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Verification of authenticity and fraud detection in South African honey using NIR spectroscopy

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ABSTRACT

The South African honey industry will benefit from a reliable method that can verify authenticity as well as detect fraudulence of honey that is on the market. This work presents the use of near-infrared (NIR) spectroscopy in combination with chemometrics as a fast and relatively inexpensive method to discriminate between authentic South African and imported and/or adulterated honey. A supervised chemometric approach was followed on NIR spectra collected from genuine South African honey, as well as intentionally adulterated honeys with sugar solutions (glucose and fructose) and also cheap imported honey. By using partial least squares discriminant analysis (PLS-DA), overall classification accuracies of between 93.3% and 99.9% were obtained when using three different NIR instruments (a laboratory instrument, as well as a portable and a mobile instruments). The usefulness of NIR spectroscopy for accurate honey classifications, regardless the instrument specifications, are demonstrated.

1. Introduction

As South Africa (SA) holds a significant floral biodiversity that hosts more than 30 000 plant species, including the Cape's Fynbos that boasts one of the most diverse flora on earth (Khan, Hill, Kaehler, Allsopp, & van Vuuren, 2014), there are abundant nectar and pollen producing plants available for honey production. Nonetheless, SA is a small honey producing country compared to other African countries such as Ethiopia, Tanzania, Kenya and Angola, with SA producing approximately 1500 tons of honey in contrast with Ethiopia that export, let alone produce, over 40 000 tons yearly (The SA Beekeeping Industry, 2008; NAMC, 2014). The limited South African production cannot fulfill the consumer demand and another 1500 tons of honey is imported yearly (NAMC, 2014; Wolfaardt, 2014). Interestingly, in SA only 10% of the beekeepers are farming on a commercial scale (800–10 000 hives) with the bulk of the beekeepers having less than a 100 hives and being seen as hobbyist beekeepers (SABIO South African Bee Industry Organisation, 2015; Wolfaardt, 2014).

Natural bee honey is superior to other sweeteners, such as refined cane sugar, beet sugar, and maize syrup due to it being a valuable source of rich nutritious compounds, its medical benefits and unique flavour (Zhu et al., 2010). Nutritional benefits include the availability of sugars, macro and micro elements and biologically active substances, phenolic compounds, minerals, proteins, organic acids, free amino acids, enzymes and vitamins present in genuine honey (Li, Shan, Zhu, Zhang, & Ling, 2012; Yilmaz et al., 2014). Additionally, benefits for human health include the treatment of wounds due to their anti-microbial and anti-inflammatory properties (Khan, Hill, Kaehler, Allsopp, & Vuuren, 2014; Robson, Dodd, & Thomas, 2009; Theunissen, Grobler, & Gedalia, 2001). Honey also possesses anti-carcinogenic and anti-viral properties (Yilmaz et al., 2014).

Generally, honey being sold comprises a mixture of floral sources, but in recent years the production of monofloral honeys has gained interest (Vanhanen, Emmertz, & Savage, 2011). Monofloral honey is produced from a single main flower source (with the presence of a single pollen type in quantities higher than 45% of the total pollen content) of a specific geographical or botanical region and has a typical smell and taste (Vanhanen et al., 2011). Monofloral

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Abbreviations

PLS-DA	partial least squares discriminant analysis
NIR	near-infrared
SCIRA	stable carbon isotopic ratio mass spectrometry
AuthSA	authentic South African honey
NAuth&Adult	non-authentic and adulterated honey
SAnc	non-commercial SA honey
SAc	commercial SA honey
Imp	imported honey
AdSug	SA honey adulterated with sugar
AdImp	SA honey adulterated with imported honey
FTNIR	Fourier transform near-infrared
SNV	standard normal variate
VIP	variable importance in projection

honey is more valuable than polyfloral honeys and certain types of monofloral honey have claimed benefits, for example manuka (*L. scoparium*) honey from New Zealand that possesses antibacterial properties (Jandrić et al., 2015; Vanhanen et al., 2011), consequently having a higher commercial value.

To identify the floral origin of honey, microscopic analysis of the pollen (melissopalynology) is required (Cen & He, 2007; Jandrić et al., 2015; Marini, Magri, Balestrieri, Fabretti, & Marini, 2004), which can be supplemented with sensory and physico-chemical analysis (Jandrić et al., 2015). These methods, however, have many disadvantages for example pollen identification requires an exceptionally specialised analyst, while the other analytical measurements are not very specific and cannot be consistently applied to all honey varieties (Jandrić et al., 2015; Marini et al., 2004). As a consequence, the need for faster, but still reliable methods to discriminate between honeys of different floral origins has led to the development of alternative approaches based on coupling the determination of multiple chemical indices with multivariate analysis techniques. For instance, Marini et al. (2004) used both discriminant and modeling tools on 15 selected chemical indices to classify Italian honeys according to their floral origin, obtaining 100% accuracy, while similar variables were used by Corbella and Cozzolino (2006) for the same purpose, but considering samples of different botanical species from Uruguay.

Honey is seen as a high-value foodstuff and is therefore a target for adulteration, which is a global concern. Honey is adulterated by either the addition of cheap sugar syrups or by diluting the high quality honey with low cost and low quality honey (Li et al., 2012; Zhu et al., 2010). Honey is imported into SA at a lower price than that of locally produced honey (NAMC, 2014), and a form of misconduct would be to label the honey as being produced in SA, but in reality it would be imported or diluted with imported honey. Not only is the consumer mislead, but the local producers cannot compete with the low pricing of these adulterated honeys. Therefore, there is a definite demand and growing interest in verifying the authenticity of honey to protect both the local SA producer, as well as the consumer.

Adulterated honey is generally detected using stable carbon isotopic ratio mass spectrometry (SCIRA) (Cabanero, Recio, & Ruperez, 2006; Cotte et al., 2007; Simsek, Bilsel, & Goren, 2012), as well as other methods such as: chromatography (Morales, Corzo, & Sanz, 2008), thermal analysis (Cordella, Faucon, Cabrol-Bass, & Sbirrazzuoli, 2003) and nuclear magnetic resonance (NMR) (Cordella, Militão, Clément, Drajnudel, & Cabrol-Bass, 2005; Cotte et al., 2007). Although these methods have been demonstrated as being useful for assessing adulteration in honey, they are expensive, time-consuming, destructive (apart from NMR which is also a non-destructive technique) and requires the operator to be considerably skilled. Again there is a need for a fast, non-destructive/non-invasive, easy to use and low cost classification method to detect adulteration in honey (Herrero Latorre, Pena Crecente, Garcia Martin, & Barciela Garcia, 2013; Li et al., 2012; Zhu et al., 2010). Moreover, if fraud is detected, the use of a non-destructive/non-invasive technique preserves the integrity of the evidence and allows the sample to be re-analysed after some time or, if necessary with an alternative technique.

Near-infrared (NIR) spectroscopy, in combination with chemometrics, has previously been used, either to determine the floral origin of honey (Etzold & Lichtenberg-Kraag, 2008; Liang, Li, & Wu, 2013; Schievano, Stocchero, Morelato, Facchin, & Mammi, 2012), or to authenticate the geographical or botanical origin thereof (Chen et al., 2012; Herrero Latorre et al., 2013; Kelly, Petisco, & Downey, 2006; Toher, Downey, & Murphy, 2007; Woodcock, Downey, Kelly, & O'Donnell, 2007). Classification models of multi-national honey samples produced 96, 100, 100, and 100% correct classifications for the Argentinean, Czech, Hungarian, and Irish honey samples, respectively (Woodcock et al., 2007). The detection of fraud due to unclaimed sweetener addition using NIR spectroscopy and chemometrics is not as commonly found, however this was studied by Zhu et al. (2010) who obtained good accuracies (95.1%) when classifying pure and adulterated honeys. Li et al. (2012) also detected sweetener adulteration, but using another vibrational spectroscopic method, Raman spectroscopy, in combination with chemometrics.

In this study, South African honey samples were characterised and the authenticity of these were verified and fraudulent honeys detected, using NIR spectroscopy. The effectiveness of three different types of NIR spectroscopy instruments, differing in wavelength range, resolution and portability, were systematically compared. The main objective of this work was to develop a fast, non-destructive NIR spectroscopy classification method for detecting either imported or adulterated South African honey, while simultaneously detecting the addition of sugars.

2. Materials and methods

2.1. Honey samples

The sample set (n = 84) consisted of two main categories of honey, i.e. authentic South African honey (AuthSA), as well as non-authentic and adulterated honey (NAuth&Adult) (Table 1). Sub-categories of the AuthSA honey comprised non-commercial SA honey (SAnc), as well as commercial SA honey (SAc), whereas the three sub-categories for the NAuth&Adult contained imported honey (Imp), SA honey adulterated with sugar (AdSug) and SA honey adulterated with imported honey (AdImp).

The SAnc samples (n = 32) were collected between 2009 and 2013, from beekeepers representing four South African provinces (Northern Cape, Eastern Cape, Free State and Southern Cape). These samples were considered by the beekeepers to be monofloral honey, based on the nature of the vegetation of the area that hosted only one floral species suitable for honey production at that given period. After collecting and before analysis, the samples were stored in a dark room at ambient temperature in air-tight plastic or glass containers. The samples were kept unfiltered. Prior to spectral collection, the samples were incubated in an air-oven (SMC Scientific Manufacturing cc, Killarney Gardens, South Africa) overnight at 55 °C to dissolve any crystals present. The samples were left to equilibrate to ambient temperature for approximately 1 h before spectral acquisition. All the samples were also adjusted to a standard soluble solids (SS) content of 70 °Brix in order to sidestep

Table 1
The different categories, as well as sub-categories, with their respective abbreviations for the honey sample set.

Main category	Abbreviation	Sub-category	Abbreviation
Authentic South African (n = 44)	AuthSA	Non-commercial SA honey (n = 32)	SAnc
		Commercial SA honey (n = 12)	SAc
Non Authentic and Adulterated (n = 40)	NAuth&Adult	Imported honey (n = 12)	Imp
		SA honey adulterated with sugar (n = 14)	AdSug
		SA honey adulterated with imported honey (n = 14)	AdImp

spectral difficulties from naturally-occurring variations in the SS and to avoid false classification on the basis of variations in SS content between honeys. The SS content of each sample was measured with an Atago bench top refractometer (Atago Co., Ltd, Japan) and subsequently adjusted to 70 ± 0.5 °Brix with distilled water. The SAc samples consisted of 12 honeys that were purchased from five different retail stores (Spar, Checkers, Pick-*n*-Pay, Woolworths and Clicks) in South Africa and these samples were also adjusted to 70 °Brix prior to spectral collection. Additionally, imported honey samples (n = 12) (Imp) were purchased from the same retail stores and were also adjusted to 70 °Brix for standardised spectral collection.

This study also involved the extension of the sample set by adulterating some of the SAnc (n = 4) and SAc (n = 3) samples with fructose:glucose combinations, or by adding Imp (n = 1) to these AuthSA honeys. All these adulterated honeys (n = 28) were categorised as being NAuth&Adult, and additionally sub-categorised as AdSug (n = 14) or as AdImp (n = 14) (Table 1). To prepare the AdSug samples, fructose:glucose mixtures were prepared by dissolving fructose (Tongaat Hulett, Cape Sweetners, Cape Town, South Africa) and glucose (Orley Foods (Pty) Ltd, Montague Gardens, South Africa) in distilled water in the following ratios: 0.7:1, 1.2:1 and 2.3:1 w/w (Zhu et al., 2010). The 7 AuthSA samples were then adulterated at a 10% w/w level with each of the three glucose:fructose solutions, respectively. For the AdImp samples, the Imp sample was also added at a 10% w/w level to the same 7 AuthSA samples that were used for the sugar adulteration. Both the adulterant solutions were also produced at 70 °Brix.

Spectra from the entire sample set (n = 84) were collected using laboratory and portable NIR spectrophotometers, whereas the sample set for which spectra were collected on a mobile instrument did not include all the SAnc samples. Due to technical problems spectra of only 20 (and not 32) non-commercial SA honey samples were acquired, reducing the respective sample set to only 72 samples for the mobile instrument.

2.2. NIR spectral acquisition

Three different NIR instruments were used for spectral collection, i.e. a benchtop laboratory instrument, a small hand-held portable instrument, as well as a mobile modular instrument. Table 2 summarises some aspects of these respective instruments. In all three cases, two analytical replicates were acquired for each sample, for a total of 168 (laboratory and portable intruments) and 144 (mobile instrument) spectra, respectively.

2.2.1. Laboratory instrument

A BÜCHI NIRFlex-500 Fourier transform near-infrared (FTNIR) spectrophotometer (BÜCHI Labortechnik GmbH, Flawil, Switzerland) coupled with a liquid cell and using NIRLabWare (version 3.0) (BÜCHI Labortechnik GmbH, Flawil, Switzerland) measurement software was used to collect spectra of Brix-adjusted honey samples. The samples were presented to the instrument in a Hellman quartz cuvette (Sigma-Aldrich, Munich, Germany) with a 0.5 mm path length, and the NIR spectra were collected from 1000 to 2500 nm at a nominal resolution of 8 cm^{-1} in transmission mode. Each spectrum was the average of 32 scans.

2.2.2. Portable instrument

Spectra were collected using a MicroNIR 1700 (JDSU Corporation, Santa Rosa, CA, USA) miniature NIR spectrometer fitted with a 128-pixel InGaAs detector. The reflectance spectra were measured in the NIR region (908–1680 nm) of the electromagnetic spectrum at 6.2 nm intervals. An InGaAs detector was used to achieve a resolution of 30 × 250 μm/50 μm. Each spectrum was the average of 64 scans. The MicroNIR is fitted with a LVF filter which can be compared to a scanning Fabry-Perot interferometer that scans with position instead of time (Macleod, 2010; O'Brien, et al., 2012). Two tungsten lamps allowed for illuminating a spot (3 mm in diameter) on the sample with the optimal focal distance being 3 mm from the sample. The external white reference was a 99% diffuse reflectance standard (JDSU Corporation, Santa Rosa, CA, USA) measured once every 15 min during the sample acquisition period. The Brix-adjusted samples were presented in a hollowed-out Teflon (PFTE) disk (Maizey's (Pty) Ltd, Kuils River, South Africa) which the MicroNIR's connectable collar could cover completely during scanning. MicroNIR software (JDSU Corporation, Santa Rosa, CA, USA) was used to perform the spectroscopic measurements.

2.2.3. Mobile instrument

A mobile fiber type NIRQuest model 512–2.5 (OceanOptics, USA) modular spectrophotometer was used for spectral collection of honey samples presented in a Teflon cup from 860 to 2500 nm in reflection mode. Each scan was the average of 64 scans and an integration time of 20 ms was achieved along with scanning intervals of 6 nm. OceanView software (OceanOptics, USA) was used to perform the spectroscopic analysis. Before sample measurement and every 15 min during the sample acquisition period, a reference scan was taken from a 99% diffuse reflectance standard (JDSU Corporation, Santa Rosa, CA, USA).

2.3. Software

For all three NIR instruments, spectral data were saved in Microsoft Excel (Microsoft Corporation, Seattle, WA) spread sheet format and then transferred to MATLAB (v.8.5 R2015b; The

Table 2
Summary of selected specifications with respect to the different NIR instruments.

Manufacturer and model	Instrument type	Wavelength range (nm)	Spectral interval (nm)	Acquisition mode	Sample presentation
BÜCHI NIRFlex	Laboratory scale benchtop	1000–2500	0.4	Transmission	Quartz cuvette (0.5 mm)
JDSU MicroNIR	Portable and hand held	908–1680	6.2	Reflection	Teflon cup
OceanOptics NIRQuest	Mobile and modular	860–2500	6	Reflection	Teflon cup

MathWorks, Natick, MA, USA) for further chemometric analysis, performed by means of in-house written functions.

3. Results and discussion

In order to verify the authenticity of South African honey samples, chemometric classification models have been built based on the collected NIR spectra, using the partial least squares discriminant analysis (PLS-DA) approach (Barker & Rayens, 2003; Sjöström, Wold, & Söderström, 1986; Ståhle & Wold, 1987; Wold et al., 1984).

At first it was necessary to split the available samples into training and test sets; the former to be used for model building and the latter for the validation stage. It is well known that, since building a chemometric model would require the selection of optimal values for a set of parameters or meta-parameters (the choice of which is normally based on the minimisation of an error criterion over the training samples), the use of the same individuals to evaluate the reliability of the model itself would introduce a bias and lead to an overoptimistic estimate (Westad & Marini, 2015). Accordingly, an independent (external) set of samples is normally left aside to constitute the so-called test set for the validation of the reliability of the optimised model in the prediction of new (unknown) individuals. Indeed, test samples are treated as unknowns by the model during the validation stage but, since their true class is known, by comparing the predicted category to the actual one it is possible to assess in an unbiased way how reliable the model is for future objects.

Based on these considerations, it is obvious that the validity and generalisability of the results obtained heavily relies on how properly the whole validation stage was carried out and on whether the test samples truly represent the actual set of individuals which one expects to be analysed in the future. With these premises, to ensure the independency and the representativeness of the test set, the following procedure was adopted to split the samples. At first it was decided that all the AdImp samples had to be put in the test set, in order to verify whether a model built using only authentic SA and pure imported honeys (together with specimens subjected to another kind of adulteration) could be able to identify the presence of small amount of foreign products. As a consequence, all the authentic and imported samples used to produce the adulterated ones were also removed from the training set and added to the test set to be used for the validation stage. Lastly, in order to have a higher number of individuals in the test set, duplex algorithm (Snee, 1977) was then applied separately to each of the sub-categories (with the only exception of AdImp, which, as already stated, was entirely used for validation). Duplex algorithm is a variant of the Kennard-Stone one (Kennard & Stone, 1969), which has the property of alternatively adding samples to the training and test sets, so that the two maintain the same diversity.

Operationally, to allow a proper comparability of the results of the different instruments and to account for the fact that not all the samples could be analysed with one of them (mobile instrument), Duplex was applied on the matrix obtained by concatenating the first 5 PCs (accounting for more than 99% variance) extracted from the laboratory and the portable instruments' data, after averaging the two replicate measurements for each individual. It is obvious that, to meet the independency requirements listed above, all the replicate measurements collected on a particular sample should stay in the same set (be it either training or test). As a result of the data splitting stage, 52 samples were included in the training set (20 SAnc, 9 SAc, 9 Imp and 14 AdSug, for a total of 104 spectra) and 32 in the test set (12 SAnc, 3 SAc, 3 Imp, 7 AdImp and 7 AdSug, for a total of 64 spectra). Of course, for the mobile instrument, keeping the same splitting scheme, only the data from the samples which were actually measured were included in the two sets (41 samples in the training and 27 in the test set, corresponding to 82 and 54 spectra, respectively).

3.1. Data collected on the laboratory instrument

The first data set to be analysed was the one made up of the spectra collected on the laboratory (benchtop) instrument. Although the spectra were acquired on a wider region (up to 2500 nm), since after 1415.6 nm no significant absorption was registered, only the 1000.0–1415.6 nm interval (735 variables) was used for the chemometric data analysis. PLS-DA was applied to the resulting data set to build a model for the discrimination of authentic SA and non-authentic and adulterated honeys. During the model building phase, two meta-parameters had to be optimised, i.e. the complexity of the bilinear projection (the number of latent variables, LVs) and the spectral pretreatment. In particular, for the latter, different options were evaluated: none, standard normal variate (SNV (Barnes, Dhanoa, & Lister, 1989)), first and second derivative (calculated according to the method of Savitzky & Golay (1964) and SNV plus either first or second derivative. Each pretreatment was followed by mean centering prior to analysis. The optimal combination of preprocessing and model complexity was chosen as the one leading to the highest correct classification rate in 8-fold cross-validation (of course, keeping the two replicate measurements for each sample in the same cancelation group) and resulted to be no pretreatment (i.e., mean centering only) and 5 latent variables. The characteristics and the performances of the optimal PLS-DA model are summarised in Table 3.

The optimal PLS-DA model showed performances in calibration and cross-validation with 93.1% and 100.0% of the authentic and non-authentic/adulterated samples correctly classified, respectively, in both stages. When applied to the independent test set, comparable accuracy was obtained (93.3% for AuthSA

Table 3
Classification accuracy of the models built on the different NIR instruments.

Instrument	Range	Pretreat-ment	LV	Class	Modeling (%)	CV (%)	Prediction (%)
Laboratory	1000.0–1415.6 nm	Mean centering	5	AuthSA[a]	93.1	93.1	93.3
				NAuth&Adult[b]	100.0	100.0	100.0
				Overall	96.2	96.2	96.9
Portable	908.1–1453.2 nm	SNV	7	AuthSA[a]	93.1	89.7	93.3
				NAuth&Adult[b]	93.5	84.8	94.1
				Overall	93.3	87.5	93.7
Mobile	861.8–2514.8 nm	SNV + der2 (SG21:2)	8	AuthSA[a]	100.0	97.2	90.0
				NAuth&Adult[b]	97.8	89.1	82.3
				Overall	99.0	94.2	87.5

[a] AuthSA = Authentic South African honey.
[b] NAuth&Adult = Non Authentic and Adulterated honey.

and 100% for NAuth&Adult). This result can also be visually appreciated in Fig. 1a, where the projection of the training and test samples onto the first three latent vectors of the model (scores plot) is reported. Moreover, by looking at Fig. 1b, where the individuals were colour-coded according to their sub-category, it is possible to see that, in the model space, there are rather distinct clusters corresponding to the different groups of samples within each class, indicating the possibility (should one have enough specimens to ensure representativeness) of a more detailed differentiation.

Once having reliably assessed that the optimal PLS-DA model was able to accurately verify the authenticity of honeys, interpretation of the observed results in terms of identifying which spectral regions contributed the most to the discrimination of the samples was carried out by inspection of the variable importance in projection (VIP) scores (Fig. S1).

VIP (Wold, Johansson, & Cocchi, 1993)) is an index quantifying the contribution of the individual variables to the bilinear (PLS) model and it is normalised in a way that a "greater than one" criterion can be applied to assess the relevance of the predictors. In particular, the plot in Fig. S1 shows that a significant contribution to the model is given by the intervals: 1000.0–1038.6 nm, 1097.5–1132.8 nm, 1167.1–1199.6 nm, 1274.2–1283.4 nm, 1328.4–1355.0 nm and 1375.9–1386.6 nm. The first band is related with the

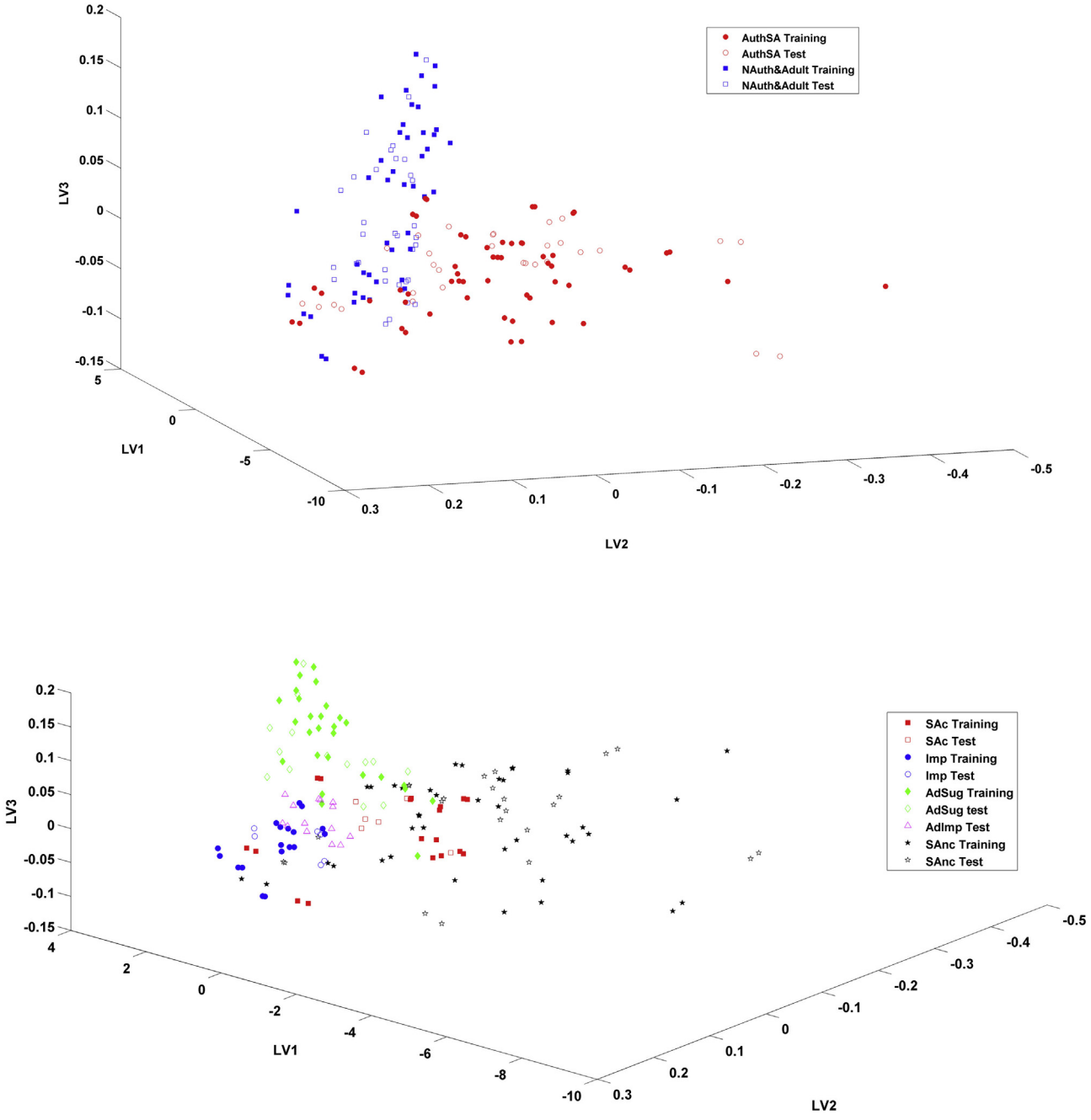

Fig. 1. PLS-DA model built on the data collected from the laboratory instrument: projection of the training and test samples onto the space spanned by the first three latent variables of the model. (a) Samples coloured according to the two class scheme used for modeling (AuthSA/NAuth&Adult); (b) Samples coloured according to their respective sub-classes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

combination of the second O-H and N-H stretching overtones, whereas the rest of the intervals relate to the second overtone of the C-H and C-C stretching bands (Osborne, Fearn, & Hindle, 1993). The typical absorbance peaks that are associated with aqueous solutions of fructose and glucose, i.e. 1460, 1935, 2095 and 2280 nm (Woodcock et al., 2007) was not included in the wavelength range used in this study. Nonetheless, overall good classification accuracies were achieved, even with respect to the fructose and glucose adulterated samples. It seemed that the variation associated with the protein structures presented between 1020 and 1030 nm allowed for sufficient variation.

3.2. Data collected on the portable instrument

Successively, the same chemometric approach was followed to process the data from the measurements made with the handheld portable instrument. Since the data were collected in reflectance mode, prior to the analysis they were converted to pseudo-absorbance (log(1/R)) and the spectral region above 1453.2 nm was cut, as the instrument could not record any meaningful signal in that interval. Also in this case, in the model building phase, the optimal combination of preprocessing (to be chosen among those already detailed in Section 3.1) and complexity was chosen on the basis of 8-fold cross-validation and resulted to be standard normal variate pretreatment and 7 latent variables. The results obtained using the optimal model (see Table 3) were very good and comparable (even if slightly worse) to those from the model built on the measurements from the laboratory instrument operating in the same spectral range. Indeed, a correct classification rate of 93.1% and 93.5% in calibration, 89.7% and 84.8% in cross-validation, 93.3% and 94.1% in prediction (on the test set) was obtained for the AuthSA and NAuth&Adult samples, respectively.

The almost perfect discrimination between the two classes can also be observed in Fig. 2a, where the projection of the training and test samples onto the space spanned by the first three latent vectors of the model is displayed.

It is evident from Fig. 2a that the Authentic South African (AuthSA) samples and the imported and adulterated (AdImp) ones

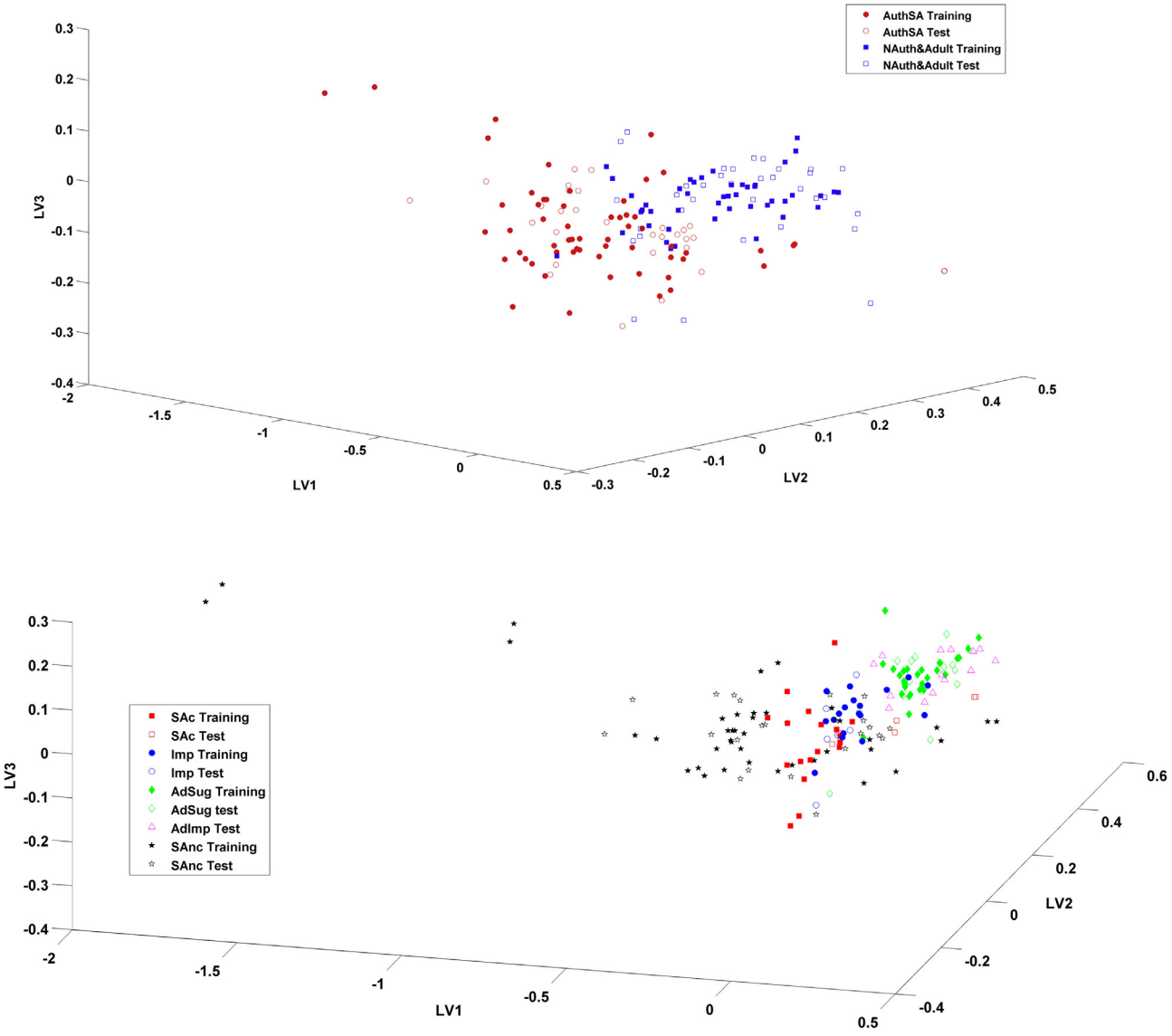

Fig. 2. PLS-DA model built on the data collected from the portable instrument: projection of the training and test samples onto the space spanned by the first three latent variables of the model. (a) Samples coloured according to the two class scheme used for modeling (AuthSA/NAuth&Adult); (b) Samples coloured according to their respective sub-classes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

are mapped onto distinct regions of the latent variable space and that the test samples fall well within the regions occupied by the training objects from the same categories. Moreover, as already evidenced in Section 3.1, when considering the subcategories of the samples (Fig. 2b), a rather distinct clustering can be observed.

As for the previous model, interpretation in terms of the spectral regions contributing the most to the observed discrimination could be accomplished through inspection of the VIP scores, reported in Fig. S2.

The plot in Fig. S2 indicates that the variables which are mostly significant in defining the bilinear projection all correspond to chemically meaningful spectral intervals. The two bands, 1143.5–1162.1 nm and 1447.0–1453.2 nm relate to aromatic structures with C-H second overtone stretching bonds, whereas the first band (908.1–976.2 nm) relate to protein and CH_2 structures with C-H deformations (Osborne et al., 1993). The other significant band (1205.4–1267.4 nm) is related with the second overtone C-H bonds.

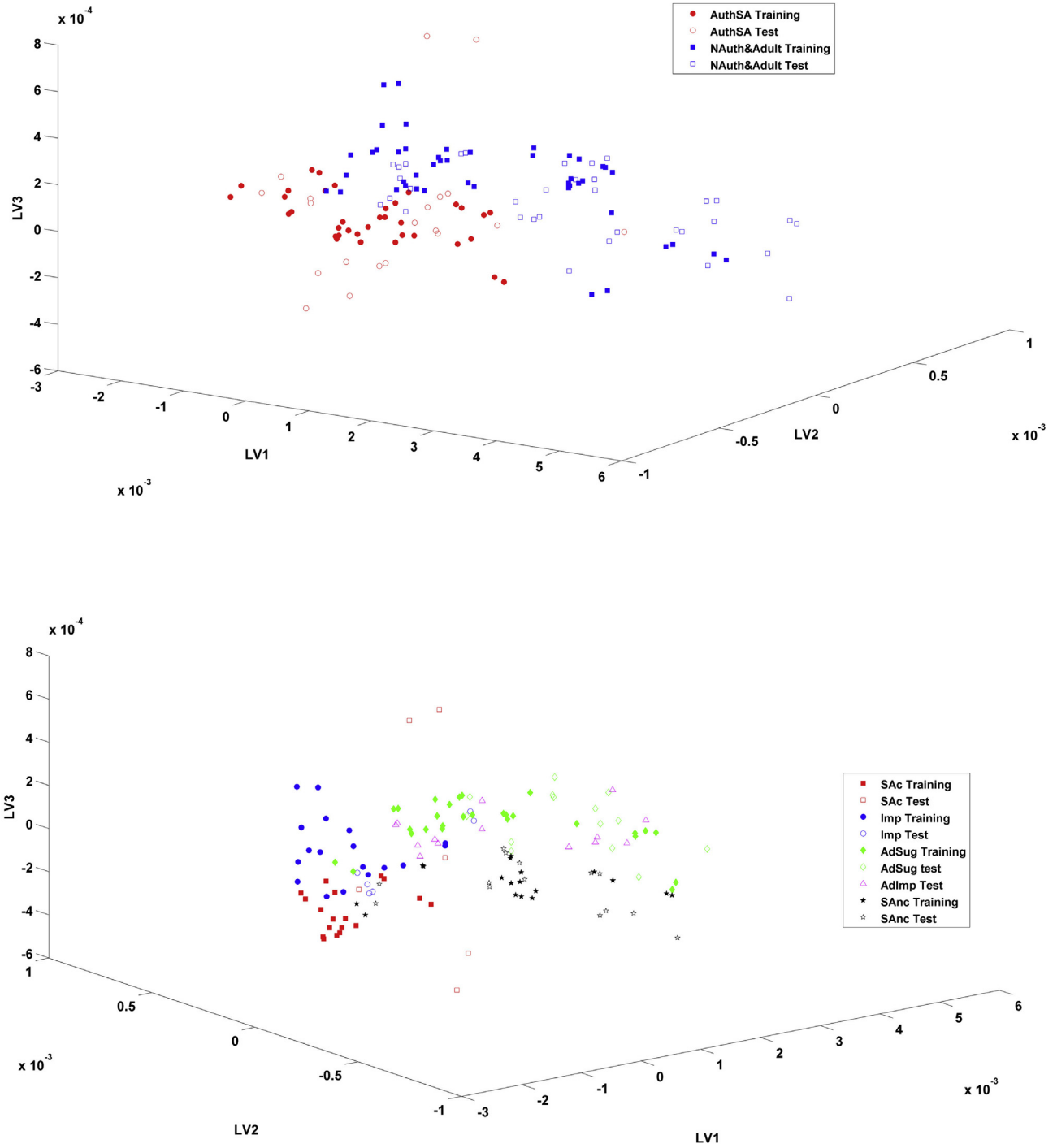

Fig. 3. PLS-DA model built on the data collected from the mobile instrument: projection of the training and test samples onto the space spanned by the first three latent variables of the model. (a) Samples coloured according to the two class scheme used for modeling (AuthSA/NAuth&Adult); (b) Samples coloured according to their respective sub-classes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Data collected on the mobile instrument

The last data matrix to be analysed was the one made up of the spectra recorded using the mobile (modular) instrument. As known, with respect to the two other data sets, this last one included less spectra (82 training and 54 test). As in the two previous cases, the training samples were used to build the model, whose optimal complexity and preprocessing methods were optimised through an 8-fold cross-validation strategy. In particular, SNV followed by second order derivative (21 point window and 2nd order polynomial) was found to be the best preprocessing while 8 latent variables appeared to be the optimal complexity. The corresponding results obtained by applying the optimal model to the training and the test samples are summarised in Table 1. Here it must be stressed that, being evaluated only on a subset of the test set used for the other data sets, the results reported in Table 3 are not fully comparable to those described in Sections 3.1 and 3.2, but they nonetheless allow formulating some general considerations. Despite the lower resolution of the instrument and the higher impact on the signal of spurious sources of variation, the PLS-DA model built on the data still results in a very good overall classification accuracy (99.0%, 94.2% and 87.5% in calibration, cross-validation and prediction, respectively), only slightly worse than the models obtained using the other instruments. This good classification accuracy can also be visualised by inspecting the scores plot reported in Fig. 3a, which shows how the two categories are quite well separated in the space spanned by the first three latent variables of the model. When considering the sub-categories of the samples (Fig. 3b), more distinct clustering can be observed.

Investigation on the relevance of the experimental variables in defining the PLS-DA model, accomplished through inspection of the VIP scores (Fig. S3) indicated that the spectral regions which contribute most significantly to the discrimination among the categories are: 861.8–888.8 nm, 955.9–989.3 nm, 1356.7–1363.1 nm, 1504.9–1530.6 nm, 1594.7–1754.4 nm, 1786.3–1856.5 nm, 2003.5–2035.4 nm, 2131.6–2196.0 nm, 2234.6–2273.4 nm, 2423.0–2514.8 nm. Most of these regions relate with O-H, N-H and C-H deformations. Spectral regions that coincide roughly with a former NIR honey study (Woodcock et al., 2007) are 1594–1754 nm that is related to glucose and starch, 2131–2196 that is related to amino-acids and 2234–2273 nm that is related to -CHO.

4. Conclusion

A non-destructive method that allows simultaneously authentication of honey samples, not only in terms of addition of a non-SA honey but also in terms of addition of sugars, has been proposed in this study. Honey NIR spectra contain useful information for the development of authenticity verification procedures in combination with chemometrics. In particular, PLS-DA applied to the spectroscopic fingerprints revealed that not only is it possible to verify the authenticity of South African samples, by reliably differentiating them from honey of other geographical origin, but also to detect adulteration with glucose, fructose and imported honey, even at relatively low concentration. In this respect, the results are even more promising. On the one hand it was shown that, despite their coming from diverse regions and floral origins, authentic SA samples present shared characteristics that allowed differentiating them from imported and adulterated honeys. On the other hand it was also evident that distinct clusters can be observed within each category, indicating the potential, if more samples will be available, of discriminating even among subgroups. As a compliment for the South African honey industry, all the commercial honey samples used in this study were true to their label claims.

From an analytical standpoint, in contrast with other approaches, NIR is advantageous as the measurements are fast, non-destructive and non-invasive and, especially when using a portable instrument, easy to perform. Moreover, by inspection of the parameters of the different chemometric models, it was possible to interpret the results obtained in chemical terms, identifying the spectral bands, which were related to the observed discrimination among samples. Depending on the model, wavelengths corresponding to the vibrational transitions of the main functional groups in proteins/amino-acids, sugars and aldehydes have proved to contribute significantly.

Lastly, another relevant outcome of this study was that very good classification accuracies could be obtained, irrespective of the type of NIR instrument used. This suggested that, for field or industrial applications, the choice of portable or at least mobile NIR instruments, which are easier to handle or even more suited, would not result in a significant loss of reliability.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodcont.2016.11.002.

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