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Discrimination of stored and aged cachaça using synchronous fluorescence spectroscopy and supervised classification

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Abstract

Why was the work done: Brazilian legislation requires 'aged' cachaça to be matured for a minimum of one year in wooden barrels with a maximum capacity of 700 L. 'Stored' cachaça is stored for less than a year in wooden barrels that are bigger than 700L. Discrimination between these categories is required to mitigate against economic fraud, as the maturation process increases production costs and market value. The development of robust analytical methodologies for discrimination assures labelling integrity and protects the commercial value of the spirit.
How was the work done: Synchronous fluorescence spectroscopy (SFS) was used to analyse 212 samples of cachaça that were either aged or stored in barrels made of oak, umburana, bálsamo, jequitibá, and amendoim. To differentiate the two classes, supervised classification models based on partial least squares discriminant analysis were developed. Savitzky–Golay smoothing with the first derivative was used to preprocess the spectral data before splitting them into two datasets: a training set (141 samples) and a test set (71 samples) for respectively building and then validating the model.
What are the main findings: Using synchronous fluorescence spectra recorded at a $\Delta\lambda$ (λ_{em} - λ_{exc}) of 30 nm, the model differentiated 88% of the cachaça samples as either stored or aged.
Why is the work important: The method is simple, fast and inexpensive. It establishes a robust analytical methodology to differentiate aged and stored cachaça. This study enables the validation of cachaça, providing regulatory agencies with a tool for authentication and quality control.

Keywords

cachaça; distilled spirit; synchronous fluorescence spectroscopy; supervised classification; storage; aging time; wood.

Introduction

Maturation of beverages in wooden containers contributes to the aroma, taste, and colour of whisky, rum, brandy, wine, and beer. The maturation process promotes changes in chemical composition through a complex array of reactions, including the extraction of wood compounds and the degradation or formation of compounds (Mosedale and Puech 1998). These changes depend on the barrel (wood species, toasting treatment, and size) and maturation conditions (temperature, humidity and time).

With commercialisation and high prices, the falsification and adulteration of distilled beverages are common worldwide. Fraudulent declaration of longer aging occurs as maturation is the longest process in the production of alcoholic spirits. This has resulted in several studies. Martí et al (2005) described a quantitative method - with an accuracy of about one month - for determining the aging of sugarcane spirits aged in oak barrels between two to 23 months using an electronic nose coupled to a headspace mass spectrometry (HS-MS) and partial least squares (PLS). Chen et al (2021) presented a combination of headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) and PLS to detect the aging of baijiu (Chinese white liquor) between one and 22 years, with a root mean square error of prediction (RMSEP) of 0.244 years. Roullier-Gall et al (2020) used Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) combined with partial least squares discriminant analysis (PLS-DA) to discriminate Scotch whiskies with a maturation time of six to 18 years, with a standard deviation between 0.69 and 1.57 years. Li et al (2020) developed models based on PLS using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-ion mobility spectrometry (GC-IMS) to age brandy (two to 13 years) and its volatiles, with a root mean squared error of cross-validation (RMSECV) of 0.673 years.

Cachaça is only produced in Brazil, and is a spirit from the distillation of fermented sugarcane juice, containing 38–48% ethanol by volume at 20°C (Brazil 2022). Brazilian legislation classifies aged cachaça into three types: ‘aged’, ‘premium’, and ‘extra-premium'. This is related to the duration of ageing, capacity of the wooden barrels, and amount of aged cachaça. Cachaça is considered aged when 50% of the cachaça is aged for at least one year in a wooden barrel, with a maximum capacity of 700 litres. Premium cachaça is aged for at least one year but contains 100% aged cachaça in a wooden barrel of ≤ 700 L. Extra-premium cachaça is 100% cachaça aged in a wooden barrel of ≤ 700 L for at least three years. ‘Stored cachaça’ is aged for less than one year in wooden barrels of > 700 (Brazil 2022). A further variable is that cachaça is aged in oak (*Quercus* sp.) barrels along with other native Brazilian woods.

During the maturation of cachaça, the concentration of phenolic compounds and maturation-related congeners increases (Dias et al. 1998; Miranda et al. 2008; dos Anjos et al. 2011; Alcarde et al. 2014; Santiago et al. 2014; Bortoletto et al. 2016; Santiago et al. 2017; Carvalho et al. 2020; Castro et al. 2020; Castro et al. 2021), volatiles (Miranda et al. 2008; Alcarde et al. 2014; Bortoletto et al. 2016), dry extract and colour (Miranda et al. 2008; Bortoletto et al. 2016; Santiago et al. 2017).

Silvello et al (2021) evaluated the concentration of lignin-derived products (LDPs) in cachaça aged in different barrels using high performance liquid chromatography coupled to a ultraviolet/visible detector (HPLC/UV–Vis). They proposed separation into maturation classes based on use, toast level, and aging time by applying two distinct clustering algorithms: k-means and Gaussian mixture model (GMM). Rodrigues et al (2014) developed methods using artificial neural networks (ANN) and k-nearest neighbour (k-NN) coupled to a computer vision system and chemical information for the discrimination of aged cachaças by type (aged, premium, and extra-premium), with 97% accuracy. In another study, Rodrigues et al (2016) captured image signals from samples and an intelligent classification technique using an ensemble of classifiers to recognise aged cachaça types, achieving an accuracy of about 86%.

Of the techniques used to test the authenticity of spirits, synchronous fluorescence spectroscopy (SFS) has been used coupled with different chemometric methods. There are two main benefits of molecular fluorescence: ease of use and low cost, as there is no need for sample preparation or reagents. SFS has been for the analysis of brandy (Urícková et al. 2013; Ziak et al. 2014; Jakubíková et al. 2018; Sádecká et al. 2019), vodka (Facci et al. 2020), and fruit spirit drinks

(Sádecká et al. 2015; Tomková et al. 2015; Sádecká et al. 2016). In a previous study, SFS was used to discriminate between commercial cachaças and rums (Silveira and Barbeira 2022) and to discriminate aged commercial cachaça in barrels of different woods employed in the aging process (Silveira and Barbeira 2022). Using SFS and PLS-DA, this current study sought to distinguish between cachaça aged and stored in barrels made of different woods; amendoim (*Pterogyne nitens*), bálsamo (*Myroxylon peruiferum*), jequitibá (*Cariniana estrellensis*), oak (*Quercus* sp.), and umburana (*Amburana cearensis*).

Materials and methods

Cachaça - 212 samples

- Commercial samples (143) were from local liquor stores which were stored or aged by the manufacturers in barrels of amendoim (*Pterogyne nitens*), bálsamo (*Myroxylon peruiferum*), jequitibá (*Cariniana estrellensis*), oak (*Quercus* sp.), or umburana (*Amburana cearensis*), as identified on their labels.
- Laboratory samples (69) were from a single white cachaça sample stored for at least two months in barrels made of the same five woods.
- The distribution of the total samples by wood barrel was oak (65), bálsamo (44), umburana (39), jequitibá (38), and amendoim (26).
- Samples were further categorised by the duration of the contact with wood: 'S' (145 stored less than one year) and 'A' (67 aged at least one year). All samples were kept at room temperature and were not subject to any preparation prior to analysis.

Synchronous fluorescence spectroscopy

The synchronous fluorescence spectra of cachaça were obtained using a Xenon lamp equipped Shimadzu RF-5301PC spectrofluorometer (Duisburg, Germany). The spectra resolution was set to 1 nm, and the excitation and emission slits were set to 10 nm. Synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromators at the emission wavelength (λ_{em}) range of 230-650 nm, with constant wavelength intervals ($\Delta\lambda = \lambda_{em} - \lambda_{exc}$) between them. Spectra were recorded at $\Delta\lambda$ intervals ranging from 10 to 100 nm in steps of 10 nm. Fluorescence measurements were performed in triplicate for each sample in a 10 × 10 × 45 mm quartz cell, and the fluorescence intensity of the media was plotted as a function of λ_{em}.

Data analysis

Chemometric models were developed using MATLAB software, version 7.10 (R2010A) (The Math Works, Natick, USA), and PLS Toolbox, version 5.2.2 (Eigenvector Technologies, Manson, USA).

Preprocessing

The synchronous fluorescence spectra were arranged in a two-dimensional matrix (samples × λ_{em} (nm)) for each $\Delta\lambda$. The mean centre and first derivative of the Savitzky-Golay smoothing method were used to preprocess the fluorescence spectra. The response from the face-centred central composite designs was the number of classification errors, determined for different filter widths and polynomial orders. This metric, representing the proportion of incorrect predictions made by a PLS-DA model when attempting to assign samples to different classes, was recorded for the training, cross-validation, and test sets. Using the desirability function to visualise how multiple response variables are optimised simultaneously, the filter width and polynomial order resulting in the fewest classification errors was selected.

Supervised classification models based on PLS-DA

Initially, each class was split into two sets, a training set (141) and a test set (71) of samples using the Kennard-Stone algorithm (Kennard and Stone 1969). The models were constructed and validated with the training (Stored, n = 100; Aged, n = 41) and test sets (Stored, n = 45; Aged, n = 26). The **X** block contained fluorescence intensities, and the **Y** block consisted of a matrix with values of 1 (and 0) if a sample belonged (or did not belong) to a specific class. Decision criteria for determining if a predicted y value belonged (or not) to each modelled class was based on the Bayesian thresholds.

The **X** and **Y** blocks were simultaneously decomposed into latent variables (*LV*) that maximise the covariance between the two data sets. The number of *LV* was selected based on the lowest value of the cross-validation classification error (*CVCE*) estimated using Venetian blinds cross validation, by selecting data points through a systematic, interleaved sampling pattern to maintain a representative distribution across validation folds. Samples presenting Q residues and Hotelling's T^2 above the critical values simultaneously at a 95% confidence level were excluded.

To estimate the quality of the PLS-DA models, the sensitivity (*SEN*), specificity (*SPE*), and efficiency (*EFF*) were calculated. *SEN* is the ability of the model to correctly identify samples belonging to a specific class, *SPE* is the ability of the model to correctly exclude samples that do not belong to that class, and *EFF* is the proportion of correct classifications in relation to the total number of samples. These are the main figures of merit used to evaluation the quality of PLS-DA models.

Results and discussion

Synchronous fluorescence spectroscopy

Figure 1 reports the synchronous fluorescence spectra recorded at Δλ of 30 nm for the same cachaça sample stored in amendoim, bálsamo, jequitibá, oak, and umburana barrels for periods of one, three, six, 12, and 24 months.

The synchronous fluorescence spectra exhibited bands at λ_{em} of approximately 480 and 550 nm. The most intense band for the five different wooden barrels was at λ_{em} 480 nm and showed an increase in intensity of fluorescence up to six or 12 months of aging, followed by a decrease in intensity. The band at λ_{em} 550 nm increased in fluorescence intensity with time of aging. The spectra of the samples aged in amendoim (A) and oak (C) barrels showed more bands (350 and 380 nm) than those of the other woods. The bands at λ_{em} of approximately 350 and 380 nm decreased in intensity of fluorescence as a function of aging.

These observations indicate that the transfer of compounds from wooden barrels to distillates is not constant and homogeneous over maturation time, in agreement with the work of Roullier-Gall et al (2020).

The spectra of the samples differed by the wood used in the maturation process and by different storage or aging times. This reflects the higher concentration of fluorophores released from wooden barrels, the presence of highly fluorescent components, the quenching of excited state reactions, molecular rearrangements, energy transfer, and complex formation in ground states.

Spectra preprocessing

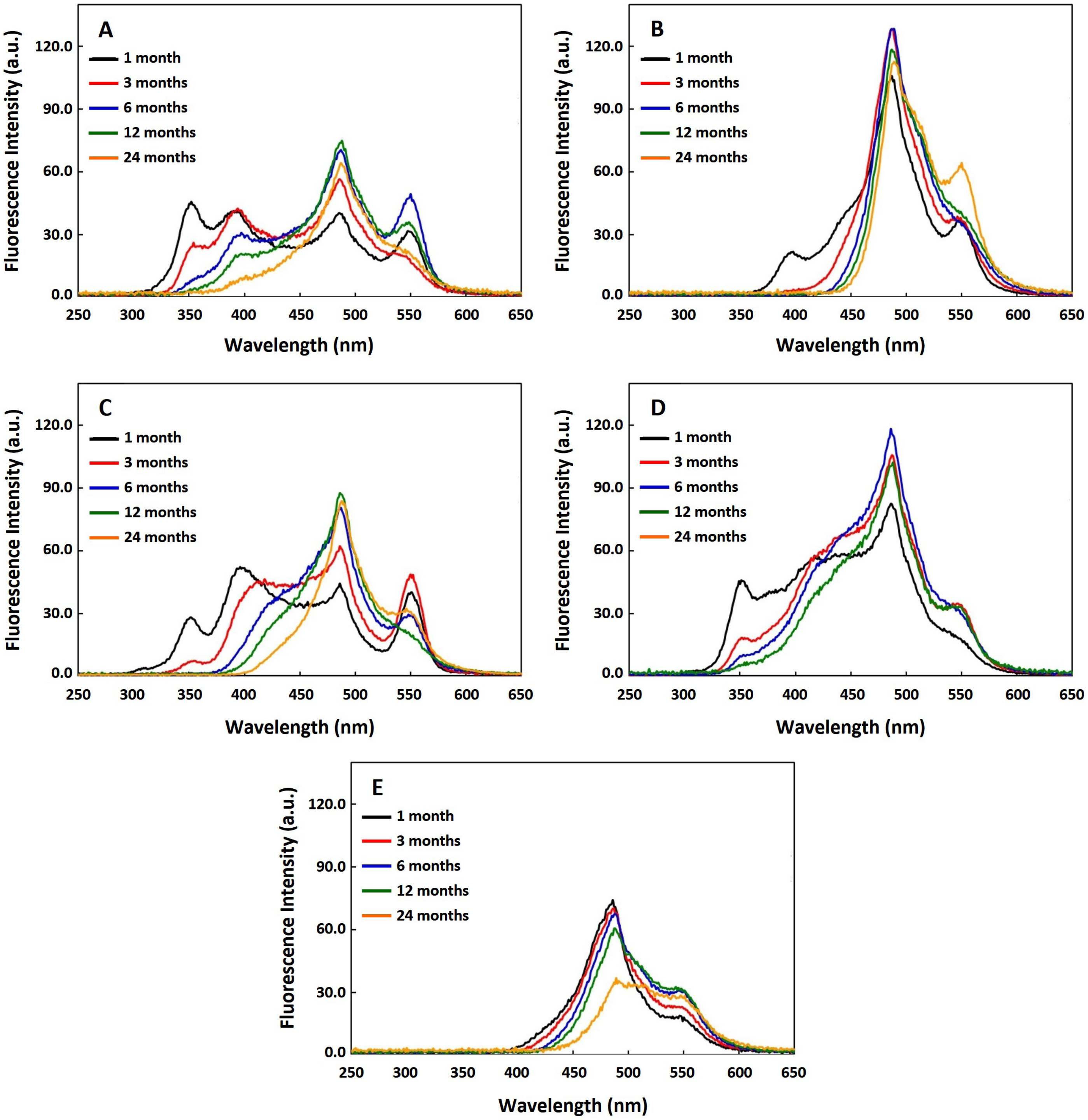
Data preprocessing through first-derivative Savitsky-Golay smoothing and mean-centring effectively reduced spectral noise and baseline shifts. The optimisation via face-centred central composite design allowed for the identification of the ideal smoothing parameters (filter width and polynomial order) for each Δλ. These optimised parameters were critical in minimising classification errors across the training, cross-validation, and test sets. The response surface of the spectra recorded at various Δλ values is shown in Figure 2 and illustrates the effect of choosing these smoothing parameters on the PLS-DA models. The figure also emphasises the optimal smoothing combination for each Δλ, highlighting the significance of the optimisation process.

Variations in training and test errors for optimised models of different values of Δλ (Figure 3) show that the model obtained for a Δλ of 30 nm has the lowest classification errors and the highest percentage of variance explained in the **X** and **Y** blocks (Table 1).

Table 1. Filter width, polynomial order, LVs, and variance (%) captured for each Δλ PLS-DA models (* best condition)

PLS-DA model *Δλ* (nm)	Filter width	Polynomial order	Latent Variables	Variance captured (%)	
				X block	**Y** block
10	15	6	6	93	36
20	11	3	5	69	40
30*	11	6	7	71	72
40	11	2	11	98	42
50	11	2	8	95	31
60	19	6	10	98	53
70	17	2	9	99	36
80	15	3	9	98	46
90	11	2	7	99	21
100	11	6	4	84	26

Figure 1. Synchronous fluorescence spectra recorded at Δλ = 30 nm of cachaça aged in amendoim (A), bálsamo (B), oak (C), jequitibá (D), and umburana (E) barrels for one, three, six, 12, and 24 months. Sensory assessment of cachaça obtained from different distillation methods.



Supervised classification models

The best PLS-DA model, using the spectra recorded at Δλ of 30 nm, was validated using Venetian blinds cross-validation. The number of *LV* was seven, accounting for 71 and 72% of the variance in the **X** and **Y** blocks, respectively, and the threshold value was 0.54 (Figure 4). This model presented the following performance parameters: *EFF* was 88%, *SEN* and *SPE* were 94% and 100% in the training set, and 78% and 69% in the test set, respectively.

However, the model displayed some classification errors (Figure 4a). Five *stored* cachaça samples were identified as *aged* cachaças in the training set. Similarly, *eight* aged cachaças were

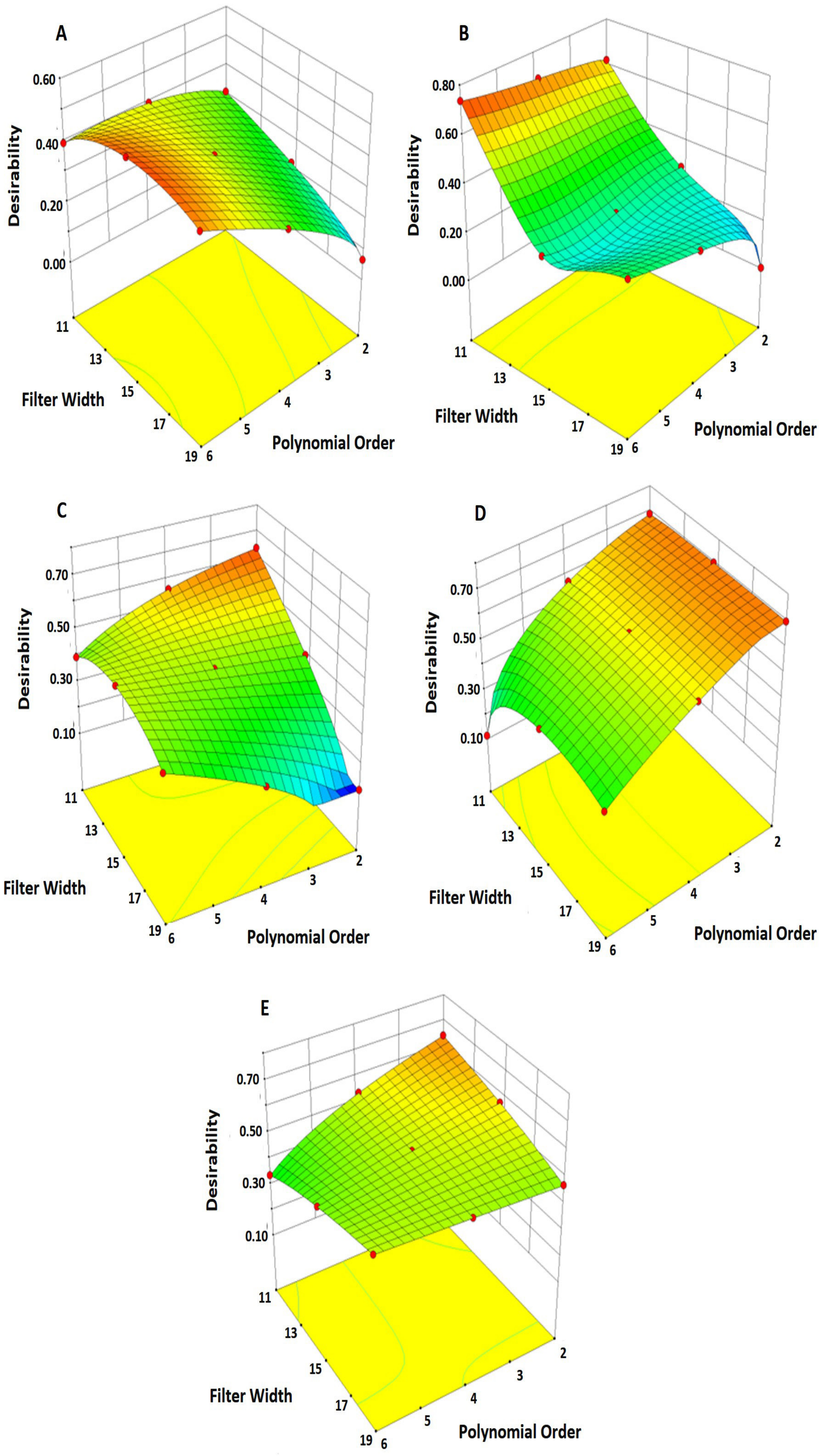


Figure 2. Desirability surface plots for minimum classification errors in the training, cross-validation, and test sets for synchronous fluorescence spectra recorded at Δλ = 10 nm (A), 30 nm (B), 50 nm (C), 70 nm (D), and 90 nm (E).

classified as stored cachaças, and *ten* stored cachaças were classified as aged cachaças in the test set. The explanation for these these classification errors may be due to the commercial samples being falsified or cachaça being blended without correct labelling or aged in different wooden barrels for different periods. Blending, which includes combining various aged batches to enhance their complexity and identity, is widely used in the production of cachaça.

The variable importance in projection (*VIP*) scores (Figure 4b) highlight the wavelengths that contributed the most to the classification of stored and aged cachaça samples. However, the assignment of these specific wavelengths is difficult as SFS is not a fingerprinting technique.

Compared to other methods for discriminating cachaça, the approach reported here is simpler, faster, and less expensive, as no sample preparation is necessary (Rodrigues et al. 2014; 2016). Although the models developed by Rodrigues and colleagues showed better qualitative performance parameters, they were constructed and validated using non-commercial samples, which limits their applicability and robustness owing to the reduced data variance (Rodrigues et al. 2014; 2016). In contrast, this work used 212 samples with a range of storage and aging times, from two months to 12 years. Additionally, a single model was developed using cachaça aged and stored in five different species of wooden barrel.

Figure 3. Classification errors in the training and test sets for each Δλ PLS-DA model.

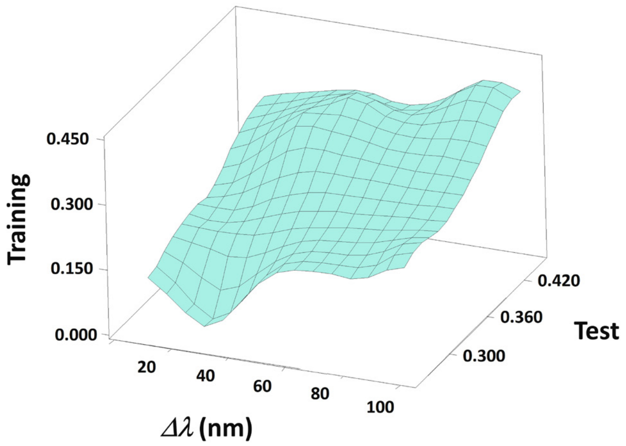
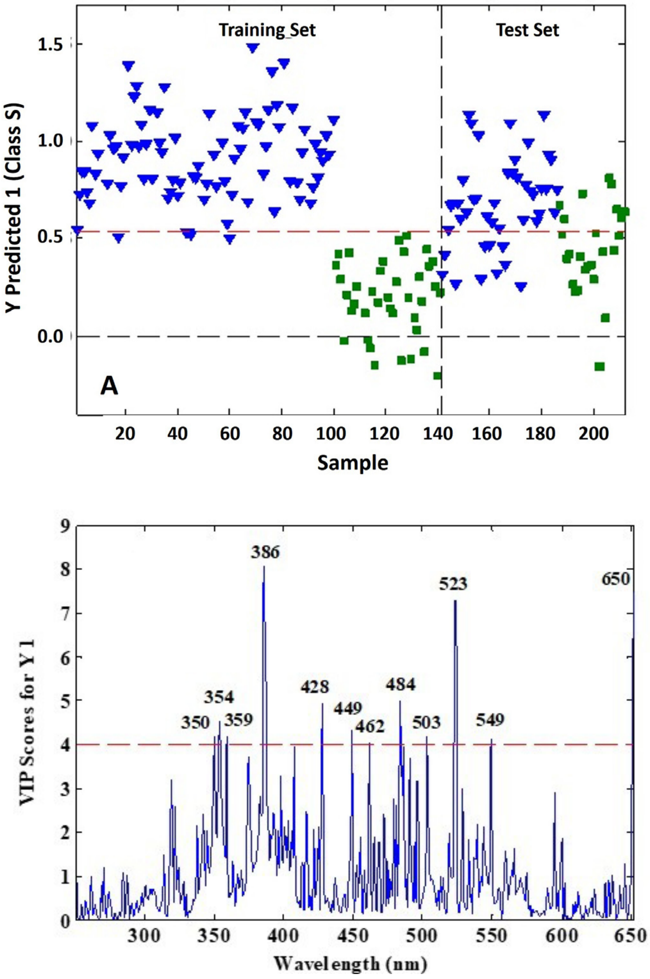
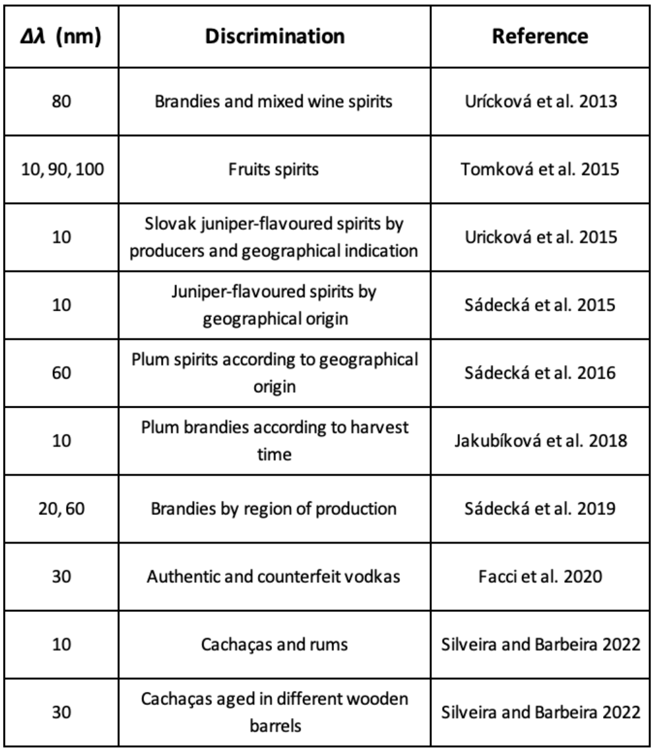


Figure 4. Synchronous fluorescence PLS-DA predictions for stored cachaça class (Δλ = 30 nm). a) Vertical dashed lines separate the training and test samples, and horizontal dashed lines indicate the threshold class, blue down triangles for stored cachaça, and green squares for aged cachaça. b) VIP scores.



Because the shape and intensity of the synchronous fluorescence spectra depends on the Δλ, their analysis according to specific purposes is notable. A review of the literature on spirit discrimination using SFS (Table 2), shows that the Δλ employed is variable based on different factors, such as the type of spirit and the objectives of the analysis.

Table 2. Methods for the discriminant analysis of spirits using synchronous fluorescence spectroscopy.



$\Delta\lambda$ (nm)	Discrimination	Reference
80	Brandies and mixed wine spirits	Urícková et al. 2013
10, 90, 100	Fruits spirits	Tomková et al. 2015
10	Slovak juniper-flavoured spirits by producers and geographical indication	Uricková et al. 2015
10	Juniper-flavoured spirits by geographical origin	Sádecká et al. 2015
60	Plum spirits according to geographical origin	Sádecká et al. 2016
10	Plum brandies according to harvest time	Jakubíková et al. 2018
20, 60	Brandies by region of production	Sádecká et al. 2019
30	Authentic and counterfeit vodkas	Facci et al. 2020
10	Cachaças and rums	Silveira and Barbeira 2022
30	Cachaças aged in different wooden barrels	Silveira and Barbeira 2022

Conclusions

Using a combination of synchronous fluorescence spectra (SFS) and Partial Least-Squares Discriminant Analysis (PLS-DA), a simple, fast, and inexpensive analytical method was developed to distinguish between commercial cachaça samples aged and stored in five different wooden barrels (made of amendoim, bálsamo, jequitibá, oak, and umburana). The building and validation process was used to increase the model robustness and variability, as well as to demonstrate its applicability to commercial samples. The Savitsky-Golay smoothing conditions (filter width and polynomial order) that resulted in the lowest classification errors were determined using the desirability function. This analysis is simple and requires no sample preparation or analytical reagents, which reduces the analysis time compared to other techniques. This supervised classification approach (PLS-DA) allowed for the separation of aged and stored cachaças from one another, as well as the identification of the key wavelengths that differentiated these classes. Accordingly, synchronous fluorescence spectroscopy. offers a promising approach for routine screening of samples of commercial cachaça.

Author contributions

Amanda Lemes Silveira: methodology, investigation, formal analysis, writing (draft, review and editing).
Paulo Jorge Sanches Barbeira: conceptualisation, investigation, writing (review and editing), supervision.

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Conflict of interest

The authors declare no conflicts of interest.

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