A SEMI-QUANTITATIVE ONE-CLASS PARTIAL LEAST SQUARES MODEL FOR DETECTING HONEY ADULTERATION USING TD-NMR SPECTROSCOPY

Authors' names

Glòria Rovira[a], Carolina Sheng Whei Miaw Botelho[b], Laura Lima de Oliveira [c], Marcus Vinicius de Oliveira Andrade [d], Poliana Macedo Santos[e], Marcelo Martins Sena [f], Scheilla Vitorino Carvalho de Souza [b], Maria Pilar Callao[a], Itziar Ruisánchez [a,*]

[a] – Chemometrics and Sensorics for Analytical Solutions (CHEMOSENS) Group, Department of Analytical and Organic Chemistry, Rovira i Virgili University, Marcel·lí Domingo s/n, 43007, Tarragona, Spain.

[b] - Food Science Graduate Program (PPGCA), Faculty of Pharmacy (FAFAR), Federal University of Minas Gerais (UFMG), Av. Antônio Carlos, 6627, Campus da UFMG, Pampulha, 31270-010, Belo Horizonte, MG, Brazil.

[c] - Faculty of Pharmacy (FAFAR), Federal University of Minas Gerais (UFMG), Av. Antônio Carlos, 6627, Campus da UFMG, Pampulha, 31270-010, Belo Horizonte, MG, Brazil.

[d]- Brazilian Federal Police, Regional Superintendence in Minas Gerais, 30441-170, Belo Horizonte, MG, Brazi.

[e] - Chemistry and Biology Department (DAQBI), Federal Technological University of Paraná (UTFPR), Rua Dep. Heitor Alencar Furtado, 5000, Cidade Industrial, 81280-340, Curitiba, PR, Brazil.

[f] - Chemistry Department, Institute of Exact Sciences (ICEx), Federal University of Minas Gerais (UFMG), Av. Antônio Carlos, 6627, Campus da UFMG, Pampulha, 31270-010, Belo Horizonte, MG, Brazil. Instituto Nacional de Ciência e Tecnologia em Bioanalítica (INCT-Bio), Campinas, SP, 13083-970, Brazil.

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Abstract

This study developed a screening method to detect honey adulteration with inverted sugar syrup using two types of honey samples; eucalyptus (EUC) and wild (W). For each type, 35 authentic and 42 adulterated samples were analyzed using Time-Domain Nuclear Magnetic Resonance (TD-NMR). One-class Partial Least Squares Discriminant Analysis (OCPLS) models were built from the authentic samples of each honey type, with performance evaluated via sensitivity and specificity. Semi-quantitative performance parameters were estimated by Performance Characteristic Curves (PCC). Decision limits (CCα) were set at 5% (EUC) and 2% (W) adulteration, below which adulterated samples could not be distinguished from authentic ones.

Two class limits were defined for unknown sample predictions, creating an uncertainty region (UR). Samples within the UR were assigned as inconclusive and should be submitted for further analysis. Among non-adulterated samples, 17% (EUC) and 43% (W) were classified as inconclusive, avoiding false adulteration assignments. For adulterated samples, 19% (EUC) and 7% (W) fell in the UR, avoiding false non-adulteration assignments. This approach eliminated classification errors, ensuring 100% reliability in identifying both non-adulterated and adulterated samples. The method offers a robust tool for accurate honey authentication, obtaining inconclusive samples that require further confirmation.

1. Introduction

According to the Standard for Honey CXS 12-1981 from the Codex Alimentarius, honey is defined as the natural sweet substance produced by honeybees from the nectar of plants or secretions of living parts of plants (Codex Alimentarius, 2001). Honey is widely recognized for its good and attractive organoleptic properties and high health benefits (Se et al., 2019; Huang et al., 2020).

The price of high-quality honey has gone up due to the rising demand and limited availability. Consequently, honey is susceptible to adulteration (Ciursă et al., 2021). This type of fraud violates the standards established by Codex Alimentarius (Codex Alimentarius, 2001) and regulatory bodies such as the European Commission (De Souza et al., 2021; European Commission, 2018) and the Brazilian Ministry of Agriculture and Livestock (Instrução Normativa Nº 11, 2000), due to the possible adverse health impact of the consumption of adulterated honey (Fakhlaei et al., 2020).

There are different types of fraud in honey but the most common is the direct addition of syrups. The most used syrups are high-fructose corn syrup (Başar et al., 2018; Li et al., 2017), rice syrup (Li et al., 2020; Limm et al., 2023), inverted sugar (Ciursă et al., 2021), cane sugar (De Souza et al., 2021; Dias et al., 2022), and other sugar syrups (Huang et al., 2020; Brar et al., 2023; Egido et al., 2024).

In this work, a strategy was established to detect the adulteration of honey by the addition of inverted sugar syrup, one of the most common adulterants reported in fraud (Ciursă et al., 2021). The official reference method for the identification of syrup-adulterated honey is based on stable carbon isotope ratio analysis (AOAC, 1998). This method is time-consuming, sample-destructive, and requires expensive instruments. In recent years, an effort has been made to implement faster, environmentally friendly, and non-destructive techniques, such as molecular spectroscopy (Huang et al., 2020). To cite some examples of spectroscopic techniques combined with multivariate analysis in honey adulteration: near-infrared (NIR) (Huang et al., 2020; Fakhlaei et al., 2020; Guelpa et al., 2017; Biaswas et al., 2024), UV-visible (De Souza et al., 2021), Fourier-transform infrared-attenuated total reflectance (FTIR-ATR) (Ciursă et al., 2021; Cárdenas-Escudero et al., 2023; Sahland et al., 2019), spectrofluorimetry (Antônio et al., 2022), Raman (Oroian et al., 2018), and nuclear magnetic resonance (NMR) (Rachineni et al., 2022; Ribeiro et al., 2014; Biaswas et al., 2023). In recent years, a type of NMR technique named Time-domain

nuclear magnetic resonance (TD-NMR) has been investigated in food science. In TD-NMR studies proton relaxation is characterized by the longitudinal relaxation time (T_1) and transverse relaxation time (T_2). T_2 relaxation time is multiexponential and indicates the presence of water populations in food matrices (Ribeiro et al., 2014; Bertram et al., 2001; Belton, 1990; Finch et al., 1971). TD-NMR offers different information about ^1H nuclei such as characterizing bound water, free water, and their exchange between these states (Santos et al., 2016). This technique requires minimal sample preparation, presents simple and fast measurement procedures, and is non-invasive, which makes it suitable for application in food control. Some examples of its application include detecting food adulteration in products such as cheese (Curti et al., 2023; Machado et al., 2022), milk (Santos et al., 2016; Coimbra et al., 2020), honey (Ribeiro et al., 2014), fruits (Colnago et al., 2021), and oil (Cengiz, 2023; Ribeiro et al., 2021).

Qualitative methods relying on multivariate classification techniques, such as partial least squares discriminant analysis (PLS-DA) (Antônio et al., 2022; Brereton et al., 2014), soft independent modelling of class analogy (SIMCA) (De Souza et al., 2021), and one-class partial least squares discriminant analysis (OCPLS) (De Souza et al., 2021), have increasingly been employed for detecting food fraud. The validation of these classification methods typically involves assessing main performance parameters, such as sensitivity, specificity, accuracy, precision, and occurrence (López et al., 2015; Cuadros-Rodríguez et al. 2016; Jiménez-Carvelo et al., 2020). These parameters are calculated from the four binary possible responses: True Positive (TP), True Negative (TN), False Positive (FP), and False Negative (FN) indicating whether a sample belongs to or does not belong to the modelled class. To determine if a sample belongs to the modelled class, it is necessary to set the limit for this class, which is usually defined as a model distance. Samples with a distance value lower than the class limit are considered to fit within the class model (Jiménez-Carvelo et al., 2020; Rovira et al., 2023; Ruisánchez et al., 2021).

The performance parameters mentioned above refer to a binary response, e.g., the unknown sample being compliant or not. When dealing with adulteration problems, it is important to consider that the model's ability will depend on the level of sample adulteration. Therefore, in addition to strictly qualitative performance parameters, concentration-related performance (semi-quantitative) parameters should be established. Recently, the detection limit ($CC\alpha$), the detection capability ($CC\beta$), and the unreliability region (UR) have been referenced (Ruisánchez et al., 2022) with their estimates derived from performance characteristic curves (PCC) (Ruisánchez et al., 2022; Ricardo et al., 2021; Corps et al., 2021).

The strategy proposed in this article aims to establish an OCPLS classification model and characterize it by main qualitative and semi-quantitative performance parameters ($CC\alpha$, $CC\beta$, and UR) estimated from PCC. Once characterized, in the prediction stage of new samples, the main qualitative parameters give a global probability of wrong assignations while the semi-quantitative parameters give similar information but at different concentration levels.

To minimize the probability of error (wrong assignation), in this work we propose defining two class limits (lower and upper) as model distances, resulting in three distance regions: i) non-adulterated region with distance values below the lower limit, ii) adulterated region with distance values greater than the upper limit, and iii) uncertainty region with distance values falling between both limits [40]. The assignment of unknown samples will be based on the region where their calculated distance value falls. If a sample falls in the uncertainty region, it will be considered inconclusive and should be submitted for further confirmatory analysis.

Numerous studies in food analysis have utilized multivariate qualitative methods to detect potential adulteration. However, only a few of them have determined parameters related to the limits of concentration detectable for the adulterant (Ruisánchez et al., 2022; Yang et al., 2022; López et al., 2014; Fulgêncio et al., 2022; Pomerantsev et al., 2023). The strategy proposed in the present work makes a noteworthy contribution in this sense, taking advantage of PCC to

determine the decision limit ($CC\alpha$) to evaluate the model before prediction. Setting two limits eliminates the probability of error in the prediction of unknown samples but part of them may be predicted as inconclusive.

2. Experimental part

2.1. Samples

Two batches of eucalyptus (EUC) and wild (W) types of honey were obtained directly from traceable producers in Minas Gerais State, Brazil. Seven proportions of those batches were prepared in five replicates to form 35 unadulterated samples for each type. Inverted sugar syrup (ISS) was added in different quantities to the seven formulated batches of unadulterated samples to obtain six levels of adulteration (1.0, 3.0, 9.0, 15.0, 21.0, and 27.0% w/w), resulting in 42 adulterated samples.

All samples were weighed using a Shimadzu AUX 220 analytical balance with a calibrated scale in 300 g flasks and in 15 mL or 50 mL Falcon tubes, followed by manual homogenization and storage at room temperature (19ºC to 25ºC) until the moment of the analysis. In the case of a honey sample crystallized, it was placed in a water bath at 39-40ºC for 5 minutes.

2.2. Instrumental measurements

Time-domain NMR analysis was performed using the Minispec ND mq-20 from Buker Biospin GmbH (Rheinstetten, Germany), operating at a ^{1}H frequency of 20 MHz (0.47 T) with a 10 mm probe head. The Transverse relaxation decays (T^2) were measured by a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with a 90° and 180°pulse length of 2.76 and 5.42, respectively, an echo time of 500 µs, a recycle delay of 2 s, and 54 dB of gain. A homogenized sample portion (approximately 5 mL) was placed on a crystal NMR tube and heated in a thermostatic bath at

40ºC. The tube was introduced into the Minispec and analyzed with three readings of 16 scans each, generating a relaxation curve of 500 data points for each sample.

2.3. Software

The data obtained were processed using MATLAB, version 8.0.0.783-R2012b (Natick, MA, USA), and PLS Toolbox 7.0.2 (Eigenvector Research Inc., Wenatchee, WA, USA). The Matlab routine for OCPLS was provided by Lu Xu (Xu et al., 2014). For the Inverse Laplace Transform the webapp rmn-ilt.streamlit.app was used, developed by Tiago B. Moraes (Moraes et al.).

3. Theoretical background

3.1. One-class partial least squares classifier (OCPLS)

OCPLS classifier is based on partial least squares (PLS). For each sample, two statistical parameters emerge once the number of latent variables (LV) is fixed. The first, Hotelling's T^2, relies on the score distance (*SD*), quantifying the distance from an object to the center of the class. The second is the absolute centered residual (*ACR*), which is a measure of dispersion or residuals in the projection onto the vector of the OCPLS regression coefficients. A confidence limit is established for both statistics, SD_{lim} and ACR_{lim}, that corresponds to the limit of the class at a specified confidence level (typically 95%) (Xu et al., 2013; Fageerzada et al., 2020). A sample must exhibit values for both statistical parameters below these limits to be classified as belonging to a target class. Another criterion for sample assignment involves calculating the reduced distance of a sample from its class ($d_{i,r}$) which is calculated according to the following equation, Eq. (1). If that is the case, for sample prediction its distance value ($d_{i,r}$) should be compared to a reduce distance class limit. Different distance values can be used as a limit to assign a sample to a specific class, for instance, 1 (Miaw et al., 2018; Gondim et al., 2017), √2

(Bevilaqua et al., 2013; Durante et al., 2011), or an optimized distance obtained by applying ROC curves (Ruisánchez et al., 2022). Usually, it is used the distance value established at 1, so $d_{i,r} \leq 1$.

$$d_{i,r} = \sqrt{\left(\frac{SD_i}{SD_{lim}}\right)^2 + \left(\frac{ACR_i}{ACR_{lim}}\right)^2} \qquad \text{Eq. (1)}$$

where SD_i and ACR_i are the statistical parameters of a sample "*i*" and the SD_{lim} and ACR_{lim} are the corresponding statistical class limits at a determinate level of significance.

If the model is considered appropriate for the case under study, the next step is the prediction of unknown samples. Instead of just using a distance class limit (usually $d_r \leq 1$), it is suggested to define two distance class limits, the upper (d_{upper_lim}) and the lower (d_{lower_lim}) class limits. Recent articles have also proposed the use of two decision limits (Rovira et al., 2023; Quintanilla-Casas et al., 2020). In the sample prediction step, the sample is assigned according to $d_{i,r} \leq d_{lower\_lim}$, $d_{i,r} \geq d_{upper\_lim,}$ or in between both ($d_{lower\_lim} \leq d_{i,r} \leq d_{upper\_lim}$).

3.2. *Semi-quantitative figures of merit*

Sensitivity, specificity, and accuracy (López et al., 2015; Cuadros-Rodríguez et al., 2016; Ballabio et al., 2018) are the main performance parameters for qualitative method validation. When detecting food adulteration, these parameters can be related to the percentage of adulterant present in the sample. This dependency can be observed by establishing PCC. PCCs have been referred to by different names, such as the probability of detection (Wehling et al., 2011), performance curves (Song et al., 2001), and probability of identification (LaBudde et al., 2012), among others. PCC illustrates the probability (frequency) of obtaining a positive result (P(X)) against the concentration level of the analyte (Wehling et al., 2011; Macarthur et al., 2012). A

positive response will be obtained when the sample is predicted as adulterated or, in other words, the sample does not belong to the model established for unadulterated/authentic samples. So, when the adulterant is absent, the probability of obtaining a positive result should be zero or close to zero (P(X)=0%). But when the concentration of the adulterant increases, the probability should increase until it reaches 100%. PCC is obtained according to Eq. 2, estimating the parameters by minimizing the root mean square of the residuals (RMSE) (López et al., 2015).

$$P(X) = \frac{a}{1 - e^{(b+(c*x))}} + d \qquad \text{Eq. (2)}$$

where *P(X)* is the probability of a positive model outcome, *x* is the level/percentage of adulterated present in the sample, and *a, b, c* and *d are* the parameters to be estimated.

From PCC, three additional semi-quantitative parameters can be obtained (López et al., 2015; Gondim et al., 2017; Trullols et al., 2005):

- The decision limit ($CC\alpha$) is the minimum concentration of the analyte (adulterant) that can be reliably detected or identified in a sample with a low statistical certainty, usually 5%. This value is obtained from the intersection of the PCC with a horizontal line at a certain value of P(X), usually 5%.
- The detection capability ($CC\beta$) is the concentration of the analyte (adulterant), from which its presence can be reliably detected or identified in a sample with a statistical certainty of 95%. The $CC\beta$ is obtained from the intersection of PCC with an upper horizontal line at P(X)= 95%.
- The unreliability region (UR) is the range between $CC\alpha$ and $CC\beta$, if a sample falls into UR, it will be assigned as inconclusive and should undergo a confirmatory analysis.

3.3. *Receiver Operating Characteristic Curve*

The Receiver Operating Characteristic curve is a graph that illustrates the performance of a classification model for a considered parameter, for example, the class distance limit. For each value of the parameter studied, the curve plots sensitivity against 1-specificity (Ruisánchez et al., 2021; Gondim et al., 2017).

The curve allows us to identify different points based on user interest. Normally it is used to obtain the optimal value of the parameter under study which is considered as the point that balances sensitivity and specificity (Ruisánchez et al., 2021). In this work, it is proposed to use the ROC, to set the two class limits, which correspond to the class limit at which sensitivity reaches 100%, regardless of specificity, and the class limit at which specificity reaches 100%, regardless of sensitivity.

4. Results and discussion

Fig. 1 shows the relaxation curves of the non-adulterated EUC honey samples and adulterated EUC honey with inverted sugar at different percentages (1-27%). The intensity of the signals of mean curves of maximum normalized T^2 relaxation profiles obtained with the CPMG pulse sequence increases with the level of adulteration (Fig. 1a). Similarly, Fig. 1b shows the inverse Laplace transform (ILT) applied to these CPMG decays, where can be observed the distributed T^2 relaxation time in the logarithmic scale for non-adulterated and adulterated samples. Non-adulterated (dark green line) and adulterated samples at 1% (orange line) and 3% (blue line) have relaxation water populations between 4.76 – 4.85 ms, from the start of their bands (Fig. 1b), as can be seen in Table 1. As the level of adulteration increases, these bands are shifted reaching relaxation water populations between 6.85 – 7.37 ms (Table 1) for adulteration at 21% (black line) and 27% (light green line). Therefore, the relaxation time depends on the percentage of adulteration, indicating that water mobility was lower for non-adulterated than for adulterated honey. Similar plots (Figure 1S and Table 1S) were obtained for W samples.

---------- Figure 1 ----------

---------- Table 1 ----------

The nature of these differences in Time-domain NMR (TD-NMR) signals allowed the problem to be addressed through a semi-quantitative classification approach. Fig. 2 schematically shows a flowchart of the steps involved in the development and validation of a multivariate classification model. The first step was the selection of the proper classification method. As the aim was to detect whether a sample is adulterated or not, a one-class classification model was chosen, namely OCPLS [Xu, L. et al. 2014 and Moraes, T.B. et al., 2023].

---------- Figure 2 ----------

Two independent one-class models were built for each of the two origins of honey samples, EUC and W. Due to the reduced number of authentic samples, models were built with all 35 non-adulterated samples. Four and three LVs were selected for EUC and W models respectively, based on the minimum cross-validation classification error. Figs. 2S shows the OCPLS model assignations plots of SD versus ACR obtained in the prediction for each type of honey, considering the reduced SD and ACR values and class limit at $d_r \leq 1$.

Following the flowchart (Fig. 2), once the model is built, the next step is to validate it by estimating its performance parameters, which gives a global performance of the model. In the prediction of the non-adulterated EUC and W samples, considering the class limit at $d_r \leq 1$, the sensitivity was 94.3%. This means that both models wrongly assign as adulterated 2 out of 35 samples when they are not. For the EUC and W models, 57.1% and 66.7% specificity were achieved, meaning that 18 and 14 samples out of the 42 adulterated samples respectively, were wrongly assigned as non-adulterated when indeed they were.

As stated, the specificity provides overall information about the prediction of the adulterated samples. However, it does not provide information about the model behaviour for each level of

adulteration. When there are samples available at different adulteration levels, useful information can be obtained related to the level of adulteration from PCC. Fig. 3 shows the PCC estimated from OCPLS models for EUC (Fig. 3a) and W (Fig. 3b) honey samples. In addition to authentic samples, 42 samples adulterated with inverted sugar syrup (7 samples for each level of adulteration, between 1% and 27%) were used for testing each model. Table 2 presents the equations and parameters related to fitted PCC.

---------- Figure 3 ----------

---------- Table 2 ----------

CCα values were obtained from the intersection of the PCC curve with the horizontal line fixed at P(X) equal to 5% and 10% for W an EUC honey PCC curves, respectively. From Fig. 3, *CCα* is equal to 2% (w/w) for W honey and 5% (w/w) for EUC honey. Samples adulterated at percentage levels below or close to *CCα* cannot be differentiated from the non-adulterated samples. CCβ values were obtained from the intersection of the PCC curve with the horizontal line fixed at a P(X)=95%. *CC*β was estimated at 12% (w/w) for both models. So, above 12% of adulterant content, all samples will be assigned as adulterated with 95% certainty. In addition, in both cases, above 15% of adulterant content, they can be detected with 100% of certainty.

It is important to emphasize that although α and β values are usually set at 0.05 (5%), the user can define other values, which will change *CC*α, *CC*β, and consequently the uncertainty interval. In addition to *CC*α, *CC*β, other probability values could be worth exploring. For instance, the adulteration level at which there are 50% of properly obtaining a positive assignation (sample is adulterated). From Fig. 3 (grey dotted lines), these values correspond to a percentage of adulteration of 10 % and 7% for EUC and W, respectively.

Once the PCCs have been evaluated, confirming that samples adulterated at 1% and 3% do not differ from the non-adulterated samples, the sensitivity and specificity values have been

recalculated without them, also considering the class limit at $d_{i,r} \leq 1$. The sensitivity value remains the same since the non-adulterated samples are consistent, resulting in a value of 94.3% for both models. Specificity was 85.2% and 96.3% for the EUC and W models respectively.

Following the scheme in Fig. 2, if the model is considered appropriate for the case under study, the next step is the prediction of unknown samples. Instead of just using the class limit, usually established at $d_r \leq 1$, it is suggested to define two class limits, the upper limit (d_{upper_lim}) and the lower limit (d_{lower_lim}). Both limits were established with 100% confidence obtained from the ROC curves (Fig. 4). Fig. 4a shows the ROC curves from OCPLS models for EUC and Fig. 4b for W honey samples. They have been built without considering adulterated samples with 1% and 3% adulterant since the PCC curves showed that these cannot be differentiated from the non-adulterated samples. The d_{upper_lim} was obtained from the maximum distance value at which sensitivity is 100% (indicated on ROC plots with a red circle). Similarly, the d_{lower_lim} was obtained from the minimum distance value at which the specificity is 100% (indicated on ROC plots with a green circle). The d_{upper_lim} was set around 1.3 in both EUC model W model, while the d_{lower_lim} was established at 0.68 and 0.61 for EUC and W models, respectively.

---------- Figure 4 ----------

In Fig. 5, the two-class limits (d_{upper_lim} and d_{lower_lim}) for each type of honey are presented. It should be noted that adulterated samples hardly appear in it because their distances are greater than those in the graph (see Figures S2a and S2b). Table 3 presents the predictions of the different types of samples depending on whether two limits or 1 limit (at a distance less than or equal to 1) are considered.

---------- Figure 5 ----------

---------- Table 3 ----------

At the prediction stage, the percentage of non-adulterated samples that are correctly predicted decreases considerably with the two-limit strategy, much more so in the case of W honey. In the case of adulterated samples, the percentage of correctly predicted samples is similar in both strategies for the two types of honey.

However, as can be seen in the table, in the case of using a single limit, the prediction of the samples carries an associated probability of error, for example, there is a 5.7% probability of wrongly predicting a non-adulterated sample as adulterated. In the case of following the two-limit strategy if a sample is predicted as non-adulterated, there is a 100% probability that it is. The counterpart of not having an error in the assignment is that 17.1% of the non-adulterated samples would require considering a confirmatory analysis

The main advantage of establishing two class limits is eliminating the probability of error because the two limits permit no assignment ambiguity. Below the lower threshold are only samples assigned as non-adulterated and above the upper threshold there are only ones assigned as adulterated.

From the practical point of view, end-users have the flexibility to define the UR limits based on the specific nature of the problem. For instance, in the current study, if the end-user is aware that honey samples are not expected to be adulterated below 10%, adjusting the d_{lower_lim} to slightly higher values can effectively reduce the UR. Defining the two limits involves striking a balance between the percentage of samples requiring confirmatory analysis and the acceptable error percentage for the end user.

5. Conclusions

Two one-class models using OCPLS were developed to detect eucalyptus and wild honey adulteration with inverted sugar syrup. These models were validated by establishing main

performance and semi-quantitative parameters ($CC\alpha$, $CC\beta$, and UR) obtained from performance characteristic curves (PCC). The developed strategy allowed the examination of the model's behaviour across different adulteration levels before the prediction step. The estimate of $CC\alpha$ provided insights into which/how many samples at the lowest levels of adulteration cannot be discriminated from non-adulterated/authentic samples. The estimate of $CC\beta$ indicated the adulteration level from which samples will be identified as adulterated with certainty.

The proposed strategy of defining two class limits allowed the establishment of an uncertainty region, which implies determining with 100% certainty whether a sample is compliant or non-compliant. Consequently, the probability of error in the prediction of unknown samples is eliminated when compared to using only a single. For both types of honey, all samples adulterated at the highest levels (15%, 21%, and 27% w/w) were correctly assigned as adulterated, while all samples at 9% w/w were classified in the uncertainty region, thus demanding to be subjected to confirmatory analysis. The approach developed in this article can be easily adapted to the detection of other types of adulterants in honey.

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Figure Captions

Fig. 1. (a) Maximum normalized T^2 mean relaxation curves obtained with CPMG pulse sequence for unadulterated/authentic and adulterated (1-27% w/w) eucalyptus honey samples. **(b)** T^2 relaxation spectra of the respective honey samples obtained via ILT. Color codes: dark green for non-adulterated honey, orange for samples adulterated at 1%, blue for adulterated at 3%, red for adulterated at 9%, purple for adulterated at 15%, dark blue for adulterated at 21% and light green for adulterated at 27%.

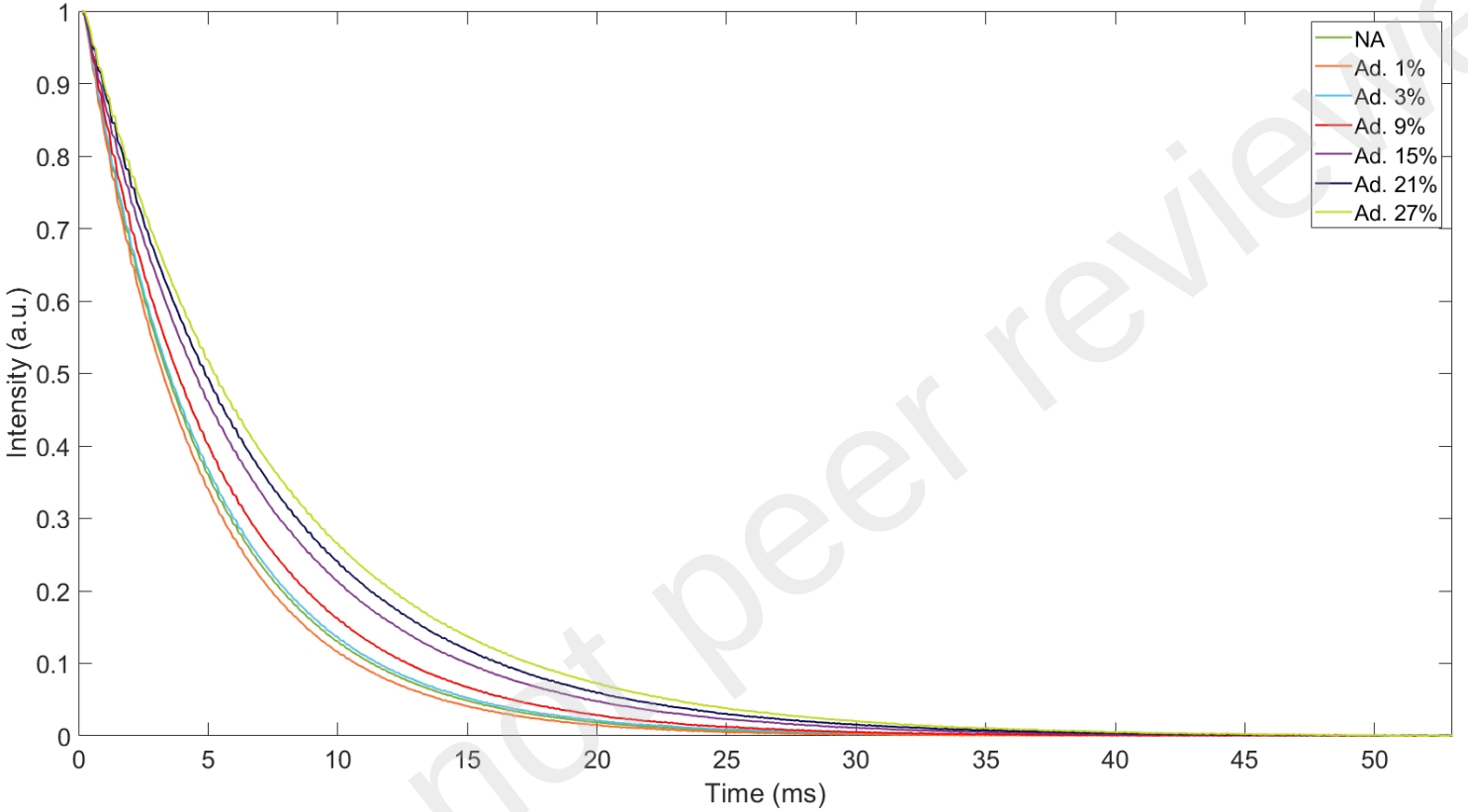
Fig. 2. Schematic flowchart describing the semi-quantitative chemometric approach developed in this work.

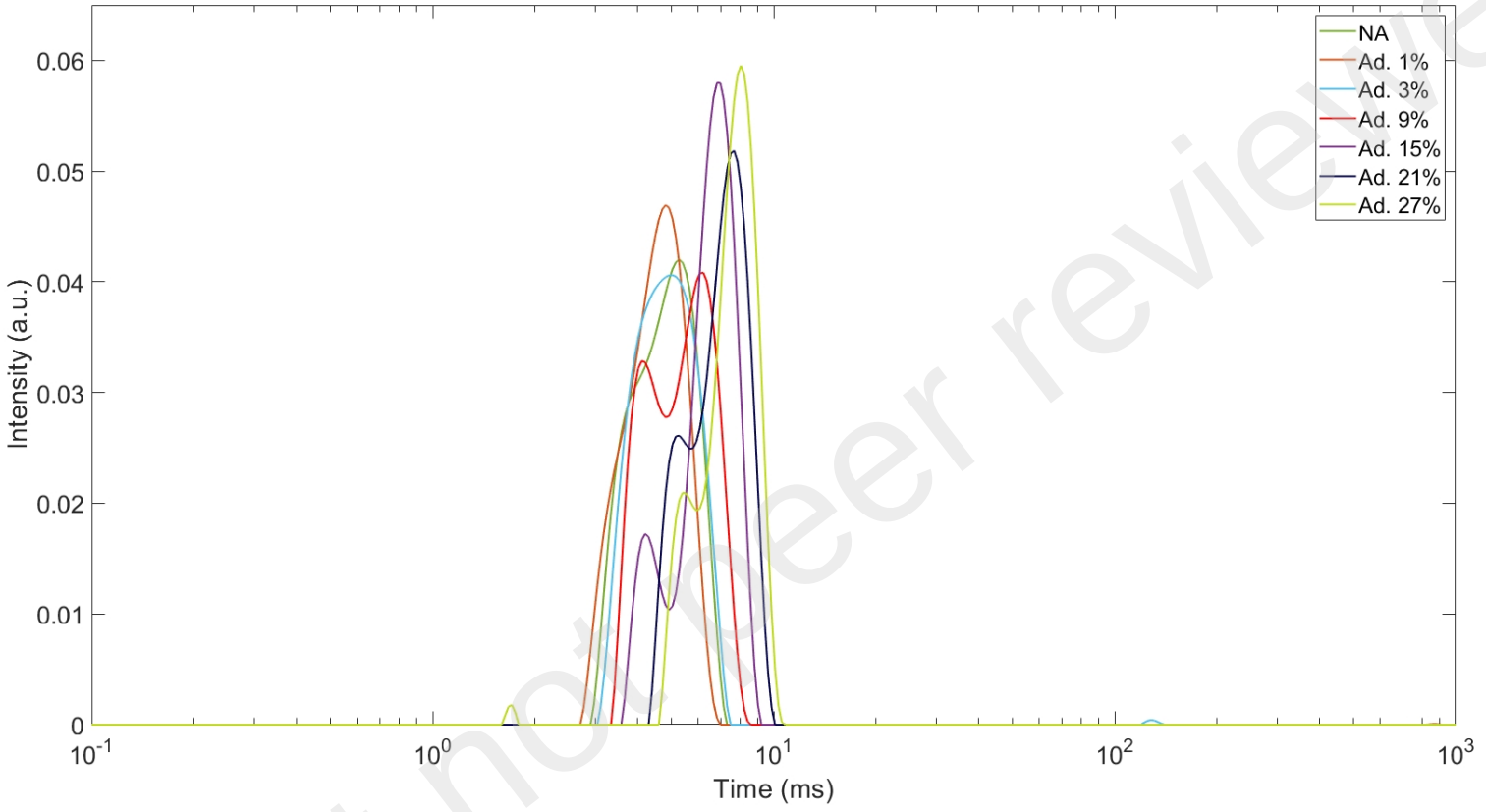
Fig. 3. Prediction results obtained by OCPLS models for detecting adulteration with inverted sugar syrup in **(a)** eucalyptus and **(b)** wild honey. Dashed lines indicate significance levels of 0.05 for both score distances (SD) and centered model residuals (ACR). Boxes inside the plots represent a zoom view of the acceptance region (SD and ACR values below the limits). Color and symbol codes: Full green circles for non-adulterated honey, empty orange triangles for samples adulterated at 1%, empty light blue triangles for adulterated at 3%, empty red triangles for adulterated at 9%, empty purple triangles for adulterated at 15%, empty dark blue triangles for adulterated at 21% and empty light green triangles for adulterated at 27%.

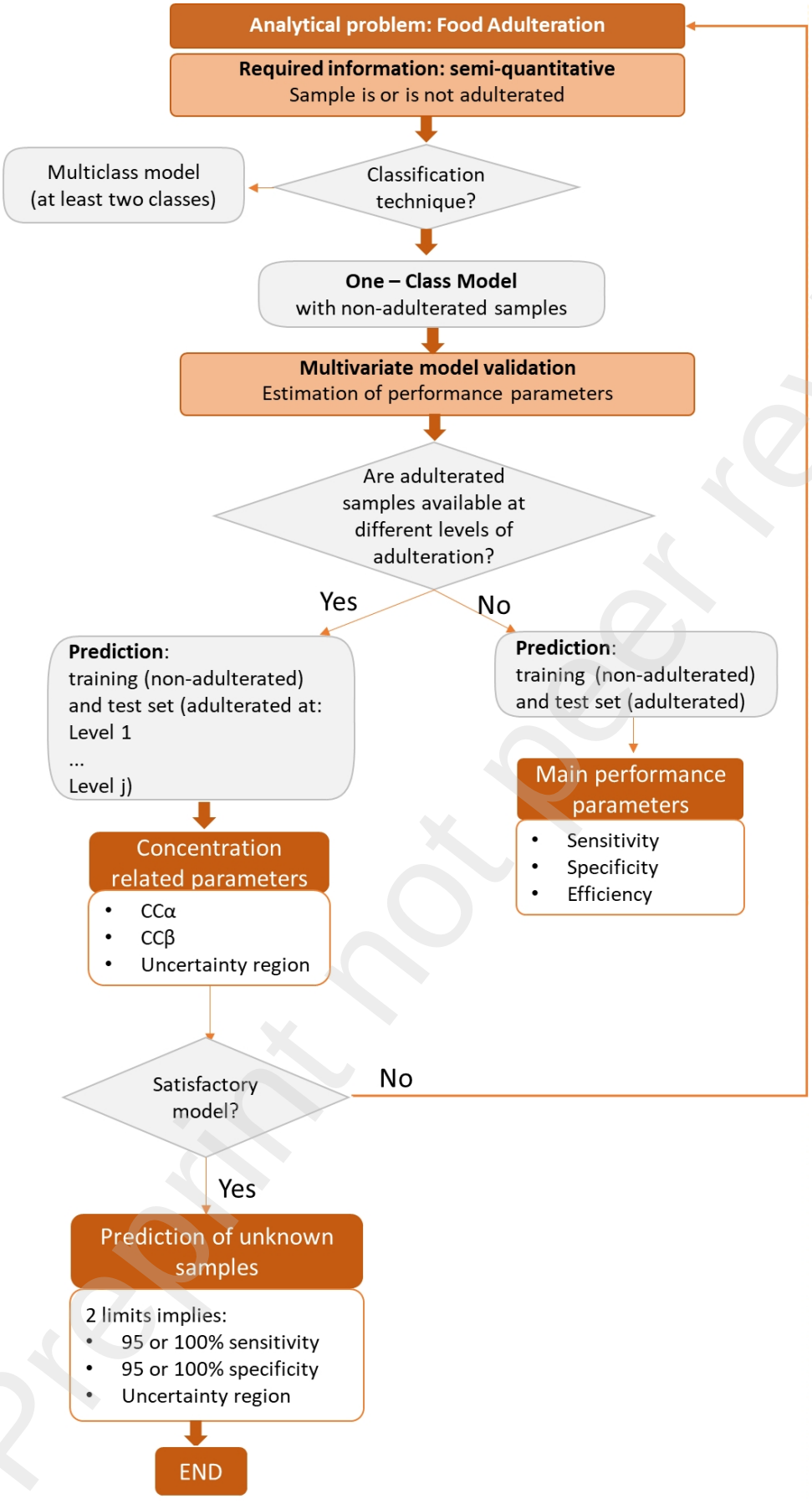
Fig. 4. Performance characteristic curves (PCC) constructed for estimating CCα and CCβ for OCPLS models built with adulterated **(a)** eucalyptus and **(b)** wild honey samples.

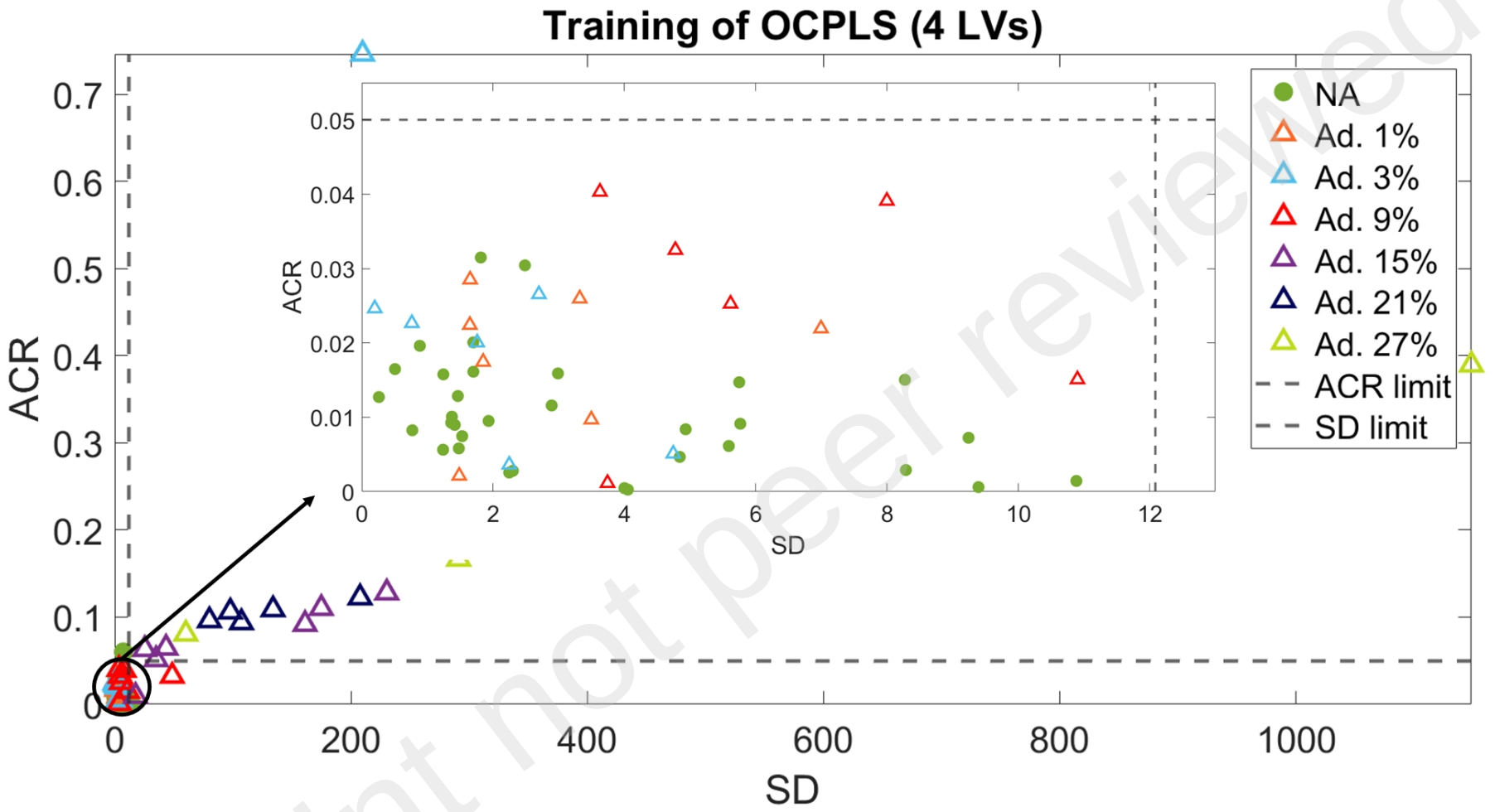
Fig. 5. Distances of all the analyzed samples to the OCPLS model. Vertical red and green solid lines represent the lower and upper limits estimated for the uncertainty region, respectively.

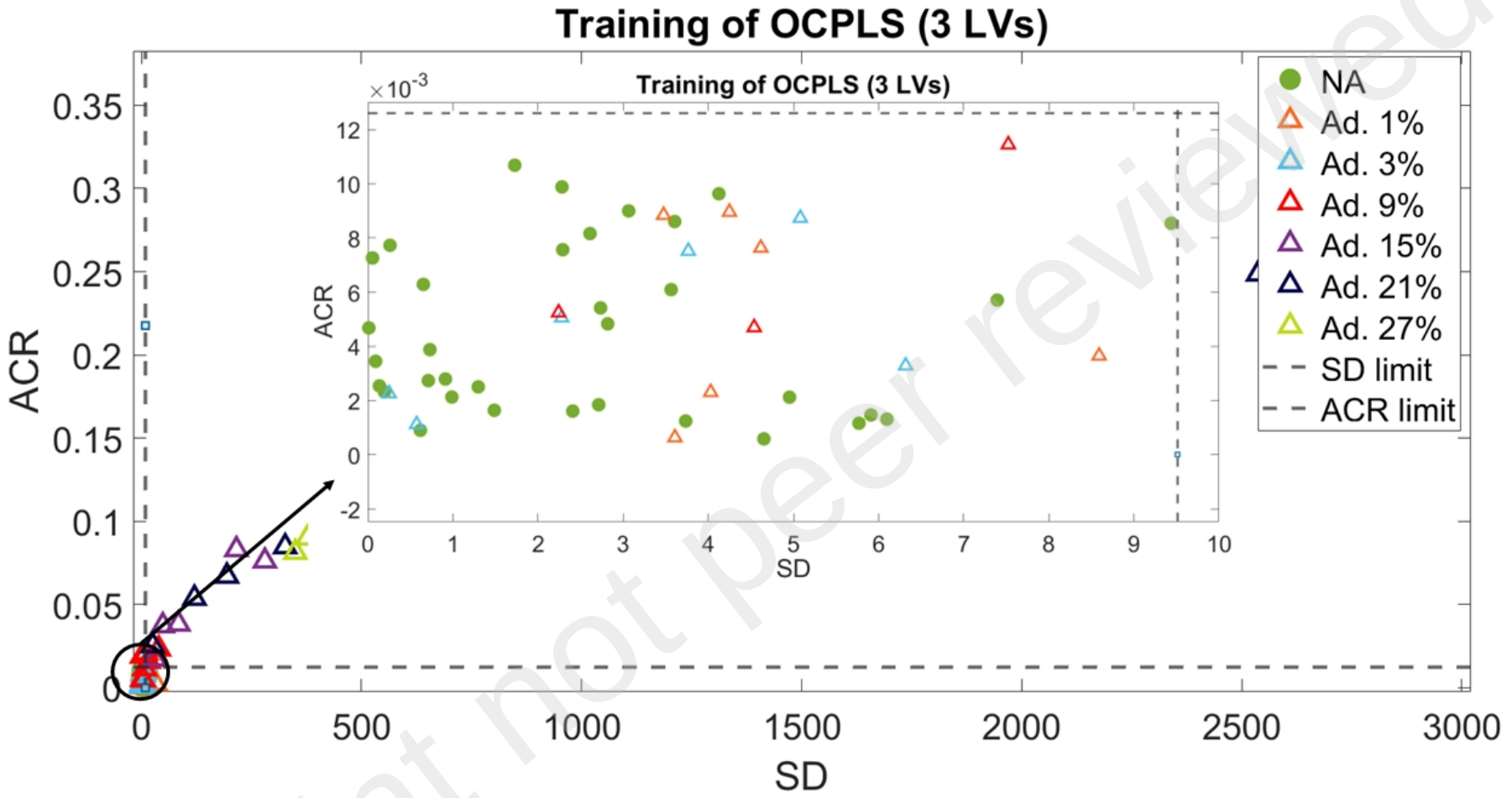
Color and symbol codes: Full green circles for non-adulterated honey, empty orange triangles for samples adulterated at 1%, empty light blue triangles for adulterated at 3%, empty red triangles for adulterated at 9%, empty purple triangles for adulterated at 15%, empty dark blue triangles for adulterated at 21% and empty light green triangles for adulterated at 27%.

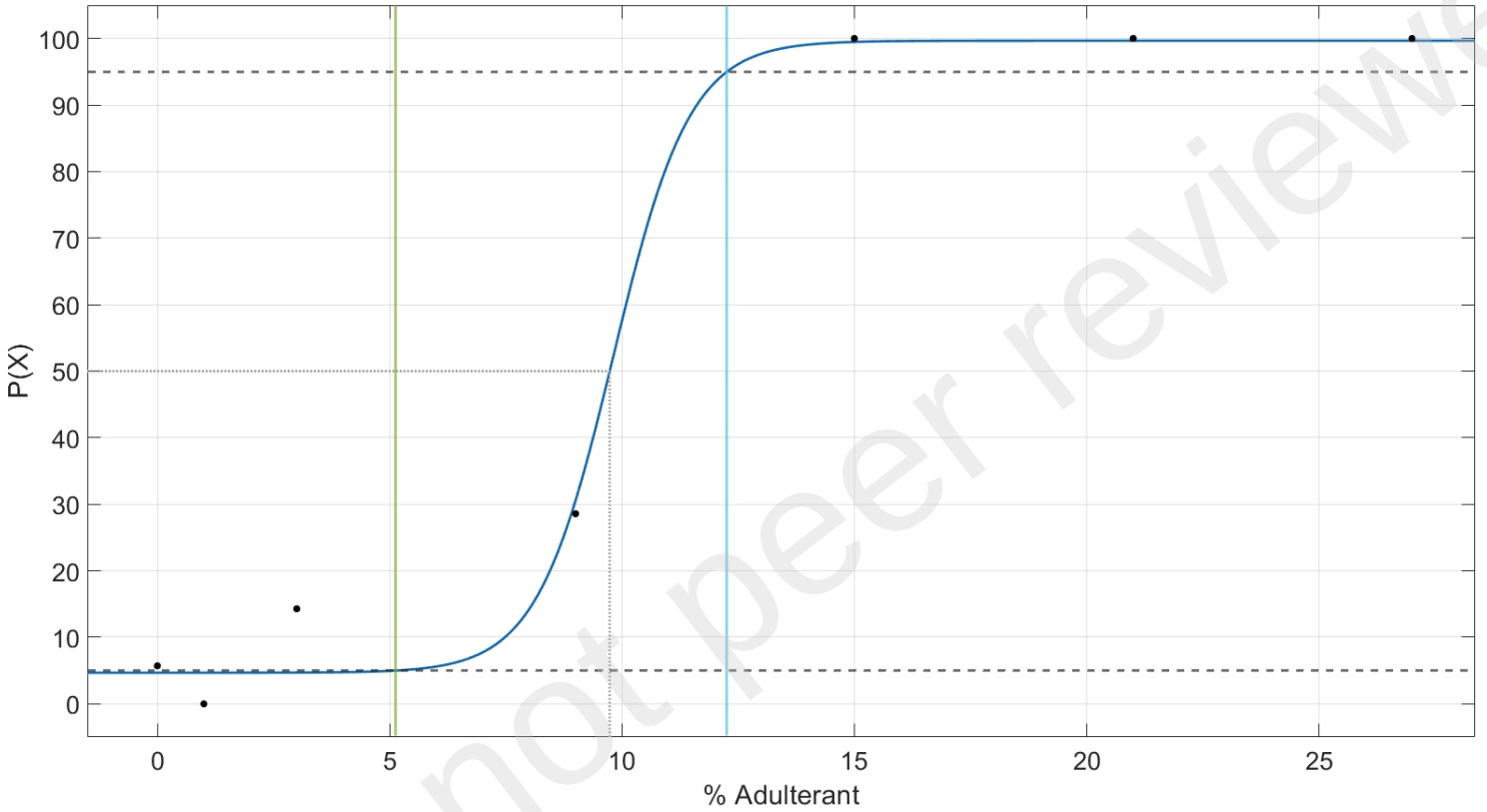


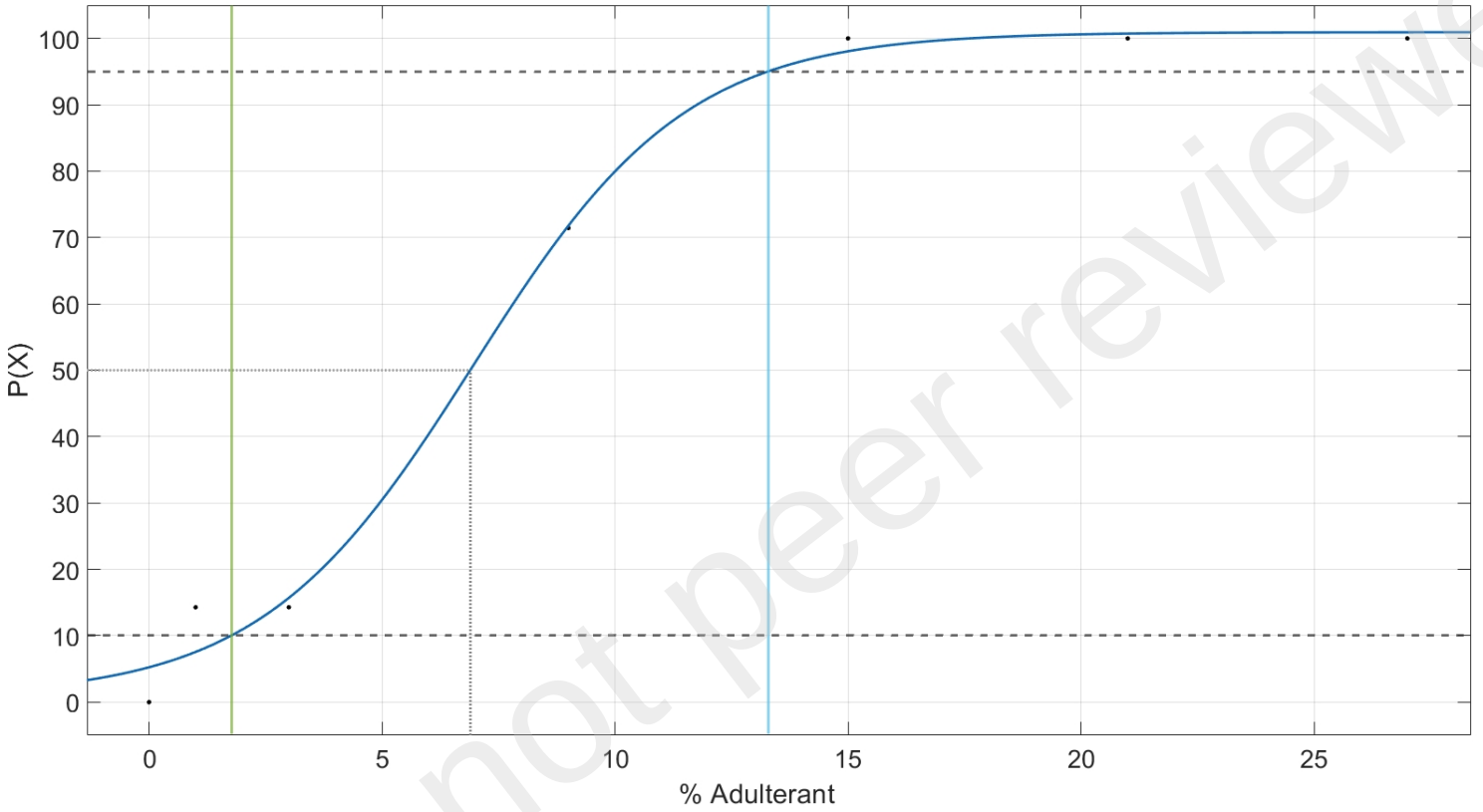












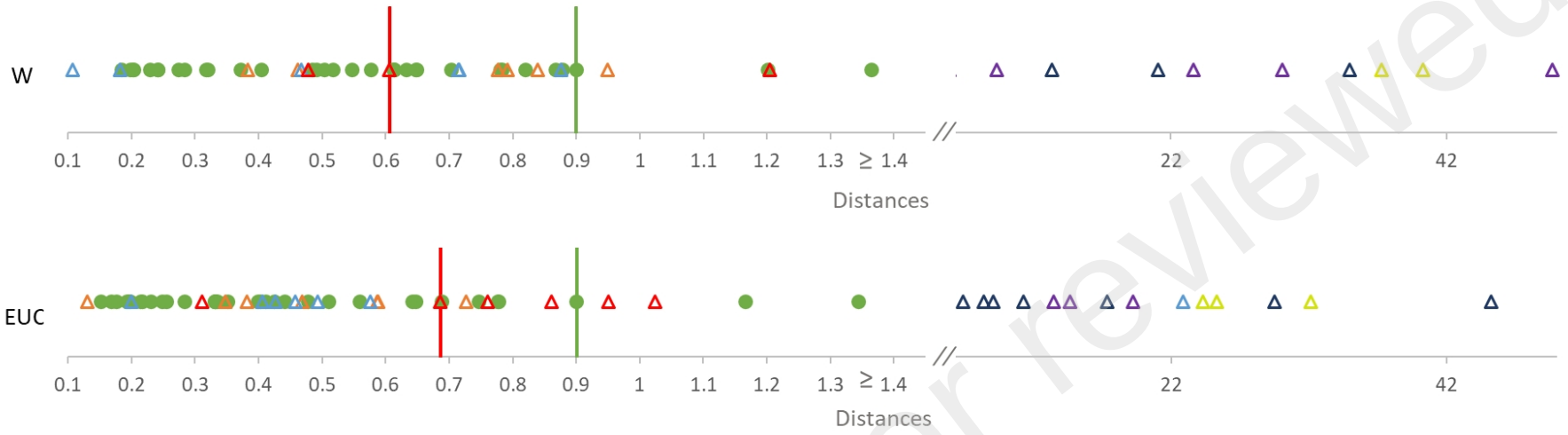


Table 1. T^2 parameters obtained in EUC honey according to level of adulteration with ISS

Adulteration level	T^2 (ms)
0%	4.76
1%	4.50
3%	4.85
9%	5.34
15%	6.34
21%	6.85
27%	7.37

Table 2. Fit parameters of performance characteristic curves (PCC) and semi-quantitative parameters for both OCPLS models (EUC and W).

Parameter	EUC Model	W Model
R^2	0.9948	0.9937
RMSE (%)	3.68	5.17
Adjusted R-square	0.9934	0.9873
Equation	$\frac{94.98}{(1+e^{(11.88+(-1.211\cdot x))})}+4.67$	$\frac{-100.2}{(1+e^{(-3.05+(0.44\cdot x))})}+100.9$
$CC\alpha$ (%)	5	2
$CC\beta$ (%)	12	12
UR (%)	5-12	2-12

Table 3. a) Uncertainty intervals and percentage of samples assigned as adulterated and as uncertain considering two class limits (d_{lower_lim} and d_{upper_lim}). b) Percentage of samples assigned as adulterated considering one class limit ($d_{i,r} \leq 1$) for eucalyptus (EUC) and wild (W) honey.

a)

		% Adulterant assignment						
Model	***Uncertainty interval (d)***	**0% (NA)**	**1%**	**3%**	**9%**	**15%**	**21%**	**27%**
EUC	**0.69-0.90**	6	0	14	43	100	100	100
		11	14	0	43	0	0	0
W	**0.60-0.90**	6	29	14	71	100	100	100
		37	43	43	14	0	0	0

b)

		% Adulterant assignment						
Model	***Uncertainty interval (d)***	**0% (NA)**	**1%**	**3%**	**9%**	**15%**	**21%**	**27%**
EUC	***d*≤1**	6	0	0	29	100	100	100
W		6	14	14	71	100	100	100