies, depending on their mass, and type of bonds (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020).

Spectroscopy in the near infrared (NIR) range is characterised by low molar absorption and scattering, allowed for an efficient and simple analysis of the sample (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020). Spectral 'signatures' in the mid infrared region (MIR) derived from the fundamental stretching, bending, and rotating vibrations of organic molecules, whereas in the NIR region they are derived from overtones and combinations tones (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020). One of the main drawbacks of the characteristic overlap and complexity in the NIR spectra make it difficult to directly interpret and quantify the NIR spectra. Frequencies in the MIR region is sharper and better resolved than those observed in the NIR range, for example the higher overtones of the O–H (oxygen-hydrogen), N–H (nitrogen–hydrogen), C–H (carbon-hydrogen) and S-H (sulphur-hydrogen) bands from the MIR are still observed in the NIR region (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020). One of the main advantages of the MIR region is that it contains the so-called fingerprint range between 1500 and 400 cm^{-1} where most of the identification of specific functional groups can occur (Oroian and Escriche, 2015; Blanco and Villaroya, 2002; McClure, 2003; Sorak et al., 2012; Herberholz et al., 2010; Karoui et al., 2010; Hashimoto and Kameoka, 2008; Bec and Huck, 2019; Li-Chan, 2010; Bec et al., 2020).

Raman spectroscopy, is based on the inelastic scattering of molecules, and is gaining widespread applications in different industries (e.g. pharmaceutical, food) (Baranska and Schultz, 2006; Baranska et al., 2004; Schulz et al., 2005). The inelastic scattered light delivers information about the vibration modes of the molecules, allowing for the elucidation of the specific characteristics and structure of a given molecule (Baranska and Schultz, 2006; Baranska et al., 2004; Schulz et al., 2005). During the analysis a small amount of the incident light is inelastically scattered, therefore the Raman effect is considered intrinsically a weak effect, the weak counterpart of the incident light energy is modified by the molecular vibrations of the scattering sample (Baranska and Schultz, 2006; Baranska et al., 2004; Schulz et al., 2005). Therefore, the observed vibrational responses provide with the relevant information about the chemical composition of the sample. In recent years, measuring the Raman spectra of complex foods has became more accessible due to the release of high-resolution and portable instruments with good detection capabilities (Baranska and Schultz, 2006; Baranska et al., 2004; Schulz et al., 2005). The combination of Raman spectroscopy with microscopy and imaging makes it possible for the identification and quantification of photochemical molecule distribution directly in the plant tissues (Baranska and Schultz, 2006; Baranska et al., 2004; Schulz et al., 2005).

The application of vibrational spectroscopy as analytical tool requires the extraction and analysis of data to develop a model (e.g. calibration, classification and pattern recognition). The family of methods and techniques utilised to extract information from these instrumental techniques have become part of so-called artificial intelligence or machine learning tools (Szymańska et al., 2015; Szymanska, 2018; Bureau et al., 2019; Callao and Ruizsanchez, 2018; Cozzolino, 2020). Machine learning tools are associated with the utilization of statistical methods to identify patterns in a data set and can be classified as unsupervised and supervised approaches (Witten et al., 2016). These techniques allow the analyst to isolate and interpret information from complex data sets, illustrate patterns within the data, as well as the development of mathematical relationships between different sources of data (e.g. calibration and validation models) (Szymańska et al., 2015; Szymanska, 2018; Bureau et al., 2019; Callao and Ruizsanchez, 2018). Principal component analysis (PCA) is the most widely adopted variable-reduction method utilised in many of these applications in the food sector (Bro and Smilde, 2014). In addition to PCA other classification or pattern recognition techniques have been utilised and

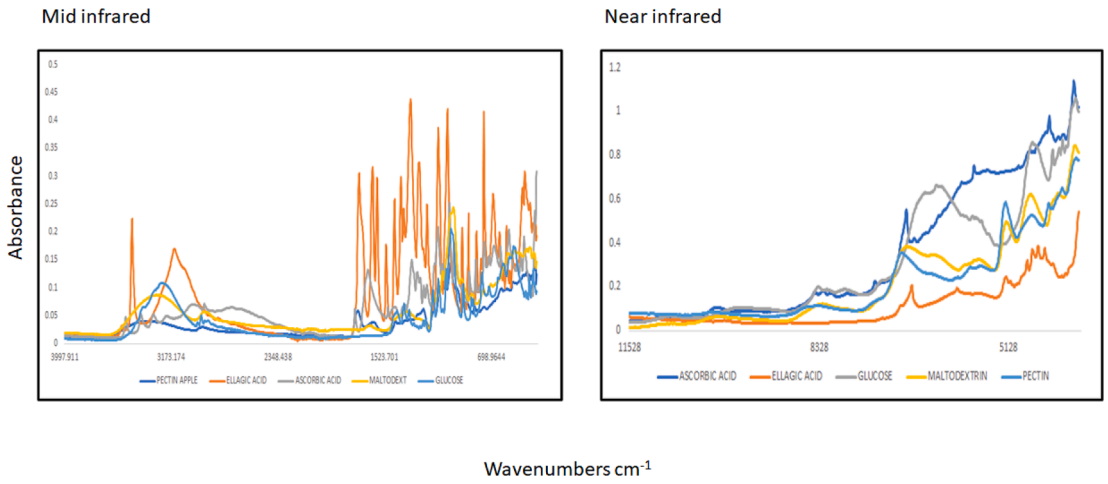

Fig. 2. Mid and near infrared spectra of ascorbic acid, pectin (from apple), glucose, ellagic acids and maltodextrin.

reported, including the use of discriminant analysis methodologies (e.g. supervised and unsupervised) such as cluster analysis, K-nearest neighbours (k-NN), linear discriminant analysis (LDA), factorial discriminate analysis (FDA), partial least squares discriminant analysis (PLS-DA), quadratic discriminant analysis (QDA), artificial neural networks (ANN), soft independent modelling of class analogy (SIMCA), and more recently the group of machine learning methods and techniques [e.g. support vector machines (SVM)] (Szymańska et al., 2015; Szymanska, 2018; Bureau et al., 2019; Callao and Ruizsanchez, 2018). The utilisation and combination of data analytics alongside the adoption of sensing technologies has make possible the development of a wide range of applications targeting issues detailed in the following sections.

3. Measurement of metabolites in fruits

3.1. Applications of mid and near infrared spectroscopy

The assessment of several metabolites in kiwifruit fruit samples was evaluated using MIR spectroscopy (Park et al., 2012). These authors quantify the concentration of polyphenols (POLY), flavonoids (FLAV), flavanols, and tannins (TAN) in different cultivars (e.g. Bidan and Hayward) (Gorinstein et al., 2010). The ability of MIR spectroscopy to measure compounds that have high antioxidant ability in a wide range of fruit samples such as durian, mango and avocado was also reported in this study (Gorinstein et al., 2010). Total POLY, FLAV, TAN and flavanols were determined using routine analytical methods. The authors of this study concluded that this method could determine the bioactivity in a wide range of phytochemical and plant derived compounds (Gorinstein et al., 2010).

MIR spectroscopy was also used to quantify fruit ANTACT (Lam et al., 2005). Blueberry, grape, and blackberry extracts (methanol–-water-formic acid) having high concentration of flavonoids were analysed for their ANTACT by conventional methods and spectroscopy (Lam et al., 2005). PLS regression was used to develop the predictive models yield a R^2 of 0.97 for and RMSE of 5.35 for ANTACT (Lam et al., 2005). The prediction of compounds with high antioxidant ability (e.g. lycopene and POLY) in tomato was achieved using visible (VIS) and NIR spectroscopy (500 and 1000 nm) (Szuvandzsiev et al., 2014). The quantification of vitamin C (VIT-C), POLY and SUG in different apple genotypes was attempted using NIR spectroscopy and least squares support vector machine (LS-SVM) as regression method (Pissard et al., 2013). Low SEP and high RPD values, demonstrated the high precision of the models to predict POLY and SUG content (Pissard et al., 2013). MIR spectroscopy coupled with ATR was also evaluated to determine several bioactive compounds in *Momordica charantia* extracts (Khatib et al., 2017).

The prevalence of natural compounds and metabolites because of sun exposure of Granny Smith apple samples was evaluated using VIS-NIR spectroscopy (Torres et al., 2016). The NIR spectra was analysed using discriminant PLS models (PLS-DA) and interval PLS-DA (iPLS-DA) (Torres et al., 2016). Variations in the VIS-NIR spectra were associated with variations in carotenoids (CAROT) and FLAV (Torres et al., 2016). The flesh of kiwifruit samples was evaluated for its nutraceutical value using NIR spectroscopy (POLY, CAROT, ANTACT). According to the authors good prediction performances were obtained for both total flavans (R^2: 0.81, RMSEP: 0.07) and VIT-C (R^2: 0.87, RMSEP: 6.04) (Ciccoretti et al., 20).

Total POLY and CAROT content in blackberries was quantified using NIR spectroscopy (Toledo-Martin et al., 2018). The RPD and SEP values reported by the authors were $1.5 < RPD < 2.5$ and RER values (ratio of the range in the reference data to SEP) were 5.92 for POLY and 8.63 for CAROT content (Toledo-Martin et al., 2018). According to the authors of this study such values indicated that the models developed were suitable for screening purposes (Toledo-Martin et al., 2018). The loadings derived from the PLS models highlighted that SUG, chlorophyll, LIP and cellulose contributed to the models (Toledo-Martin et al., 2018).

Both NIR and MIR spectroscopy were assessed to measure total soluble solids, acidity, SUG and VIT-C measured as ascorbic acid in citrus fruit samples (Oliveira-Folador et al., 2018). Although the models were acceptable for most of these parameters, the prediction models reported for VIT-C were not considered acceptable (Oliveira-Folador et al., 2018). Both UV–VIS and MIR spectroscopy were evaluated to measure the content of CAROT, FLAV, and POLY in citrus (Song et al., 2018). The predictive models developed yielded R^2 higher than 0.90 (Song et al., 2018). These authors concluded that accurate quantitative predictions can be achieved using MIR spectroscopy and this can be used in selection and breeding (Song et al., 2018). The combination of VIS and NIR spectroscopy were evaluated to measure CAROT in processed tomato (Saad et al., 2017). Eight CAROT compounds were measured using HPLC and the data used to develop calibration models based on the spectra. The best PLS models developed were for beta CAROT $R^2 = 0.88$, for *9-cis* lycopene (LYCO) $R^2 = 0.86$, for total CAROT $R^2 = 0.84$, for *13-cis* LYCO $R^2 = 0.83$, for *5-cis* LYCO $R^2 = 0.80$ and $R^2 = 0.80$ for zeaxanthin (Saad et al., 2017).

Total soluble solids (TSS), LYCO and total CAROT content were measured in intact watermelon fruits using NIR spectroscopy (Tamburini et al., 2017). The PLS calibration models were able to predict LYCO ($R^2 = 0.877$ and SECV = 15.68 mg kg^{-1}), beta CAROT ($R^2 = 0.822$ and SECV = 0.81 mg kg^{-1}), and TSS ($R^2 = 0.836$ and SECV = 0.8%). The PLS models were also validated using an external data set (Tamburini et al., 2017).

The ability to predict CAROT in tomato fruit using NIR spectroscopy was reported. The calibration models for beta CAROT content of tomato samples was $R^2 = 0.89$; RMSECV = 0.174 μg g^{-1} (Deak et al., 2015). The NIR method was also performed for the determination of all-trans LYCO content $R^2 = 0.75$; RMSECV = 6.88 μg g^{-1} (Deak et al., 2015). The content of beta CAROT in mango fruit was attempted using NIR spectroscopy (Rungpichayapichet et al., 2015). The MLR models reported a $R^2 > 0.80$ and SEP = 11.642–20.2 RE 100 g^{-1} EP (Rungpichayapichet et al., 2015). The authors also compared different wavelength region and concluded that long wavelengths have better predictive ability compared with short wavelengths (Rungpichayapichet et al., 2015). The concentration of POLY, ANTHO and VIT-C were predicted using FTNIR spectroscopy in whole pomegranate fruit samples (Ardense et al., 2018). The PLS models reported by the authors were as follows POLY (R^2 = 88.0, RPD = 2.91) and VIT-C ($R^2 = 76.2$, RPD = 2.06) and total ANTHO (R^2 = 62.6, RPD = 1.64) (Ardense et al., 2018). The quantification of LYCO and beta CAROT content in intact tomato fruit samples was attempted by VIS-NIR spectroscopy. Calibration models were developed using PLS regression had R^2 0.85 for LYCO and R^2 0.77 for CAROT, respectively (Tilahun et al., 2018).

3.2. Applications of Raman spectroscopy

Raman spectroscopy has been evaluated to quantify TAN in pomegranate fruit samples (Khodabakhshian, 2019). The Raman spectra was acquired from the cross-sections of the fruit (100–3000 cm^{-1}) where Spectral Information Divergence (SID) was used as method to monitor the different maturity stages of the pomegranate fruit samples (Khodabakhshian, 2019). It was concluded that Raman spectroscopy has a potential of non-destructively analysing TAN in the surface of the pomegranate maturity (Khodabakhshian, 2019). Unfortunately, the authors did not report any SEP or RPD values for the models developed.

Mangoes sourced from Pakistan (cultivars *Rawl* and *Chaunsa*) were analysed for beta CAROT using Raman spectroscopy as proxy for ripening (Ullah et al., 2019). The Raman spectra of pulp from different postharvest stages, displayed specific absorbances at 1003, 1150 and 1515 cm^{-1} (Ullah et al., 2019). The content of CAROT was quantified using Raman spectroscopy in processed *Bunchosia glandulifera,* a fruit that originated from Brazil. The authors used PCA and PLS regression to identify and quantify total CAROT in *B. glandulifera* (Carvalho et al., 2019).

The combination of microscopy and Raman spectroscopy were used to evaluate different metabolites in different tissues of tomato at different ripening stages were reported (Sharma et al., 2019). The Raman data of the different tissues of the fruit (exocarp, mesocarp and endocarp) using the region $< 2000\ cm^{-1}$ using confocal Raman instrument (785 nm excitation laser) (Sharma et al., 2019). The Raman spectra display signatures of cuticular wax which is detected only in the exocarp, while signatures of LYCO, CAROT, PHE, SUG and CHO were observed in all of tomato tissue samples with changing concentration while amino acid and pectin were observed in the mesocarp and endocarp (Sharma et al., 2019). Raman spectroscopy was used to monitor the composition of tomato fruits during ripening (Trebolazabala et al., 2017). The Raman spectra confirmed a rise of CAROT concentration from an unripe to a ripe stage, where LYCO seemed to have a peak at the ripe stage (Trebolazabala et al., 2017). On the other hand, the existence of chlorophyll and cuticular waxes decreased from the unripe to the ripe stages (Trebolazabala et al., 2017). Three excitation wavelengths (532 nm, 785 nm, and 1064 nm) were evaluated to measure the concentration of different CAROT in tomatoes (Hara et al., 2018a; Hara et al., 2018b). The authors reported that using the wavelength at 532 nm no direct relationship with the content of CAROT was observed, however, the changes in CAROT from LYCO to beta CAROT and lutein (Hara et al., 2018a; Hara et al., 2018b). Both wavelengths at 785 nm and 1064 nm showed a good correlation with CAROT content (Hara et al., 2018a; Hara et al., 2018b). Spectral data obtained using these wavelengths was used to develop PLS calibrations for this compound. These researchers found that 785 nm is the most suitable excitation wavelength for the analysis of carotenoid concentration in tomatoes (Hara et al., 2018a; Hara et al., 2018b).

Surface-enhanced Raman scattering (SERS) was evaluated as a component of a method comprised of a redox-sensitive (REDOX) and a pH-sensitive probe in different fruit varieties (e.g. apple, pear) (Sun et al., 2018). The SERS spectra of REDOX probes were applied to indicate their REDOX states, and the SERS spectra of pH-sensitive probes were used to indicate their pH values to discount the influence of pH on the redox states. The authors concluded that the SERS method is simple without any pre-treatments and utilization of reagents or chemicals (Sun et al., 2018). Both MIR and Raman spectroscopy were used to predict total PHEN content and ANTACT in blueberry samples. Good calibrations models were reported for the prediction of PHEN compounds using MIR spectroscopy (R^2 ranged between 0.63 and 0.81; RMSE ranged between 6.19 and 0.71 μmol TROLOX equivalents/g fresh weight, and 0.14 mu g GAE/g fresh weight) (Zheng et al., 2017). The content of CAROT in the skin of clementine, mandarin and tangerine species was measured by Raman spectroscopy (Nekvapil et al., 2018). Citrus freshness was found to be associated with the Raman spectra. The concentration of CAROT was correlated with the Raman spectra and found to be a good predictor of fruit freshness (Nekvapil et al., 2018). A portable NIR excited Raman instrument was used to measure LYCO in vegetable-juice. The authors reported that the Raman spectra is directly proportional with the concentration of LYCO, however scattering decreased the repeatability of intensity of the Raman peaks. The authors reported the use of mean intensity ratio (PMIR) analysis to identify LYCO in the samples analysed (Hara et al., 2018a; Hara et al., 2018b). Tomatoes and tomato-related products were measured using a portable Raman system (Fu et al., 2016). Classification models based on Raman spectra were able to correctly classify 85.6 % of the samples. The authors attempted to quantify the concentration of LYCO in the samples with poor predictive ability (Fu et al., 2016). The ability of surface-enhanced Raman spectroscopy (SERS) was evaluated to determine different polyflavones in citrus (Ma et al., 2016). The authors highlighted that the position of hydroxylation in the monohydroxylated flavonoids was significant to expose the interactions with silver dendrites, providing with relevant information for further applying SERS for molecular characterization of flavonoids in fruit samples (Ma et al., 2016).

A recent review highlighted and discussed the state of the art in applications of Raman spectroscopy and imaging techniques, including issues associated with backscattering, transmission spectroscopy, Raman chemical imaging, SERS, excitation sources, type of detectors, among other applications (Qin et al., 2019). Table 1 displays the standard error in prediction reported by different authors on the applications of vibrational spectroscopy (MIR, NIR and Raman) for the measurement of metabolites in fruits.

4. Final considerations

The implementation and adoption of VIBSPEC techniques is allowing for a fast and non-destructive means to analyse bioactive compounds, metabolites and nutraceuticals in fruits. As shown in the above sections, different authors have illustrated with a wide range of applications the significance of NIR, MIR and Raman spectroscopy combined with data mining tools to evaluate and measure a variety of bioactive compounds and metabolites in fruit samples. It has also been demonstrated in these applications that both the accuracy and robustness of the methods based in VIBSPEC are comparable to those obtained during the utilization of other routine analytical methods (e.g. LC-MS, MS, GC). It is well known that VIBSPEC generally cannot measure low concentrations, other chemical and physical effects can be evaluated in the MIR scan of a given sample (fingerprint). The introduction of modern data analysis techniques jointly with these techniques can be used to explain specific characteristics and properties related with chemical and nutritive value not easily sensed by classical targeted chemical analysis, can be considered one of the main advantages. However, the utilisation of the calibration models requires continuous updating and validation (e.g. environmental conditions, samples from different and diverse origin, appropriate spectral pre-processing). Somehow ignored or underestimated, calibration development is the critical step to develop a robust method based on VIBSPEC.

The reduction in cost, and time required during the analysis of these samples, combined with the environmentally friendly nature of VIBSPEC, make them as a very attractive set of tools in the analysis of bioactive compounds in fruits. Progress in hardware, optics and software are further positioning VIBSPEC as very effective tools to quantify simultaneously and non-destructively several bioactive compounds and metabolites in fruits. Recent advances in hand-held and portable instrumentation have allowed these techniques to be utilised for in field and high throughput applications. The last three decades, have shown how these techniques could be considered as an efficient and robust alternative for the quantification and identification of metabolites and

Table 1
Standard error in prediction reported by different authors on the applications of vibrational spectroscopy (MIR, NIR and Raman) for the measurement of metabolites in fruits.

Fruit type	Technique	Analyte	SEP	Reference
Blackberries	NIR	POLY	1.22 mg g^{-1}	(Toledo-Martin et al., 2018
		CAROT	0.77 mg g^{-1}	
Bunchosiaglandulifera	Raman	CAROT	24.7 – 27.0 mg 100 g^{-1}	(Carvalho et al., 2019)
Tomato	Raman	LYCO	14.2 – 16.2 μg g^{-1}	(Fu et al., 2016)
Apple	NIR	POLY	40.6	(Giovanelli et al., 2014)
		ANTACT	0.40	
Passion fruit	NIR	Ascorbic acid	2.57	(Manizawa et al., 2014)
Orange	NIR	VIT-C	8.10	(Magwaza et al., 2013)

SEP: standard error of prediction; POLY; total polyphenols; CAROT: total carotenoids; ANTACT: antioxidant activity; LYCO: lycopene.

other compounds in fruits. However, some barriers exist to its widespread utilization. The nonexistence of academic education and formal training in VIBSPEC (e.g. NIR spectroscopy) and the lack of integration of associated disciplines such as data mining and multivariate data analysis hinder the implementation of these methods and technologies.

5. List of acronyms

ANTACT anti-oxidant capacity; ANTHO anthocyanins; ATR attenuated total reflectance; CAROT carotenoids; CHO carbohydrates; CVD cardiovascular diseases; FLAV flavonoids / flavanols, FTNIR Fourier transform near infrared; GC gas chromatography; HPLC high performance liquid chromatography; iPLS-DA interval PLS-DA; LIP lipids; LS-SVM least squares support vector machine; LYCO lycopene; MIR, mid infrared; MLR multiple linear regression; MS mass spectrometry; NIR near infrared; PHEN phenolics; PLS partial least squares; PLS-DA partial least squares discriminant analysis; PRO protein/nitrogen; POLY polyphenols; RMSE root mean square error; R2 coefficient of determination; RPD residual predictive deviation; SEP standard error prediction; SERS, surface-enhanced Raman spectroscopy; SID spectral information divergence; SUG sugars; TAN tannins; TSS total soluble solids; VIBSPEC, vibrational spectroscopy; VIT C vitamin C.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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