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Innovative non-targeted liquid chromatography fingerprinting approach for authenticating tigernuts under Protected Designation of Origin quality seal

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Abstract

BACKGROUND: Tigernut is a typical foodstuff from a specific region of Valencia (Spain) called 'L'Horta Nord', where it is commercialized under a Protected Designation of Origin (PDO) as *Chufa de Valencia* ('Valencia's tigernut'). PDO-recognized tigernuts present unique characteristics associated with their particular production region. Increasing demand and the associated expansion of its cultivation area has made necessary an exhaustive quality control to check the geographical origin and quality seal.

RESULTS: In this work, a new multivariate analytical method capable of authenticating the PDO quality seal of tigernut samples was developed. Tigernut fat fraction was extracted under optimal conditions, applying the methodology of design of experiments. The analytical method combined fingerprinting methodology and chemometric tools to observe the natural grouping of samples using the exploratory analysis method and to develop classification models (partial least squares–discriminatory analysis; PLS-DA) to discriminate between two sample categories: (i) PDO tigernuts; and (ii) NON-PDO tigernuts.

CONCLUSION: The built PLS-DA model demonstrated 100% accuracy, high sensitivity and specificity, revealing that the tigernut fat fraction can be applied to authenticate the PDO quality seal.
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Keywords: tigernuts; food fraud; chemometrics; fingerprinting; chromatography

INTRODUCTION

Tigernut (*Cyperus esculentus* L.) is a tuberous plant known by various names such as chufa, groundnut, rush nut and earth nut. It has been reported to contain 27.44% fat, 13.35% fibre and 4.27% protein. It produces a food with high energetic and nutritive properties. This tuber is consumed as a snack or in the form of a plant-based drink, being the main ingredient of the world-acclaimed Valencian 'horchata de chufa' drink.[1-3]

This foodstuff is mainly cultivated in Africa (Niger, Nigeria, Ghana and Senegal), Spain and Turkey. In Spain, around 400 ha are dedicated as a cultivation area of this product. The largest cultivation area in Spain is located in a specific region of Valencia called 'L'Horta Nord', where the tigernut is commercialized under a Protected Designation of Origin (PDO) *Chufa de Valencia* (hereafter 'Valencia' PDO). It should be emphasized that one of the biggest problems currently facing the 'Valencia' PDO is the marketing of African tigernuts with the 'Valencia' PDO seal. In 2017, the documentary 'Mousso Faso', which uncovered this fraud, made headlines.[4] Since then, more cases of African tigernut fraud have been published, including the sale of African tigernuts in Alboraia, a relevant location of the PDO Chufa de Valencia.[5]

PDO is a legal and quality recognition granted by the European Union (EU) to protect and promote traditional and regional food products with specific characteristics. The PDO quality seal guarantees that a product is produced, processed and prepared in a specific geographical region and possesses qualities or reputation that is exclusively attributed to its geographical origin.[6,7] It should

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be note that the concept of the geographical region of origin and the specific quality of the product creates a unique identity for these foodstuffs and thus adds further value. Most food products are associated with the name of a production region in a particular geographical area. Moreover, marketing a food product under a PDO provides advantages to both producers and consumers. For producers, it signifies legal protection against imitation and unfair competition, as well as the possibility to use a label that certifies the quality and authenticity of their product. For consumers, the PDO is a guarantee of quality and origin, instilling confidence in the authenticity and specific characteristics of the product they are purchasing.

In the last years, the increasing demand for tigernut and the expansion of its cultivation areas have made necessary the verification of its geographical origin, in particular those marketed under the PDO quality seal. Within this framework and to the best of our knowledge, only two studies have been reported with the goal of evaluating the differences among tigernuts produced in diverse places. On the one hand, López-Cortés *et al.*[8] applied gas chromatography coupled to flame ionization detection to determine and quantify the content of fatty acids, squalene, sterols and tocopherols of tigernuts from four distinct geographical origins (Spain, Egypt, Nigeria and South Africa). A principal component analysis (PCA) was carried out to combine the results obtained in the fatty acid profile, squalene, sterol and tocopherol composition. It evidenced a natural grouping of the samples according to their origin. Despite this, it is important to note that this study employed an analytical method based on a target approach to evaluate the tigernut's origin. This kind of approach pre-established the specific components for analysis, which could introduce a bias based on these prior assumptions. Building upon this framework, Rubert *et al.*[9] combined an advanced analytical method-based untargeted approach and chemometrics tools to determine fatty acids, tocopherols and sterols present in tigernuts. This research applied liquid chromatography coupled to high-resolution mass spectrometry, which indicated that the main differences among analysed tigernuts were found in the fat fraction. In this context it should be noted that both studies applied a univariate approach; that is, in order to find the differences between tigernuts grown in different locations it was necessary to determine and quantify different compounds or families of compounds. In other words, a targeted approach was used to assess quality in terms of geographical authenticity.

A food matrix is a complex multi-compositional material system; therefore, the verification of its authenticity (in the quality scenario) should be carried out after the application of a non-targeted approach, in which 'the whole set' of intrinsic characteristics of the food is considered, but 'what' or 'how much' of these characteristics are present is not identified.[10]

Table 1. Geographical origin of the collected tigernut samples

Origin	Number of samples
'Valencia' PDO (L'Horta Nord)	18
'Valencia' NON-PDO (from Valencia)	7
Africa	10
Total	35

PDO, protected designation of origin.

Therefore, in the food quality and authenticity scenario, the targeted approach is not the most suitable approach to be applied as it implies a waste of resources, use of reagents and solvents, and waste generation; in short, it is not an environmentally sustainable/green approach. Therefore, the application of a non-targeted approach would be the most appropriate for quality control and the targeted approach should be used in the food safety scenario, focused on the detection of chemical compounds that may cause a problem for human health.

In this sense, fingerprinting is a multivariate analytical methodology based on a non-targeted approach, which briefly consists of obtaining a non-specific but characteristic signal of the food containing the information of interest and that, through the application of data mining tools (chemometrics), allows us to extract the chemical information of interest that permits authentication it in an unequivocal way, without the need to quantify or identify any compound.[11-13]

In this contextual framework, the goal of this current study is to authenticate the geographic origin of different tigernut samples applying the tandem 'fingerprinting' and 'chemometrics' for developing a different multivariate analytical method. The study was based on two steps: (i) The fat fraction of the tigernuts was obtained using pressurized liquid extracts (PLE). For this purpose, the design of experiments methodology (DoE) was applied in order to optimize the extraction conditions. (ii) An ad hoc analysis method was developed using liquid chromatography coupled with an ultraviolet–visible detector in normal phase mode ((NP) HPLC-UV) to acquire the instrumental fingerprint of the fat fraction of each of the samples. Different chemometric tools were then applied in order to develop the multivariate analytical method with which to authenticate the quality seal of the tigernuts. In all cases the models were evaluated by obtaining the quality parameters, which were estimated following the study published by Luis Cuadros *et al.*[14]

MATERIALS AND METHODS

Chemicals

The solvents used in the sample treatment stage were HPLC grade. *n*-Hexane was supplied by Panreac AppliChem (Barcelona, Spain) and isopropanol was provided by Honeywell (Charlotte, NC, USA). The solvents used as chromatographic mobile phase were HPLC grade. Acetonitrile were supplied by Honeywell.

Samples and sample preparation

Thirty-five tigernut samples were used in this study. Fifteen of them were provided by the 'Valencia' PDO Management Body (in Spanish: 'Consejo Regulador de la DOP *Chufa de Valencia*'). The rest of the samples were obtained from local grocery markets located in Granada and Valencia (Spain). A briefly description of the geographical origin is given in Table 1.

Prior to chromatographic analysis, tigernuts were shredded using a stainless steel grinder (Taurus, Oliana, Spain), dehydrated by drying in a sterilization oven (JP Selecta, Barcelona, Spain) and fat was extracted using a pressurized liquid extraction system (ASE 100, Dionex Corporation, Sunnyvale, CA, USA), with 34 mL steel extraction cells. The extraction procedure was optimized using an experimental design with the Statgraphics Plus 5.1 software (The Plains, VA, USA) package for statistical treatment and interpretation of collected data.[15] To remove the remaining solvents after extraction a Büchi RE-124 rotatory evaporator

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equipped with vacuum pump V700 (Büchi, Flawil, Switzerland) was used.

Chromatography equipment and conditions

(NP)HPLC-UV analysis was performed using a Dionex UltiMate 3000 HPLC + Focused chromatography system (Thermo Scientific, Waltham, MA, USA) equipped with an RS autosampler and column compartment. Detection was performed with an RS variable wavelength detector recording UV spectra at 218 nm. Chromeleon version 7.0 software used to visualize and export the obtained data. A LiChrospher 100 CN instrument (250 × 4 mm, i.d., 5 µm) at 30 °C was used for HPLC analysis. The composition of the mobile phase was *n*-hexane–isopropanol (96:4, v/v) at flow rate of 1.2 mL min^{-1}. The injection volume was 10 µL and run time was 10 min.

Multivariate data analysis: chemometrics

The raw data files from each chromatogram sample were obtained as a CSV (comma-separated values) file, and then converted to MATLAB format (R2013b version, Mathworks Inc., Natick, MA, USA). An intensity data vector was obtained from each sample, which were merged into a single data matrix described by 35 samples × 1201 variables.

In order to extract useful signal information, the raw chromatograms were submitted to mathematical transformation in order to remove the variability and maximize the comprehensive interpretation of chemical information extracted. This preprocessing was carried out using a home-made MATLAB function named 'MEDINA' (version 07). This function used MATLAB Bioinformatics Toolbox software and 'icoshift' algorithm (version 1.2) to improve the quality of raw chromatographic data and to align the signals.[16] The data preprocessing consisted of the following stages: (i) grouping and overlaying of the chromatogram; (ii) selection of the interval of interest in the chromatogram; (iii) de-noising and smoothing the signal using a least-squares digital polynomial filter; (iv) baseline correction using the 'msbackadj' MATLAB function to eliminate the baseline variance from each signal. This variance could be caused by various factors such as pressure or temperature in the chromatogram detector; (v) mean-centring of the dataset.

Once the chromatographic data preprocessing was carried out, it was possible to use pattern recognition methods; principal component analysis (PCA)[17] was employed as unsupervised methods, and partial least squares–discriminant analysis (PLS-DA)[18] was employed as supervised method. The pattern recognition methods were explored using PLS_Toolbox (version 8.7, Eigenvector Research Inc., Wenatchee, WA, USA).

For multivariate method development purposes, the samples were split into two subsets: (i) training set; and (ii) external validation set, considering the two classes PDO and NON-PDO. Approximately 70% of the samples from each class were randomly chosen for the training step and the remaining ones were employed for the external validation step.

RESULTS AND DISCUSSION

Optimization of the fat extraction process

The instrumental and analytical variables set involved in the PLE method should be optimized to ensure proper extraction of the tigernut fat fraction. The instrumental variables were temperature, heating time, pressure, extraction time, flush volume, purge time and number of extraction cycles; the analytical variables were sample particle size, sample amount and extraction solvent.

Focusing on the analytical variables involved, the solvent extraction used was a mixture of *n*-hexane–isopropanol (3:3, v/v). This mixture further demonstrated this solvent to be the most effective, confirming a previous study.[15] ASE 100 extraction utilizes steel cells of 10, 34, 66 or 100 mL and a 250 mL collection bottle. For this case, the 34 mL cell was selected because it is suitable for accommodating 2.5 g tigernut containing around 27% of fat. Each sample was milled as a preliminary step to reduce its particle size, thereby facilitating the subsequent extraction process.

With regard to instrumental variables, there are two significant variables in the extraction procedure: the temperature and the purge time, as pointed out in the earlier referenced study.[15] The optimal value of each was studied using response surface methodology (RSM), based on the application of DoE. The values of the remaining variables – instrumental and analytical – were chosen according to the most favourable operation for the process. The selected time statistic was 5 min, flush volume was 40%, extraction time was 12 min, the number of extraction cycles was 1 and the heating period was 5 min.

A two-variable second-order polynomial model (six terms) was selected as the response surface describing the extraction process. In order to estimate the coefficients of the model, a rotating central composite design (2^2 factorial plus star) with five central points was used, resulting in a 13-run experimental matrix (see Table 2). The experimental domain of the two studied variables was selected around a central point corresponding to the optimum values of temperature and purge time to the extraction obtained in the above study.[15] The response variable is the measured fat content expressed in grams of fat per 100 g in the dried sample.

The effect of the variables was graphically evaluated by a response surface plot (see Fig. 1). The estimated response surface structure presents asymmetric 'tile' morphology. The maximum stationary point is located near the centre of the experimental domain (see Supporting Information, Fig. S1). In this scenario, the most curved part of the 'tile' represents the maximum response, meaning the highest percentage of extracted fat

Table 2. Experimental matrix and obtained fat content for each experimental run (coded values in brackets)

Run	Variable		Fat content (%, w/w)
	Temperature (°C)	Purge time (s)	
1	154 (−√2)	100 (0)	24.63
2	160 (−1)	150 (1)	25.18
3	160 (−1)	50 (−1)	17.46
4	175 (0)	170 (√2)	23.54
5	175 (0)	30 (−√2)	19.13
6	175 (0)	100 (0)	26.25
7	175 (0)	100 (0)	26.36
8	175 (0)	100 (0)	24.32
9	175 (0)	100 (0)	26.7
10	175 (0)	100 (0)	26.12
11	190 (+1)	150 (1)	25.96
12	190 (+1)	50 (−1)	14.04
13	196 (√2)	100 (0)	20.68

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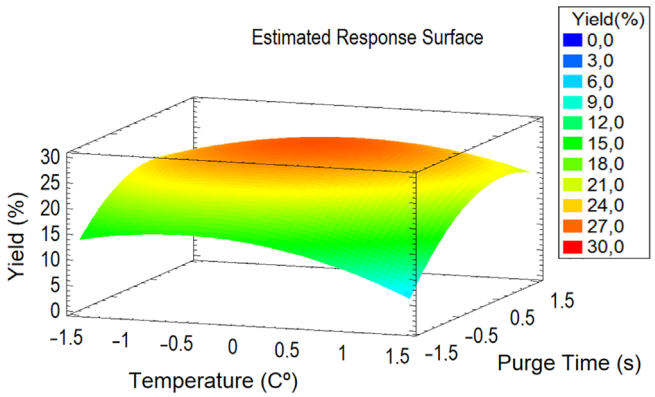

Figure 1. Response surface plot from temperature and purge time variables to obtain fat content (see Table 2 for the meaning of the coded values).

content. Accordingly, the optimum values of purge time and temperature was located at 126.8 s and 173.2 °C, respectively.

Chromatographic signals of tigernuts

As already mentioned, (NP)HPLC-UV was used to analyse the fatty acid profile of tigernut. Figure 2 illustrates the raw chromatographic signal at 218 nm of three types of tigernut samples: (i) belonging to the 'Valencia' PDO; (ii) 'Valencia' NON-PDO (grown in the 'Valencia' region but not bearing the 'Valencia' PDO quality seal); (iii) originating from an African country. These chromatograms are evidence of a possible different shape related to the PDO category. Raw chromatogram data were submitted to data preprocessing in order to extract the relevant chemical information. The resulting data were used to build the different multivariate models.

Unsupervised analysis

PCA was carried out to explore the natural grouping of the different tigernut samples (see Supporting Information, Fig. S2). Using three principal components (PCs), 93.11% of the total variance was explained. The PC2 (14.02%) *versus* PC3 (5.61%) scores plot (see Fig. 3) revealed a natural grouping of 100% of the 'Valencia' PDO samples. This means that the tigernut belonging to PDO could be a differentiating feature between the studied samples.

Note that there are four tigernut samples of Valencia origin that did not bear the 'Valencia' PDO quality seal ('Valencia' NON-PDO) and were grouped with the 'Valencia' PDO samples. These four samples originate from the Alboraya region (a municipality located in L'Horta Nord). However, they lacked the PDO quality seal, as probably they could not meet certain criteria stipulated by the regulatory authority. This grouping may therefore be due to the proximity of the cultivars. Although it is important to consider that the quality seal is not solely determined by the region where the food is grown, in addition to meeting this requirement it must also meet the rest of the specifications of the designation of origin.

Supervised analysis

Since PCA is an unsupervised technique, it is not powerful enough to detect differences between 'Valencia' PDO and 'Valencia' NON-PDO. However, this limitation is addressed by using classification techniques (supervised techniques) such as PLS-DA, which was applied in this study. PLS-DA allows a more comprehensive classification of the samples, enabling the identification of existing differences between samples from 'Valencia' PDO and 'Valencia' NON-PDO.

Analytical multivariate model building

PLS-DA was carried out to establish suitable classification models to the discrimination between two classes: PDO (target class 1) and NON-PDO (alternative class 1). Note that NON-PDO class contained both the African tigernut samples and those produced in the geographical region of Valencia that were outside of the PDO ('Valencia' NON-PDO).

A total of 23 samples having the two classes were selected to build the training set. PLS-DA method was developed by selecting nine latent variables (LVs), which explained 97.98% and 77.03% of the

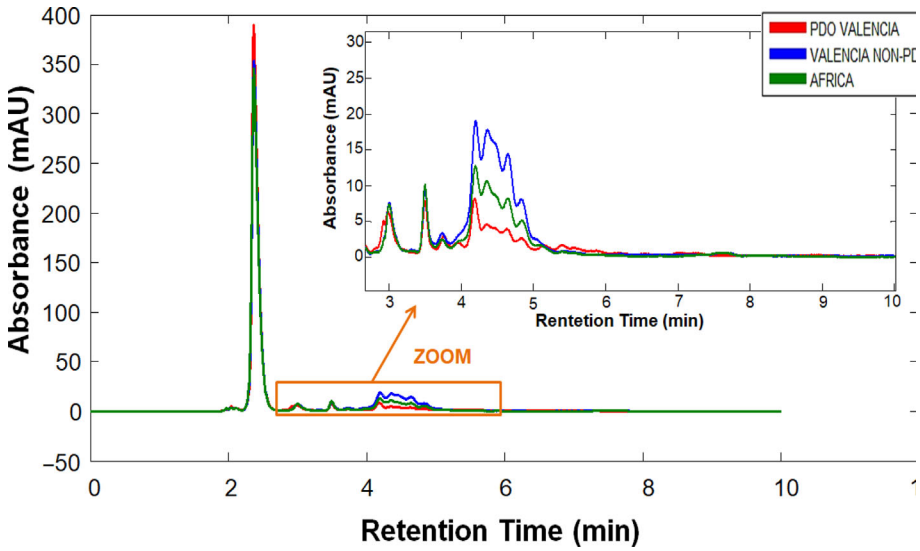

Figure 2. Superposed (NP)HPLC-UV chromatogram fingerprints recorded at 218 nm of three types of tigernut samples originating from: (i) 'Valencia' protected designation of origin (PDO); (ii) 'Valencia' NON-PDO; (iii) Africa.

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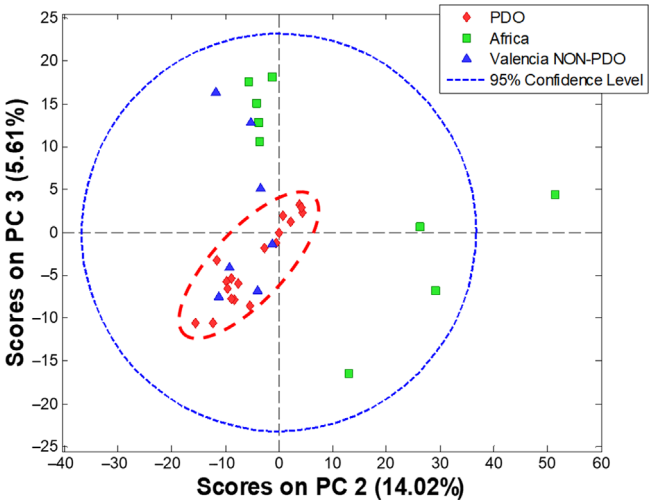

Figure 3. Scores plot of PC2 *versus* PC3 obtained using the chromatographic fingerprints of all samples (protected designation of origin (PDO), 'Valencia' NON-PDO and Africa). The red ellipse encloses the PDO tigernut samples.

accumulative variances over the *x*-variable and the *y*-variable blocks, respectively. Accordingly, the classification model was established and the remaining samples were used for the external validation model. Figure 4 shows the results of the classification model. 100% of PDO and NON-PDO samples were classified correctly; that is, no sample belonging to the PDO was considered as a NON-PDO sample and vice versa. Table 3 presents the quality performance metrics of the classification method calculated from the contingency result. The PLS-DA model obtained a value of 1.00 for all quality performance metrics, proving its suitability correct right classification of all the samples (see Supporting Information, Fig. S3).

Marker variables for of the PDO tigernut samples

In order to locate the portion of the chromatographic fingerprint containing characteristic information relating to PDO samples, the LV1–LV2 PLS-DA scores plot was evaluated, which gathered 87.75% on the overall variance (see Fig. 5). This clearly displays the sample groupings according to their class.

Figure 5 reveals that the most of the NON-PDO tigernut samples (Africa and 'Valencia' NON-PDO) have positive scores on both LV1

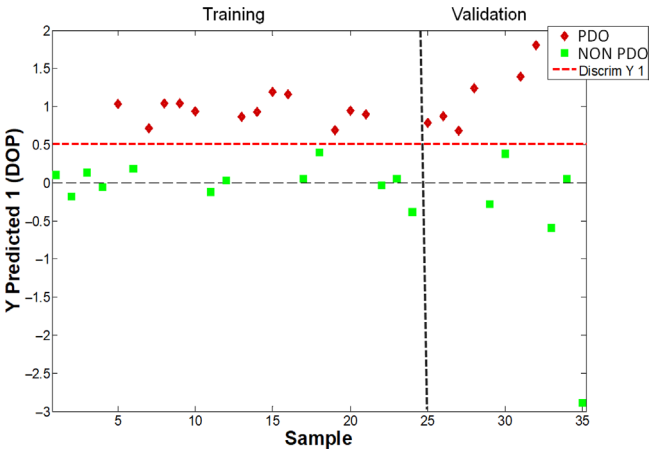

Figure 4. Classification of tigernut samples by the partial least squares–discriminant analysis model, developed with two classes: protected designation of origin (PDO) and non-protected designation of origin (NON-PDO).

Table 3. Summary of classification performance metrics from the PLS-DA model developed in this study

Classification performance metrics	Class 1 (PDO)	Class 2 (NON-PDO)
Sensitivity (SENS)	1.00	1.00
Specificity (SPEC)	1.00	1.00
False positive rate (FPR)	0.00	0.00
False negative rate (FNR)	0.00	0.00
Positive predictive value (precision) (PPV)	1.00	1.00
Negative predictive value (NPV)	1.00	1.00
Youden index (YOUD)	1.00	1.00
Positive likelihood rate [LR (+)]	—	—
Negative likelihood rate [LR (−)]	0.00	0.00
Classification odds ratio (COR)	—	—
F-measure (F)	1.00	1.00
Discriminant power (DP)	—	—
Efficiency (or accuracy) (EFFIC)	1.00	1.00
Misclassification rate (MR)	0.00	0.00
AUC (correctly classified rate)	1.00	1.00
Gini coefficient (Gini)	1.00	1.00
G-mean (GM)	1.00	1.00
Matthew's correlation coefficient (MCC)	1.00	1.00
Chance agreement rate (CAR)	0.50	0.50
Chance error rate (CER)	0.50	0.50
Kappa coefficient (KAPPA)	1.00	1.00

A dash (—) signifies that the performance feature cannot be determined, since it involves a division between zero.

and LV2 axes. In addition, the PDO samples are in turn split into two subgroups (circled in orange and green in Fig. 5); each subgroup is related to the date of harvest. This shows that it is possible not only to differentiate between PDO and NON-PDO samples,

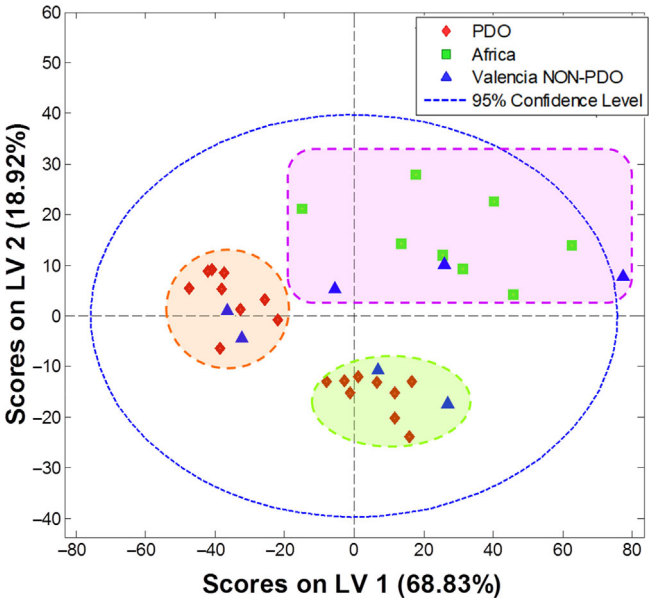

Figure 5. Scores plot obtained from the partial least squares–discriminant analysis (PLS-DA) model of protected designation of origin (PDO)/non-protected designation of origin (NON-PDO) classification. Purple shading: NON-PDO samples; orange and green shading: PDO samples.

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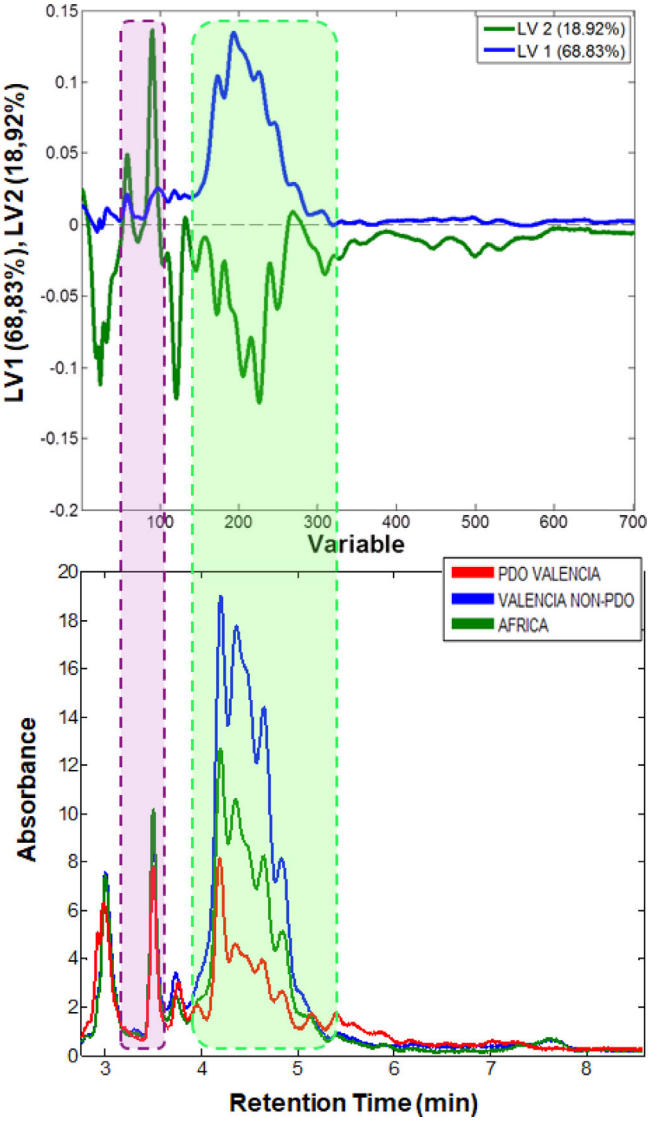

Figure 6. Specified discriminant windows concerning the classification between protected designation of origin (PDO) samples harvested in 2022 and non-protected designation of origin (NON-PDO) samples, located over the loadings plot (top), and the chromatogram (bottom). Purple shading encloses the NON-PDO samples and green shading the PDO samples harvested in 2022 (see text for a more detailed explanation).

but also between PDO samples from different harvests in 2021 and 2022. In a similar way, most of the tigernut samples harvested in 2022 have positive scores on the LV1 axis and negative scores on the LV2 one.

Accordingly, LV1 and LV2 positive loadings (purple shading at top of Fig. 6) correspond to the interval between the variables 50 and 100, which in turn match the retention times between 3.15 and 3.40 min (purple shading at bottom of Fig. 6). Thus, this first chromatogram window could be considered as the discriminant region between the two classes and specifically characteristic of the NON-PDO samples. Similarly, the zone having LV1 positive and LV2 negative scores is located between variables 140 and 320 (green shading at top of Fig. 6), which cover the retention times between 3.50 and 5.20 min (green shading at bottom of Fig. 6). Therefore, this second window could be regarded as containing the discriminating information between PDO samples harvested in 2021 and 2022. Note that, although discrimination by harvesting is not the subject of the study presented here, it could be pursued as a future perspective. In fact, this study could be of interest to detect possible fraud in the marketing of tigernuts, which could be sold indicating a false harvesting date. This could have an impact on the sensory and nutritional qualities of other food products derived from tigernuts, for example, tigernut milk (in Spanish, 'horchata').

CONCLUSIONS

The current research uncovers the optimal experimental conditions for extracting the fat fraction of tigernuts using the PLE technique. Moreover, it has been demonstrated that this fat fraction contains the relevant information for discriminating PDO and NON-PDO tigernuts. For this, in this study a multivariate analytical method has been developed 'ad hoc', utilizing the entire fat chromatographic fingerprint of the tigernuts jointly with chemometric tools for discriminating tigernuts according with their quality seal. It is important to stress that fingerprinting methodology presents an enormous potential in quality and authenticity assessment, as it has been demonstrated to be sufficiently robust, sensitive and discriminatory in verifying the quality seal of tigernuts. In addition, this strategy is less time consuming and is therefore more aligned with EU principles regarding the development of more environmentally sustainable analysis methodologies.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Follak S, Belz R, Bhoren C, De Castro O, Del Guarcchio E, Pascual Seva N *et al.*, Biological flora of Central Europe: *Cyperus esculentus* L. *PPEES* **23**:33–51 (2016).
2 Duman E, Some physico-chemical properties, fatty acid compositions, macro-micro minerals and sterol contents of two variety tigernut tubers and oils harvested from East Mediterranean region. *Food Sci Technol* **39**:610–615 (2019). https://doi.org/10.1590/fst.28018.
3 Adejuyitan JA, Tigernut processing: its food uses and health benefits. *Am J Food Technol* **6**:197–201 (2011). https://doi.org/10.3923/ajft.2011.197.201.
4 Available: https://www.eldiario.es/comunitat-valenciana/mousso-faso-documental-espanola-agricultoras_1_3047161.html [15 June 2023].
5 Available: https://www.europapress.es/comunitat-valenciana/noticia-denuncian-venta-chufa-africana-alboraia-pleno-corazon-horchata-20190404122646.html [15 June 2023].

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6 ORDER 17/2010 of Valencian Community of 18 May 2010 on Protected Designation of Origin Tigernut of Valencia and its regulatory council. 5869.
7 Regulation (EU) No 1151/2012 of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs.
8 López-Cortés I, Salazar García DC, Malheiro R, Guardiola V and Pereira JA, Chemometrics as a tool to discriminate geographical origin of *Cyperus esculentus* L. based on chemical composition. *Ind Crops Prod* **51**:19–25 (2013). https://doi.org/10.1016/j.indcrop.2013.08.061.
9 Rubert J, Hurkova K, Stranska M and Hajslova J, Untargeted metabolomics reveals links between Tiger nut (*Cyperus esculentus* L.) and its geographical origin by metabolome changes associated with membrane lipids. *Food Addit Contam Part A* **35**:605–613 (2017). https://doi.org/10.1080/19440049.2017.1400694.
10 de Araújo GA, Azcarate SM, Špánik I, Khvalbota L and Goicoechea HC, Pattern recognition techniques in food quality and authenticity: a guide on how to process multivariate data in food analysis. *TrAC Trends Anal Chem* **164**:117105 (2023). https://doi.org/10.1016/j.trac.2023.117105.
11 Cuadros Rodríguez L, Samblás Ruiz C, Valverde Som L, Pérez Castaño E and González Casado A, Chromatographic fingerprinting: an innovative approach for food 'identitation' and food authentication-a tutorial. *Anal Chim Acta* **909**:9–23 (2016). https://doi.org/10.1016/j.aca.2015.12.042.
12 Bosque Sendra M, Cuadros Rodríguez L, Ruiz Samblás C and De la Mata AP, Combining chromatography and chemometrics for the characterization and authentication of fats and oils from triacylglycerol compositional data–a review. *Anal Chem* **724**:1–11 (2012). https://doi.org/10.1016/j.aca.2012.02.041.
13 Jiménez Carvelo AM, González Casado A, Bagur González MA and Cuadros Rodríguez L, Alternative data mining/machine learning methods for the analytical evaluation of food quality and authenticity–a review. *Food Res Int* **122**:25–39 (2019). https://doi.org/10.1016/j.foodres.2019.03.063.
14 Cuadros Rodríguez L, Pérez Castaño E and Ruiz Samblás C, Quality performance metrics in multivariate classification methods for qualitative analysis. *Trends Analyt Chem* **80**:612–624 (2016). https://doi.org/10.1016/j.trac.2016.04.021.
15 Ruiz Samblás C, Cuadros Rodríguez L and González-Casado A, Pressurised liquid extraction and quantification of fat-oil in bread and derivative products. *Talanta* **83**:25–30 (2010). https://doi.org/10.1016/j.talanta.2010.08.026.
16 Jimenez Carvelo AM, Valverde Som L, Gonzalez Casado A and Cuadros Rodríguez L, Comparison of different analytical classification scenarios: application for the geographical origin of edible palm oil by sterolic (NP) HPLC fingerprinting. *Anal Methods* **7**:4192–4201 (2015). https://doi.org/10.1039/C5AY00168D.
17 Gredilla A, Ortiz F, de Vallejuelo S, de Diego A and Madariaga JM, Unsupervised pattern-recognition techniques to investigate metal pollution in estuaries. *Trends Anal Chem* **46**:59–69 (2013). https://doi.org/10.1016/j.trac.2013.01.014.
18 Lee LC, Liong CY and Jemain AA, Partial least squares-discriminant analysis (PLS-DA) for classification of high-dimensional (HD) data: a review of contemporary practice strategies and knowledge gaps. *Analyst* **143**:3526–3539 (2018). https://doi.org/10.1039/C8AN00599K.

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