



The use of multispectral imaging for the discrimination of Arabica and Robusta coffee beans

Alina Mihailova^{a,*}, Beatrix Liebisch^a, Marivil D. Islam^a, Jens M. Carstensen^c,
Andrew Cannavan^b, Simon D. Kelly^a

^a Food Safety and Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna International Centre, PO Box 100, 1400 Vienna, Austria

^b Food Safety and Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna International Centre, PO Box 100, 1400 Vienna, Austria

^c Videometer A/S, Hørkær, DK-2730 Herlev, Denmark

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ABSTRACT

Arabica coffee beans are sold at twice the price, or more, compared to Robusta beans and consequently are susceptible to economically motivated adulteration by substitution. There is a need for rapid, non-destructive, and efficient analytical techniques for monitoring the authenticity of Arabica coffee beans in the supply chain. In this study, multispectral imaging (MSI) was applied to discriminate roasted Arabica and Robusta coffee beans and perform quantitative prediction of Arabica coffee bean adulteration with Robusta.

The Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) model, built using selected spectral and morphological features from individual coffee beans, achieved 100% correct classification of the two coffee species in the test dataset. The OPLS regression model was able to successfully predict the level of adulteration of Arabica with Robusta. MSI analysis has potential as a rapid screening tool for the detection of fraud issues related to the authenticity of Arabica coffee beans.

1. Introduction

Coffee is one of the most widely traded commodities worldwide. The two main species of cultivated coffee are *Coffea arabica* L. (commonly known as Arabica), and *Coffea canephora* Pierre ex A. Froehner (commonly known as Robusta). Arabica coffee beans are highly valued among consumers for their superior smooth, mild and rich flavour and account for over 60% of global coffee production (International Coffee Organization, 2021). Arabica beans are usually sold at twice the price or even more in comparison with Robusta beans (International Coffee Organization, 2021), which yield a harsher and more bitter drink (Flament, 2001, Wang, Lim, & Fu, 2020). The significant price differential offers the potential for unscrupulous traders to make economic gain by partially or wholly substituting Arabica beans with Robusta. Severe fraud cases involving Arabica coffee have been reported in the European Union and other regions of the world over the past several years (Europol - INTERPOL, 2020).

Many different analytical techniques have been investigated to discriminate Arabica and Robusta coffee species, including molecular

genetics approaches (Combes, Joët, & Lashermes, 2018, Spaniolas, May, Bennett, & Tucker, 2006), nuclear magnetic resonance spectroscopy (Cagliani, Pellegrino, Giugno, & Consonni, 2013, Defernez et al., 2017, Monakhova et al., 2015), liquid- and gas chromatography mass spectrometry (Garrett, Vaz, Hovell, Eberlin, & Rezende, 2012, Procida, Lagazio, Cateni, Zacchigna, & Cichelli, 2020) among others. The above-mentioned methods can provide high sensitivity and thus be suitable for the tier 2 confirmatory analysis of suspect fraudulent samples, however the application of these techniques would not be feasible for the rapid tier 1 point-of-use screening and monitoring of coffee authenticity. Conversely, vibrational spectroscopy techniques are simple, non-destructive, and have been reported to be efficient for fast sample screening in order to detect potentially fraudulent samples in the supply chain. Near-infrared (NIR) spectroscopy has been applied to discriminate Arabica and Robusta species, both in green (Myles, Zimmerman, & Brown, 2006, Santos, Sarragaça, Rangel, & Lopes, 2012) and roasted (Esteban-Díez, González-Sáiz, & Pizarro, 2004, Esteban-Díez, González-Sáiz, Sáenz-González, & Pizarro, 2007) coffee.

Recent advances in spectral imaging have facilitated the

* Corresponding author.

E-mail address: a.mihailova@iaea.org (A. Mihailova).

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development of compact imaging systems with the capability to differentiate between chemical composition, surface morphology and colour in various matrices (Feng, Zhu, Liu, He, Bao, & Zhang, 2019). The rapid and non-destructive nature of these instruments, combined with no requirement for sample preparation or the use of specialised laboratory facilities and hazardous chemicals, are an advantage and mean that these systems have the potential to be used for screening applications. Spectral imaging has showed a great potential for its application in food authenticity screening over the recent years (ElMasry, Mandour, Al-Rejaie, Belin, & Rousseau, 2019, Feng et al. 2019, Liu, Xu, Liu, & Zheng, 2021; Lohumi, Lee, Lee, & Cho, 2015; Su & Sun, 2018). Depending on the number of wavebands, at which the spectral image is acquired, two main types of spectral imaging techniques have been used: hyperspectral imaging (HSI), which acquires images by utilizing a large number of wavebands leading to a continuous spectral image, and multispectral imaging (MSI), which acquires images with a few (generally up to 20) discrete wavebands (Boelt, Shrestha, Salimi, Jørgensen, Nicolaisen, & Carstensen, 2018, ElMasry et al., 2019, Su & Sun, 2018, Qin, Chao, Kim, Lu, & Burks, 2013).

The MSI offers several advantages over HSI, including shorter acquisition and processing times, which makes it applicable for screening applications (Calvini, Amigo, & Ulrici, 2017, Su & Sun, 2018). MSI has been successfully used for the discrimination of different varieties of maize, wheat, rice, soybean, vegetables and other commodities (ElMasry et al., 2019; Su & Sun, 2018). A very limited number of studies have applied the HSI or MSI for the differentiation of Arabica and Robusta coffee beans (Calvini et al., 2017).

This study aimed to develop a rapid approach for the discrimination between roasted Arabica and Robusta coffee beans based on the MSI analysis combined with chemometrics. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) with seven-fold cross-validation was used to build the discriminative model for the differentiation of Arabica and Robusta coffee beans in the training dataset. The obtained OPLS-DA model was externally validated using the test dataset. Further, a simulated adulteration experiment was conducted by adding different amounts of Robusta coffee beans to Arabica, and the selected significant spectral and morphological features from the OPLS-DA were used in the OPLS regression model to predict the adulteration level of Arabica beans. We discuss the suitability of the MSI technique coupled with chemometrics as a tool for rapid testing of the authenticity of Arabica coffee beans.

2. Materials and methods

2.1. Coffee samples

Samples of roasted Arabica ($n = 21$) and Robusta ($n = 14$) coffee beans were obtained for this study from a specialised and trusted coffee retailer (Roastmarket, Germany). The summary of the coffee sample information is presented in Table S1 (Supplementary Material). Although, without using confirmatory molecular biology techniques, there is no absolute guarantee that all of the samples analysed were authentic, we believe that this approach has minimized the chances of including in the datasets any Robusta coffee beans being fraudulently mis-sold as Arabica. Samples were kept in airtight containers in the dark at room temperature prior to analysis.

2.2. MSI analysis

Multispectral imaging system VideometerLab 4 (Videometer A/S, Hørsholm, Denmark) was used to capture multispectral images of coffee beans (Carstensen & Folm-Hansen, 2000). The system combines illumination, a high-resolution CCD camera, and computer technology with advanced digital image analysis. A schematic illustration of the VideometerLab 4 multispectral imaging system setup is presented in Fig. S1 (Supplementary Material). The instrument uses strobed light-emitting

diode (LED) technology and combines measurements at 19 different wavelengths (365, 405, 430, 450, 470, 490, 515, 540, 570, 590, 630, 645, 660, 690, 780, 850, 880, 940, and 970 nm) into a single high-resolution spectral image. The system was calibrated in respect to colour, geometry and self-illumination and was set up to operate in 100% reflection mode. The size of obtained multispectral images was 2992×2992 pixels. In addition to the spectral information, the following selected morphological and colour features were extracted from the multispectral images of individual coffee beans: area (mm^2), length (mm), width (mm), width-to-length ratio, compactness circle, compactness ellipse, beta shape a, beta shape b, CIELab L^* , CIELab A^* , CIELab B^* , saturation, and hue. The CIELab is a three-dimensional colour space that fully represents the colours visible to the human eye. It separates the ambient lighting, or luminosity (L^*), into a vertical axis, which ranges from 0 (black) to 100 (white), and the chromaticity into a xy horizontal plane. The chromaticity is represented by two parameters: a^* , representing the green-red colour component, and b^* , representing the blue-yellow opponent colours (Mendonça, Franca, & Oliveira, 2009).

The coffee beans were analysed without preparation. Analysis was carried out in standard 90-mm-diameter polystyrene Petri dishes.

2.3. Discrimination of Arabica and Robusta coffee beans

For each coffee sample, five standard 90-mm-diameter Petri dishes, each containing 20 coffee beans, were prepared, resulting in overall 175 Petri dishes. First, a region of interest (ROI) of the same size (5×4 mm) was selected for each coffee bean (Fig. 1A), and the spectra (365–970 nm) were obtained for each ROI. Then the Petri dish and the background were masked, and the binary labelled object (BLOB) tool was used to extract the individual coffee beans from the multispectral image (Fig. 1B). For each bean selected morphological and colour features were extracted (section 2.2). A total of 3500 individual coffee beans were analysed to build the discriminative model.

2.4. Adulteration of Arabica coffee with Robusta

For the adulteration experiment, ten (10) different combinations of Arabica and Robusta coffee bean samples were used. Robusta coffee beans were added to Arabica beans at 21 adulteration amounts: 0%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% and 100%. For each adulteration amount 20 coffee beans were placed in standard 90-mm-diameter Petri dishes, resulting in 210 Petri dishes overall. Spectral and morphological analysis were performed for each bean individually in the same way as described in section 2.3. A total of 4220 individual coffee beans were used for the simulated adulteration experiment.

2.5. Data pre-processing and statistical analysis

Data processing and multivariate statistical analysis were performed using VideometerLab software version 3.14.29 (7984) (Videometer A/S, Hørsholm, Denmark) and SIMCA multivariate data analysis software version 16.0 (Sartorius Data Analytics, Sweden).

MSI images of Arabica and Robusta coffee beans were initially processed using normalised canonical discriminant analysis (n -CDA) function. Further, spectral, morphological and colour features obtained from the beans in each Petri dish ($n = 20$) were averaged, pre-processed using standard normal variate (SNV) function and Unit-Variance (UV) scaling, and subjected to further chemometrics analysis.

For building the discriminative model, the full dataset ($n = 175$) was divided in randomised order into the training dataset (Arabica: $n = 70$, Robusta: $n = 47$) and the test dataset (Arabica: $n = 35$, Robusta: $n = 23$). Principal component analysis (PCA) was used to assess the data quality and evaluate the initial coffee sample groupings. A supervised chemometric approach, Orthogonal Partial Least Squares Discriminant

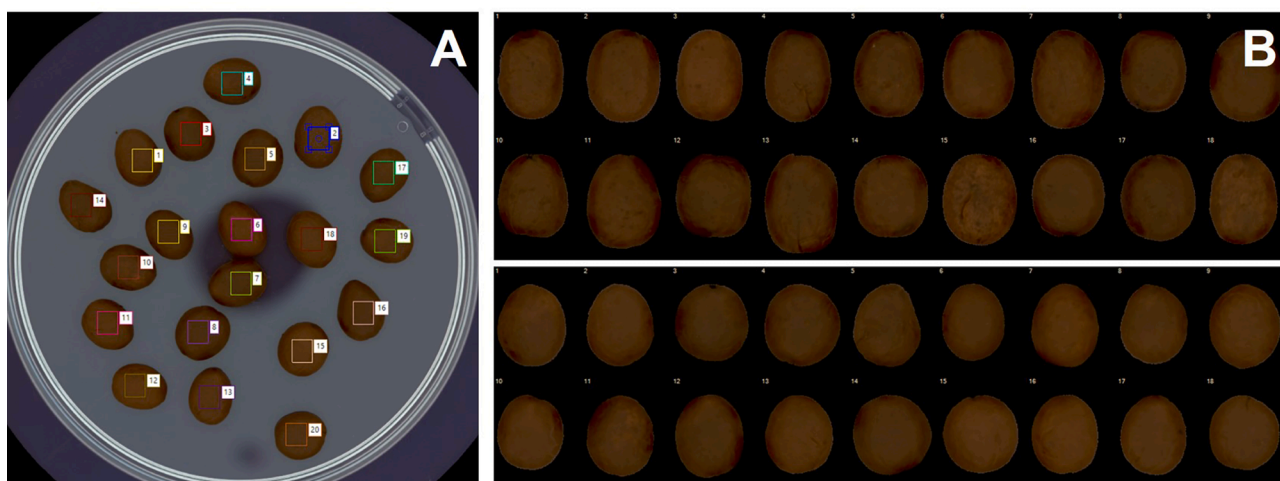


Fig. 1. MSI analysis of individual coffee beans: A – coffee beans with marked regions of interest (ROIs); B – individual Arabica (top) and Robusta (bottom) beans extracted from the Petri dish using the BLOB tool.

Analysis (OPLS-DA) with seven-fold cross-validation, was used to build the discriminative model for the differentiation of Arabica and Robusta coffee beans in the training dataset. Chemometric modelling was performed using the averaged and pre-processed a) spectral, b) morphological and colour and c) combined spectral, morphological and colour features of coffee beans in each Petri dish. The performance of the PCA and OPLS-DA models was assessed using the goodness of fit (R²) and predictability (Q²) values. The OPLS-DA model, which showed the highest discriminative power, was selected and subjected to validation using the coffee beans from the test dataset, which were not used for the generation of the model. The predictive ability of the model was assessed using the correct classification of samples from each coffee species in the test dataset. The most significant features for the discrimination of two coffee species were assessed using the variable importance in the projection (VIP) scores.

For the adulteration experiment, the full dataset, comprised of 10 combinations of Arabica and Robusta coffee at 21 adulteration levels (n = 210), was divided in randomised order into the training dataset (n = 147) and the test dataset (n = 63). Orthogonal Partial Least Squares (OPLS) regression, built using the training dataset, was used to predict the adulteration level of Arabica coffee beans with Robusta in the test dataset. The performance of the regression model was assessed using goodness of fit (R²), predictability (Q²), coefficient of determination (R²), root mean square error of estimation (RMSEE) and root mean square error of prediction (RMSEP).

3. Results and discussion

3.1. Spectral and morphological profiles of Arabica and Robusta coffee beans

The mean values of the morphological and colour features extracted from the MSI images of Arabica and Robusta coffee beans are presented in Table 1. Significant differences at the 95% confidence interval were observed in all the measured features with the exception of hue. This agrees with the previous findings that reported differences in morphology, particularly in size and shape, of Arabica and Robusta coffee beans (Mendonça et al., 2009).

The averaged reflection spectra (365–970 nm) of the individual Arabica and Robusta beans, analysed in this study, are presented in Fig. 2. It can be observed that the general trend of spectra of the two coffee species is similar, however differences can be observed between 570 and 970 nm, corresponding to visible (yellow-orange-red colour) and near-infrared regions.

Table 1

The summary of mean values and standard errors (SE) of morphological and colour features of Arabica (n = 105) and Robusta (n = 70) coffee bean samples.

Morphological/colour feature	Arabica		Robusta		p-value
	Mean	SE	Mean	SE	
Area (mm ²)	72.27	0.61	69.21	1.45	0.030
Length (mm)	10.95	0.06	10.46	0.11	0.000
Width (mm)	8.25	0.03	8.42	0.09	0.025
Width/Length ratio	0.76	0.00	0.81	0.00	0.000
Compactness Circle	0.75	0.00	0.81	0.00	0.000
Compactness Ellipse	1.00	0.00	1.00	0.00	0.000
BetaShape_a	1.42	0.00	1.50	0.00	0.000
BetaShape_b	1.38	0.00	1.45	0.00	0.000
CIELab L*	19.88	0.34	17.95	0.26	0.000
CIELab A*	12.00	0.11	11.24	0.09	0.000
CIELab B*	27.98	0.22	26.57	0.20	0.000
Saturation	29.48	0.26	27.67	0.22	0.000
Hue	1.15	0.00	1.14	0.00	0.841

3.2. Discrimination of Arabica and Robusta coffee beans

Fig. 3 shows representative MSI images of 100% Arabica (A), 100% Robusta (B) and a mixture of 50% Arabica (C, left) and 50% Robusta (C, right) beans with masked background. The respective images transformed using normalised canonical discriminant analysis (nCDA) are presented in Fig. 3A1–C1. nCDA allowed the separation of Arabica and Robusta coffee beans and demonstrated a potential for the two coffee species to be discriminated.

Further, the assessment of the performance of chemometric models, built using the averaged and pre-processed a) spectral, b) morphological and colour and c) combined spectral, morphological and colour features of coffee beans in each Petri dish, was performed (Table S2, Supplementary Material). The PCA and OPLS-DA models, built using only the spectral features (365–970 nm), were characterised by the lowest R² and Q² values and did not result in a sufficient discrimination of Arabica and Robusta coffee (Table S2, Supplementary Material). The goodness of fit (R²X(cum), R²Y(cum)) and the predictive ability (Q²(cum)) of the OPLS-DA model were 0.965, 0.387, and 0.344, respectively. The models generated using only morphological and colour features showed significantly better performance values (Table S2, Supplementary Material). The highest performance values and the best discrimination were demonstrated by the models built using combined spectral, morphological and colour features. The goodness of fit (R²(cum)) and the predictive ability (Q²(cum)) of the PCA model were 0.909 and 0.834, respectively. PCA model, built using the combined spectral,

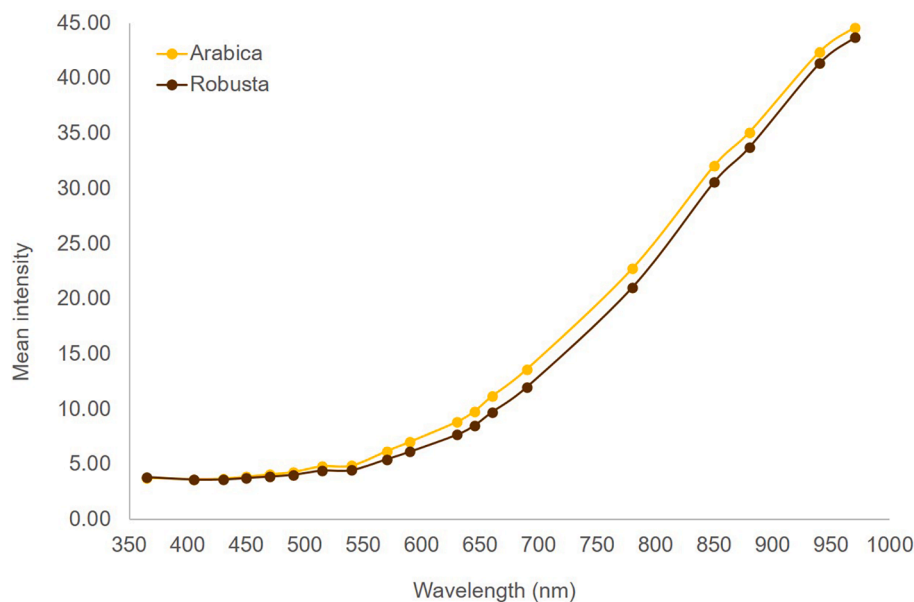


Fig. 2. Mean raw reflectance spectra of Arabica (n = 105) and Robusta (n = 70) coffee bean samples.

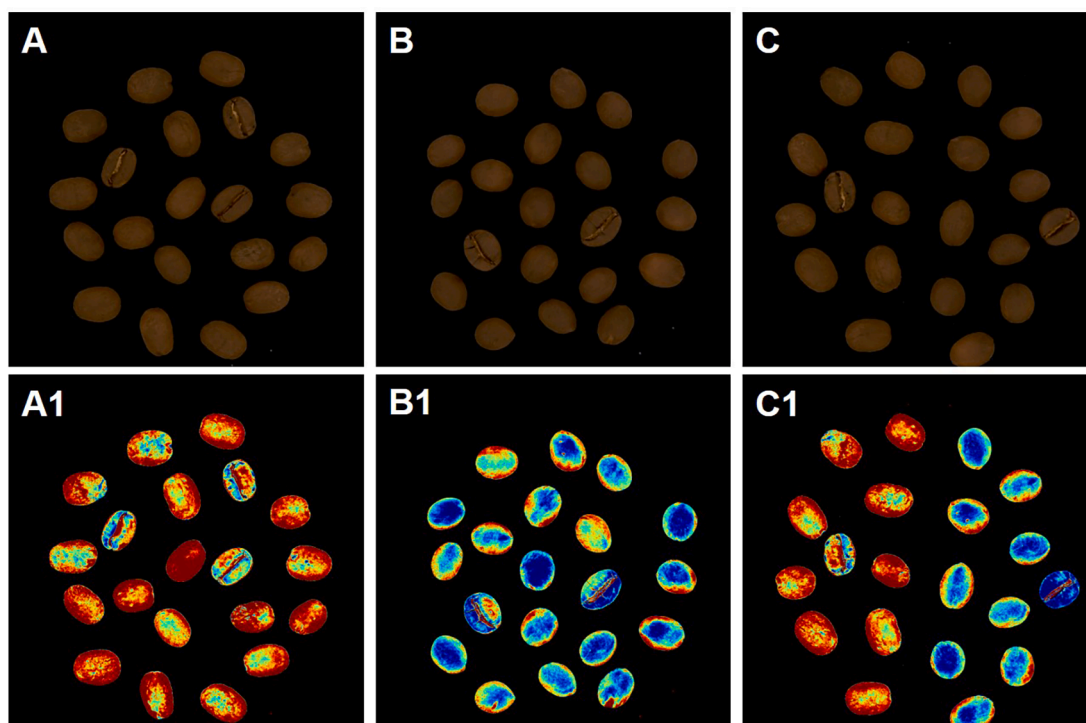


Fig. 3. Representative MSI images of 100% Arabica (A), 100% Robusta (B) and 50% Arabica (C, left) + 50% Robusta (C, right) beans with masked background; and the respective nCDA images (A1-C1).

morphological and colour features showed a tendency of samples to group according to the coffee species (Fig. 4A). OPLS-DA allowed a clear differentiation between Arabica and Robusta coffee beans (Fig. 4B). The goodness of fit ($R^2X(\text{cum})$, $R^2Y(\text{cum})$) and the predictive ability ($Q^2(\text{cum})$) of the OPLS-DA model were 0.922, 0.912, and 0.897, respectively.

External validation of the OPLS-DA model, built with the combined spectral, morphological and colour features, was performed using the test dataset comprising samples that were not used in the construction of the model (Arabica: n = 35, Robusta: n = 23). The model achieved 100% correct classification of both Arabica and Robusta coffee species in the

test dataset. The summary of the prediction results is shown in Table S3 (Supplementary Material).

The variable importance in the projection (VIP) scores were used to assess the most significant features for the discrimination between Arabica and Robusta coffee beans (Fig. S2, Supplementary Material). Five morphological features, beta shape b (VIP score = 1.82), beta shape a (VIP score = 1.81), compactness circle (VIP score = 1.46), width to length ratio (VIP score = 1.43) and compactness ellipse (VIP score = 1.42), were among the most significant features responsible for the discrimination. The visible and near-infrared spectral features as well as other morphological and colour features, although contributed to the

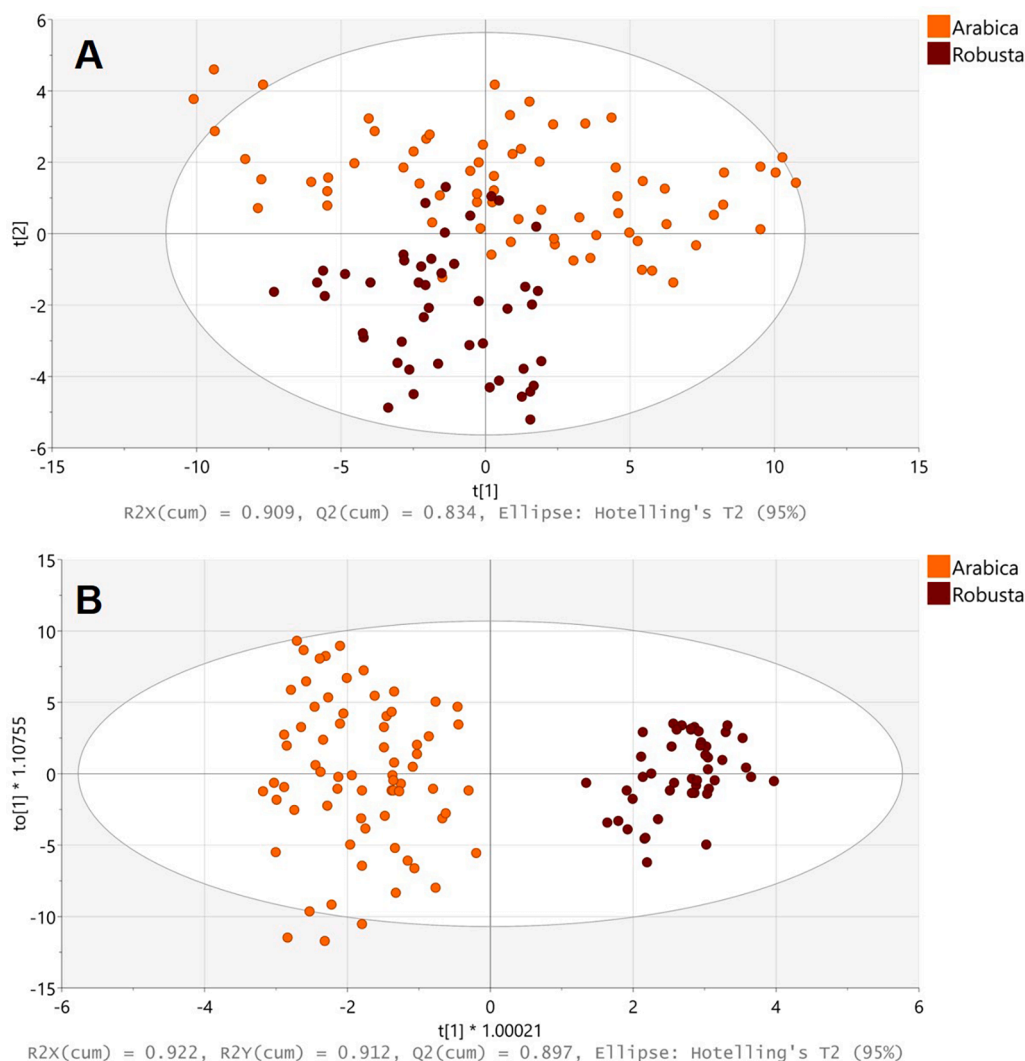


Fig. 4. A: PCA model of the Arabica and Robusta coffee bean samples in the training dataset using combined spectral, morphological and colour features (Arabica: $n = 70$, Robusta: $n = 47$); B: OPLS-DA model of the Arabica and Robusta coffee bean samples in the training dataset using combined spectral, morphological and colour features (Arabica: $n = 70$, Robusta: $n = 47$).

discrimination, were of less significant importance. Coffee beans from the Arabica species are generally oval, have a pronounced centre crease and are larger than Robusta beans. Spectral profiles of coffee beans, that can be affected by roasting, would not be a reliable parameter alone to differentiate the two coffee species in the case of retail samples. The discriminative approach would need to account for the possible variability in the roasting grades and other processing factors as well as the effects of geographical origin of beans. The combination of the selected morphological features with the spectral data offered an advantage and showed a good separation of the two coffee species and resulted in a successful prediction of the coffee species in the test dataset.

These results support the finding of [Calvini et al. \(2017\)](#) who applied HSI and MSI for the classification of green Arabica and Robusta coffee. The study applied PLS-DA and concluded that the selection of most effective combinations of spectral channels led to satisfactory classification performances (100% correct prediction of coffee species in the test set). Several other studies showed a potential of the MSI analysis using VideometerLab to discriminate different crop varieties. [Liu et al. \(2021\)](#) applied MSI for the differentiation of four rice varieties and the authentication of Thai Jasmine rice. A combination of the analysis of spectral and morphological features was coupled with chemometrics (PCA, PLS, least squares-support vector machines (LS-SVM) and back-propagation neural network (BPNN)). The study achieved up to 92%

correct classification rate using BPNN and concluded that MSI with chemometric methods can be successful in the rapid and non-destructive authentication of Thai jasmine rice. [Shrestha et al. \(2015\)](#) used VideometerLab to differentiate different varieties of tomato. PCA, PLS-DA and nCDA were used in the study and achieved successful varietal discrimination. The study concluded that MSI can be a good tool for the identification/discrimination of plant varieties. [Wilkes et al. \(2016\)](#) applied MSI and HSI for the discrimination of durum and common wheat. The study used VideometerLab MSI system and demonstrated its capability to rapidly distinguish between durum and adulterant common wheat. The results obtained had low bias and good repeatability estimates which compared well with the data published using real-time PCR. The study reported the potential of MSI to be used for seed/grain adulteration testing to augment standard molecular approaches for food authentication.

3.3. Adulteration of Arabica coffee with Robusta

The OPLS regression model, built using the combined spectral, morphological and colour features in the training dataset ($n = 147$), was able to successfully predict the adulteration level in the test set ($n = 63$) that was not used in the construction of the model ([Fig. 5](#)). The goodness of fit ($R2X(cum)$, $R2Y(cum)$) and the predictive ability ($Q2(cum)$) of the

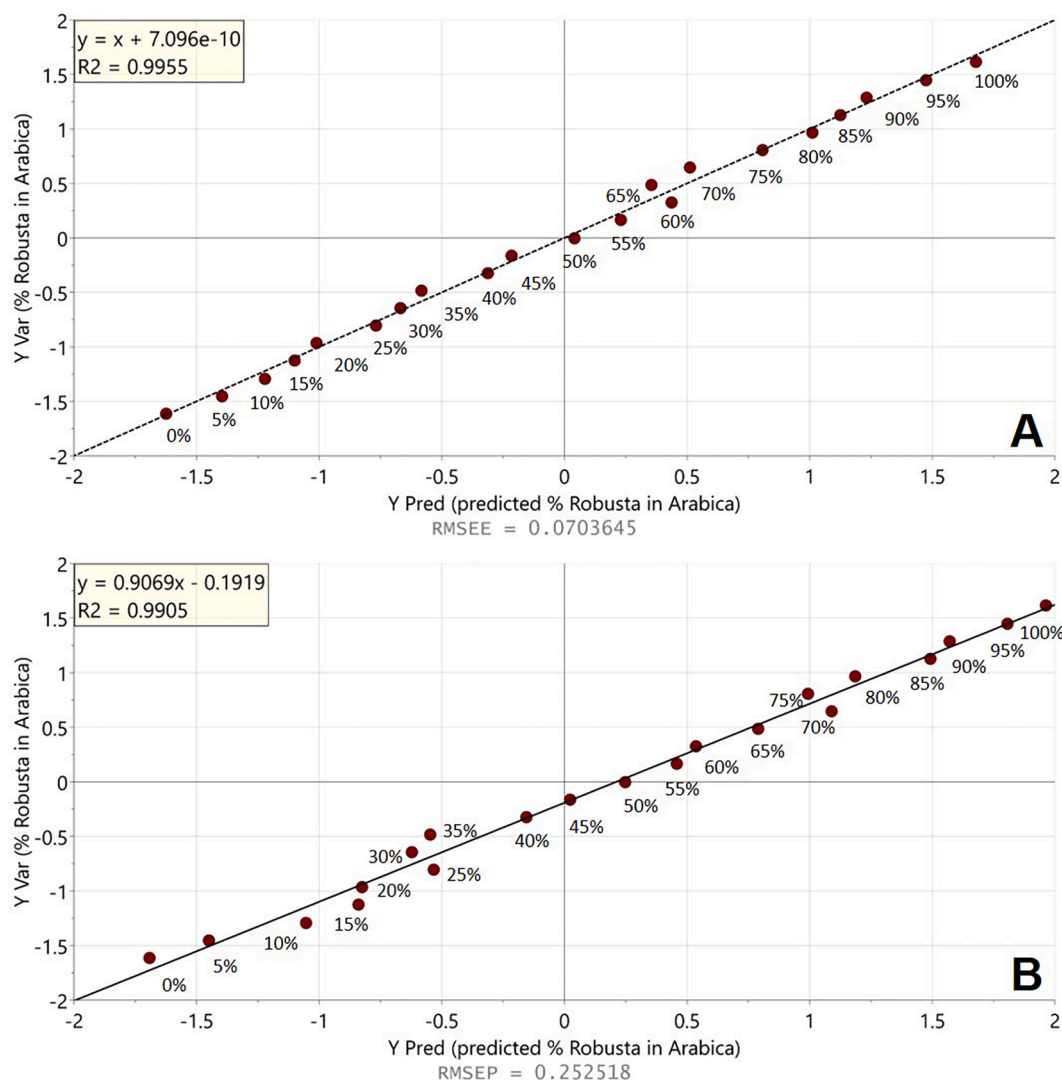


Fig. 5. OPLS regression models of the Arabica coffee bean adulteration with Robusta: A - training dataset (n = 147), B - test dataset (n = 63). Both models use combined spectral, morphological and colour features.

OPLS model are 0.922, 0.912, and 0.897, respectively. The coefficient of determination (R^2) and root mean square error of estimation (RMSEE) of the OPLS model for the training dataset were 0.996 and 0.070, respectively (Fig. 5A). The R^2 and root mean square error of prediction (RMSEP) for the test dataset were 0.991 and 0.252, respectively (Fig. 5B).

The major advantage of MSI analysis of coffee beans using VideometerLab 4 system is its rapidness, it does not require sample preparation and can be applied outside the laboratory. This opens up the possibility for these types of devices to be used for cost-effective sample screening, by which means suspect fraudulent samples can be rapidly detected thus allowing an early and more rapid detection of issues related to the authenticity of Arabica coffee. The use of VideometerLab 4 system as a portable point-of-use instrument, is, however, limited due to its relatively large footprint and therefore perhaps more suited for a mobile laboratory, for example. In addition, even though the actual analysis is quick and does not require extensive training, the rapidity of this approach will be affected by the requirement of building a robust model (i.e. sufficiently large reference sample database) prior to the application of this technique to real sample analysis. The generation of a robust model and its subsequent external validation would require good technical knowledge and would need to take into account as many factors, which may affect the variability in the data, as possible, e.g., the

geographical origin, varietal, seasonal and annual variability, roasting of beans. This often presents a practical challenge when it comes to obtaining authentic samples and would, consequently, impact upon the rapidity of the approach.

This preliminary study provides evidence for the proof of principle of the application of MSI using VideometerLab 4 system for the discrimination of Arabica and Robusta coffee beans. More comprehensive studies using larger numbers of authentic coffee samples from a wider range of geographical locations, from both roasted and green coffee beans, are required to build more robust classification/prediction models and to validate their ability to reliably classify unknown samples.

4. Conclusions

Development of reliable and rapid non-targeted screening methods is extremely important in identifying and preventing evolving fraudulent practices in the trade of Arabica coffee. This study has demonstrated that multispectral imaging analysis using VideometerLab 4 system, combined with OPLS-DA, is a promising analytical tool for the differentiation of roasted Arabica and Robusta coffee. Arabica coffee beans were differentiated from the Robusta bean samples based on the combination of morphological, colour and spectral features. The OPLS-DA model was

validated with the samples from the test dataset, which were not used for the generation of the models. The OPLS-DA model achieved 100% correct classification of the test dataset, for each of the coffee species respectively.

Arabica coffee beans were adulterated with Robusta at 21 different amounts (from 0 to 100% at 5% incremental steps) and the OPLS regression was able to successfully predict the percent of adulteration of Arabica beans with Robusta. R^2 , RMSEE and RMSEP of the OPLS were 0.996, 0.070 and 0.252, respectively.

Adapting and using MSI as a rapid screening technique is a promising way forward for the early detection of fraud at the farm/import/retail level. The main advantages of these techniques over the traditional mass spectrometry or nuclear magnetic resonance spectrometry methods are the rapidity and the ease of use in routine operations/surveillance, significantly lower cost, the non-destructive nature of the techniques and no sample preparation. This approach allows for rapid pre-screening to identify suspect coffee samples before committing to more sophisticated and time-consuming techniques for confirmatory or orthogonal analysis. Further work using a wider range of authentic coffee samples from different geographical origins is required to demonstrate the robustness of this approach.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2022.100325>.

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