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Authentication of geographical origin of palm oil by chromatographic fingerprinting of triacylglycerols and partial least square-discriminant analysis

Cristina Ruiz-Samblás [a,*], Cristina Arrebola-Pascual [a], Alba Tres [b,1], Saskia van Ruth [b], Luis Cuadros-Rodríguez [a]

[a] Department of Analytical Chemistry, University of Granada, c/Fuentenueva s.n., E-18071 Granada, Spain
[b] RIKILT Wageningen University and Research Centre, Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands

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ABSTRACT

Main goals of the present work were to develop authentication models based on liquid and gas chromatographic fingerprinting of triacylglycerols (TAGs) from palm oil of different geographical origins in order to compare them. For this purpose, a set of palm oil samples were collected from different continents: South eastern Asia, Africa and South America. For the analysis of the information in these fingerprint profiles, a pattern recognition technique such as partial least square discriminant analysis (PLS-DA) was applied to discriminate the geographical origin of these oils, at continent level. The liquid chromatography, coupled to a charged aerosol detector, (HPLC–CAD) TAGs separation was optimized in terms of mobile phase composition and by means of a solid silica core column. The gas chromatographic method with a mass spectrometer was applied under high temperature (HTGC–MS) in order to analyze the intact TAGs. Satisfactory chromatographic resolution within a short total analysis time was achieved with both chromatographic approaches and without any prior sample treatment. The rates of successful in prediction of the geographical origin of the 85 samples varied between 70% and 100%.

1. Introduction

Palm oil is the second largest edible oil produced in the world. One of its species (*E. guineensis*) originates from West Africa. It was first introduced to Brazil and other tropical countries in the 15 century by the Portuguese. However, its propagation did not take off until the 19 century when the Dutch brought seeds from West Africa to Indonesia. *E. oleifera* originates from South America and its oil is characterized by a high oleic acid content [1]. The origin of the oil will give an indication of its identity and quality characteristics. Nowadays, the major centre of production of palm oil is in Southeast Asia with Malaysia and Indonesia being together the leading producers and exporters [2]. This reason is attributed to many factors, which include favorable climatic conditions, skills and technology for palm oil cultivation. It can be expected that in the future palm oil will continue to outpace the growth of other oils, since in most of the tropical countries around the equator there are large expansion programs underway [3]. The production of sustainable palm oil has emerged as an answer to concerns about environmental impact in the areas of production. Several certification systems have been implemented in order to assure sustainable production of palm oil. Intrinsic markers of palm oil may provide information on palm oil provenance, and their analytical verification may complement the certification process (audits, inspections and paper trailing).

In the context of the authenticity of edible oils and fats, some authors have defined three main areas to be differentiated according to economic adulteration, (i.e. blending of cheaper oils with commodities of higher economic value), minimally processing of oils (crude, non-refined and cold-pressed) and characterization and verification of geographical origin [4]. The quality and chemical composition of a palm oil depend on various factors, including varieties, pedoclimatic conditions, processing technology and storage, etc. For this purpose, as well as to identify different palm oil fruit varieties, molecular markers have been also studied by several authors [5,6] and DNA fingerprinting [7]. The classification according to area of production of vegetable oils exploits the information content of minor constituents as well as subtle differences in the concentration of major components which are influenced by climate, soil, and the predominant variety used in a specific geographic region. The interested reader is referred to a recently published chapter on this topic where the main analytical techniques used for authentication of palm oils are reviewed [8].

* Corresponding author. Tel.: +34 958240797.
E-mail address: crsamblas@ugr.es (C. Ruiz-Samblás).
[1] Present address: Nutrition and Food Science Department, Faculty of Pharmacy, University of Barcelona, Avinguda Joan XXIII s/n, 08028 Barcelona, Spain.

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In literature fatty acids (FA) have received the most attention for vegetable oil authentication purposes, due to the fact that they are the most important group of compounds. The triacylglycerols (TAGs) are in chemical terms glycerol molecules, each esterified with three FAs. Most of the FAs in oils are present as TAGs, being tripalmitin the major TAG in palm oil. [9,10]. There are also other minor components of palm oil such as phosphatides, sterols, pigments, tocopherols and trace metals [1,11]. The advantage of using TAG for authentication purposes, as compared to FA profiling, is that the genetically controlled stereo-specific distribution of the FA moieties on the glycerol backbone is preserved and, thus, the information content of intact TAGs is usually higher. However the analysis of TAGs in an oil is a challenging task since the enormous number of TAGs which are possible due to the large number of FA combinations on the glycerol backbone [12].

Chromatographic techniques, such as gas chromatography (GC) and high performance liquid chromatography (HPLC) are the most frequently used for the analysis of TAGs in vegetable oils. The official method for TAG analysis in vegetable oils by HPLC (reversed-phase polarity) established by the International Olive Council [13] uses a differential refractometer as detector. However the proposed by the American Oil Chemists' Society, AOCS Official Method Ce 5b-89 [14] gives three detection options: (a) differential refractometry, (b) UV absorption, and (c) mass spectrometry. This method is applicable to all vegetable oils containing triglycerides of long-chain fatty acids, but especially to the detection of the presence of small quantities of semidrying oils (rich in linoleic acid) in vegetable oils containing oleic acid as the predominant unsaturated fatty acid, such as olive oil, palm oil, and peanut oil.

Another detector recently used for TAG analysis is the Corona® Charged Aerosol (CAD) [15,16]. A review about its principles and applications has been published in 2010 [17]. This detector was developed in 2004 to extend the universal response from isocratic to gradient elution conditions in HPLC [18]. CAD is capable of detecting analytes with reduced response, and has been used in combination with a variety of different separation modes including isocratic and gradient reversed-phase. There are few papers in which CAD was applied to the TAG about vegetables oils using CAD [19,20], in all these studies excellent results were obtained. In addition, an HPLC method for the analysis of palm oil using a binary pump system with CAD for detection has been found. This method requires simple sample preparation and no derivatization [21]. Comparisons were made to the AOAC official method 993.24 which uses a differential refractometry detector. CAD has offered a solution to some of the limitations that presents the refractive index detector.

In addition, GC is also extensively used for the analysis of TAG. The most common approach to the indirect knowledge of TAG was to release FA composition and perform GC after methylation. The progress in separation efficiency of TAG has been achieved by using capillary columns coated with more polar polysiloxane phases containing a higher proportion of phenyl groups (50–65%) and low blending at high-temperature (as far as 370–400 °C). These thermo-resistant polar columns have been a powerful tool [22]. TAG separation using high temperature gas chromatography (HTGC) offers interesting possibilities (efficiency, stability, etc.). IUPAC-AOAC did adopt a method to resolve TAGs, based on the numbers of similar carbon numbers (CN) by HTGC, of solutions of oil and fat, under temperature-programmed conditions [23]. In bibliography, there are some studies related with the analysis of TAGs in palm oil [24–26], but none of them with the aim of authentication of geographical origin.

The separation of TAG by HPLC and GC has different mechanisms, and the elution order of the diverse TAG is not the same. In HPLC, TAGs are separated according to their equivalent carbon number (ECN), defined as defined as ECN=ACN–2n, where ACN is the number of carbons of the acyl chains (or fatty chains), and n is the total number of the double bonds present in the three FA. On the contrary, the GC separation just depends of the acyl carbon number (ACN). Both techniques provide non-redundant complementary information because the peak corresponding to a given TAG might be well resolved in a type of chromatography and not in the other one.

In addition, the combination of chromatography and chemometric tools has been applied for authentication purposes of fats and oils [27]. In chromatography, chemometric tools are usually applied to extract the useful information derived from the signal profile. Fingerprinting techniques allow getting analytical information of the oils in a non-selective way, such as by collecting a nonspecific instrumental record (spectrum or chromatogram), which together with an appropriate multivariate data analysis permits the characterization of oils. The use of these instrumental fingerprinting techniques, chromatography and chemometrics, to study the geographical origin of palm oil has been very limited so far [28,29]. In a previous work, the use of fatty acid profile and volatile compounds, by gas chromatography and proton transfer reaction mass spectrometry respectively, has been studied by the authors with successful results [30].

In this paper a new approach to assess the effectiveness of the TAGs chromatographic fingerprinting for the characterization of palm oil from various geographical origins is evaluated. For this, the raw chromatograms of two chromatographic techniques such as HPLC–CAD and HTGC–MS are treated by chemometrics in order to establish models to predict the provenance of the palm oil samples and compare both results.

2. Materials and methods

2.1. Chemical and reagents

The following reagents were used: acetonitrile (HPLC grade) and chloroform (99%, reagent grade) were purchased from PANREAC (Barcelona, Spain), and n-hexane and 2-propanol (HPLC grade) were obtained from PROLABO (Barcelona, Spain). The nitrogen (99.9%) and helium (99.9%) were obtained from Air Liquid (Madrid, Spain). Solvents and samples, for HPLC analysis, were filtrated prior to injection using a 0.22 μm polytetrafluoroethylene (PTFE) membrane purchased from CAMEO (General Electric, Belgium).

2.2. Palm oil samples

A total of 85 crude palm oil samples, were collected from palm oil plantations, mills and traders during 2010 and 2011, from the three main producing continents 49 samples originated from Southeast Asia (Malaysia, Indonesia, Papua New Guinea and Salomon Islands), 25 from Africa (West Africa—Ghana, Guinea, Cote d'Ivoire, Nigeria and Cameroon), and 11 from South America (Brazil). The production of palm oil in South America during 2010 and 2011 was rather low, especially when compared with the production in South East Asia and Africa. Several plantations were growing, but production had not started or had just been started. For this reason, it was quite difficult to obtain authentic samples from different producers in South America. These samples were used previously for fatty acid and volatile organic compound fingerprinting with the same aim [30]. The samples were stored at −2 °C until its analysis. Before analysis and dilution, samples were melted at 45 °C.

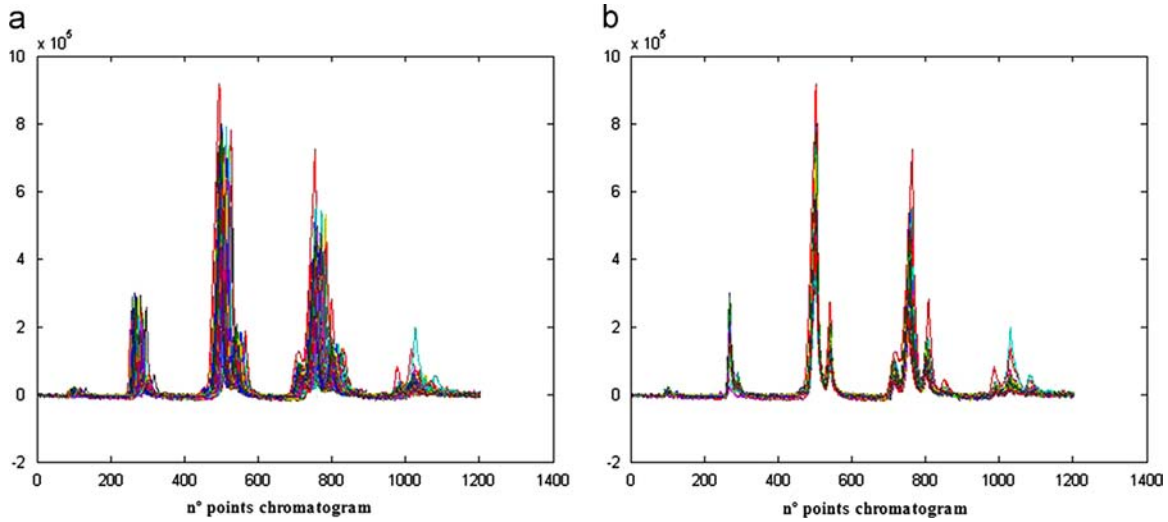

Fig. 1. HTGC–MS chromatograms of the palm oil samples: (a) before peak shifting correction, and (b) after.

2.3. Analytical determinations

Sample preparation was not needed prior to chromatographic analysis. The diluted palm oil samples were directly injected into the chromatographic system, without any other preliminary step.

2.3.1. TAG fingerprinting by HPLC–CAD

An HP Agilent HPLC 1100 Series provided with quaternary pump, degasser, automated sampler, thermostatic column compartment (Eppendorf® TC-50, Sigma-Aldrich, S.A. MA, Spain) and ChemStation software from Agilent Technologies (Santa Clara, CA, USA) were used. Detection was carried out with a Corona CAD (ESA Bioscenses Inc., Chemlsford, MA, USA). Analyses were carried out using a Poroshell 120 EC-C18 column (4.6 × 150 mm^2, 2.7 μm) purchased from Agilent Technologies (Waldbronn, Germany). The column temperature was kept at 25 °C. The injection volume used was 4 μL. The mobile phase was a binary mixture of acetonitrile and 2-propanol, 50:50 (v/v), for isocratic elution; the flow rate was 1.5 mL min^{-1}, for 12 min with a post time of 1 min. The CAD nitrogen gas pressure was adjusted to 35 psi. For CAD monitoring a 100 pA output range was used. For HPLC analysis, samples were diluted by *n*-hexane to obtain working solutions with a final concentration of 0.025% (w/w).

2.3.2. TAG fingerprinting by HTGC–MS

For the GC separations a VARIAN GC 3800 gas chromatograph (PA, USA) coupled to a VARIAN 4000 ion trap mass spectrometer (PA, USA) equipped with an electron impact source was used. A split injection with a ratio of 1:50 was used. The sample volume injected was 2 μL. The samples were introduced using an autosampler module (Combipal, CTC ANALYTICS, Switzerland). The injection port was held isothermally at 370 °C. A capillary column coated with 65% diphenyl-35% dimethylpolysiloxane stationary phase (Restek Rtx-65TG; 30 m × 0.32 mm i.d. × 0.1 μm; maximum temperature 370 °C; Restek Corp., Bellefonte, PA, USA) was used. The GC oven temperature was programmed from 315 to 350 °C at 1 °C/min. Helium (99.9%) was used as the carrier gas and its flow rate was 1.5 mL/min. The mass spectrometric conditions were as follows: ion source temperature was held at 250 °C during the GC/MS runs; the transfer-line temperature was kept at 350 °C throughout the analyses; electron energy was 70 eV and the emission current 10 μA. Chromatograms were recorded in full-scan mode. Average spectra were acquired in the *m*/*z* range of 200–1000 *m*/*z* and were recorded at a scan speed of 1.20 s. Scan control, data acquisition, and processing were performed by a MS Workstation software (VARIAN, PA, USA) [31]. For GC analysis, palm oil was dissolved in chloroform to a final concentration of 0.200% (w/w).

2.4. Chemometrics

The liquid and gas chromatographic data were exported from each respectively software to MATLAB® 7.8.0 R2009a (The Maths Inc., Natick, MA, USA) and PLS_Toolbox 7.0 (Eigenvector Research Inc., West Eaglerock Drive, Wenatchee, WA) for the data analysis.

The gas and liquid chromatographic data were arranged in two separate matrices to perform the statistical analysis for the geographical authentication. The matrix of the HPLC data was composed of 85 rows corresponding to the samples of palm oil and 834 columns corresponding with each one of the point (retention time) of the chromatogram recorded during the acquisition time. The first 4 min of the chromatograms were eliminated due to the fact that there were peaks of diacylglycerols and/or monoacylglycerols. In the case of the GC data, the matrix was composed of 85 rows as in the previous case and 1201 columns of information.

Pre-processing was applied on the data sets previously to build the models, in order to get the important information. Baseline correction, (weighted least squares) to reduce drift in the baseline, based on a second-order polynomial basis [32], was applied. Secondly, after baseline correction, peak shifting was corrected with interval correlation optimized shifting, icoshift [33], which splits the chromatogram into intervals and "coshifts" each vector to get the maximum correlation toward a target signal in that interval. Fig. 1 shows the overlapped chromatograms coming from the gas chromatographer prior and after icoshift treatment. Finally, the chromatograms were normalized and mean centered by subtracting from each signal the mean chromatogram, in order to remove the variability related to this overall offset term. The pretreatment of the data, when raw chromatogram fingerprint are used, previously to build the chemometric models is an action essential, since otherwise the results could not be representative of the data.

Subsequently the data were subjected to a supervised pattern recognition method, partial least square-discriminant analysis (PLS-DA), to estimate classification models for the origin of the palm oil samples, at a continental scale. In these methods, samples belonging to a particular class or group are known and the aim is to establish implicit or explicit classification models [28]. PLS-DA

performs a variable reduction on the data set by calculating new variables (called latent components or factors) combining the variables in the data set in order to find the maximum correlation between them and the class variable and, thus, the maximum separation among the classes. It is common in this type of studies to try previously some exploratory data analysis techniques, mainly principal component analysis (PCA), and/or some non-supervised pattern recognition method as cluster analysis. The main aim of this strategy is to assure of the sample data are appropriate to apply a subsequent classification or discrimination method. However, we consider that these trials are not necessary in this case, because we already know the samples by previous work [30].

In order to carry out a proper validation of the PLS-DA model, the initial dataset was randomly split in two sets; the training set (70% of the total samples from each class) and the external test set (30% of the remaining samples from each class). Cross validation was introduced as random subsets approach, with eight splits on the set of training samples to choose the best conditions for the model (e.g., the number of latent variables, explained variance, outliers, etc.). This cross validation procedure involves the removal of a subset of samples (one split) randomly from the set, construction of a model using the remaining samples in the dataset, and subsequent application of the resulting model to the removed samples. The removed samples are placed back into the data set, and another subset of samples (another split) is selected to be removed. The process is repeated until all samples have been removed once. The optimized model conditions were then tested on the external test set, to predict membership in samples which were not included in the training set and were not used to build the model, for a final validation [34,35].

3. Results and discussions

3.1. Optimization of the mobile phase on separation of TAGs by HPLC–CAD

Firstly, in order to obtain a fast TAG separation with sufficient resolution, for data treatment and chemometric model building, within a short time analysis, in liquid chromatographic analysis, a Poroshell 120 EC-C18 column was used. These novel columns are able of keeping high efficiencies at increasing flow rates with a consequent reduction of analysis time [36]. Randomly a South eastern Asia sample was chosen to select the adequate TAG separation in terms of mobile phase composition. Four different binary mixtures of acetonitrile:2-propanol (v:v) in isocratic elution mode were evaluated. In all cases, column temperature was set at 25 °C and flow rate was maintained at 1.5 mL min^{-1}.

As shown in Fig. 2, the 50:50 ratio offered the best chromatographic conditions; so by decreasing mobile phase polarity and increasing 2-propanol proportion, the analysis time was considerably reduced. The remaining parameters, flow and temperature, were constants. Sufficient chromatographic resolution for later chemometric analysis was achieved, within a short total run time.

3.2. Classification of geographical origin of palm oils by HPLC and PLS-DA

The models performances were evaluated by using the root mean-squared error of calibration (RMSEC) and the cross validation process, by the root-mean-squared error of cross-validation (RMSECV). The final predictions of the external test set were quantified by the root-mean-squared error of prediction (RMSEP). Also, some specific figures of merits of a classification method, as sensitivity and specificity parameters, were assessed for modelling and prediction. Sensitivity is the percentage of samples from the modelled classes that are accepted by the class model, while specificity is the percentage of samples from other classes which are rejected by the class model [37]. The results of the PLS-DA modelling on the liquid chromatographic data, with four latent components which had more than 84% of the total variance, are shown in Tables 1 and 2. These values can be obtained with the training step, cross validation and validation with external samples processes; however the reported values are referred to the cross validation of the training model (Table 1) and final validation with the external set of samples (Table 2).

Table 1 shows the results, when modelling the HPLC data and it is cross validated with random subsets and eight splits. If they are looked deeply, for samples coming from Africa, for instance, there is one sample that is rejected for this class in the model. In addition, for samples coming from South America, there are samples coming from the first class (Africa) which have been used as South American ones by the model, resulting in a model with less sensitivity than the other two (86%) Regarding to the specificity of this model, there is one sample which is not recognized as South American one when modelling the training samples. Finally, for the South eastern Asia samples, and due to the number of samples is bigger than in the other cases, the cross validated model

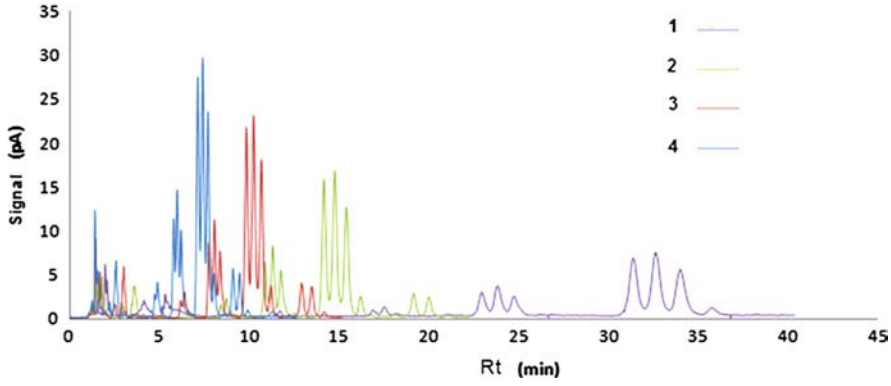

Fig. 2. Overlapped HPLC chromatograms of the mobile phase (binary mixtures of acetonitrile/2-propanol) optimization process. Chromatogram 1 (purple): 70:30 (v:v); chromatogram 2 (green): 60:40 (v:v); chromatogram 3 (red): 55:45 (v:v); and chromatogram 4 (blue): 50:50 (v:v). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Specificity and sensitivity in the PLS-DA model of the liquid chromatographic data. (Random subset cross validated).

	Africa (%)	South America (%)	South Eastern Asia (%)
Sensitivity	94	86	100
Specificity	96	91	96

Table 2
Specificity and sensitivity in validation with external test set of the PLS-DA model in the liquid chromatographic data.

	Africa (%)	South America (%)	South Eastern Asia (%)
Sensitivity	83	100	100
Specificity	93	90	100

Table 3
Specificity and sensitivity in the PLS-DA model in the gas chromatographic data (Random subset cross validated.).

	Africa (%)	South America (%)	South Eastern Asia (%)
Sensitivity	100	100	97
Specificity	100	100	96

Table 4
Specificity and sensitivity in validation with external test set of the PLS-DA model in the gas chromatographic data.

	Africa (%)	South America (%)	South Eastern Asia (%)
Sensitivity	71	100	100
Specificity	100	100	75

shows high sensitivity and specificity, with only one sample rejected by the model when it is developed.

Regarding to the prediction part with the external set, the results were very positive, as it is shown in Table 2, for all the classes. When external validation, no outliers were observed for all class models and only one sample was rejected by each of the specific category, resulting in a high sensitivity for all the models as well as specificity in prediction. Same situation than before, for the class of South America, samples coming from Africa were predicted as South American ones. This might be due to the number of South American samples that were available to optimize and to validate the model, was very low. This problem could be solved by increasing the number of samples of this class in further research. Finally, the RMSE in calibration, cross validation and prediction were lower than 0.6 for all the classes.

3.3. Classification of geographical origin of palm oils by HTGC–MS and PLS-DA

The same chemometric processes were applied in both types of chromatographic data in order to compare the results from both techniques. For the GC data, the classification model was also cross validated with random subsets and externally validated by predicting the identity of the samples with the external validation set which corresponded to the 30% of the samples of the calibration set. The RMESP was also lower than 0.6 for each class. In this case, the fitted PLSDA model on the gas chromatographic data with four latent components involved 70% of the total variance. Results of the cross validation of the training model are shown in Table 3.

When modelling on GC data was performed, for all the classes a sensitivity and specificity in calibration of 100% were obtained, since samples from other classes did not were used to build the training models. The specificity values, in cross validation, for the two first classes, Africa and South America, are significantly improved (100%) with those on the HPLC data with the exception in the South eastern Asia which is the same. The results, in external validation with the external test set, are shown in Table 4. The specificity on Africa and South America samples has improved with the GC data, even though it has decreased for the South eastern Asia samples.

According to the obtained results for all classes, they were very promising with both techniques. These results show that the geographical origin of crude palm oil samples might be verified by means of their TAG fingerprinting measured by liquid and gas chromatography. A comparable sensitivity with respect to the same kind of class modelling performed on the previous studies with the volatile compounds and fatty acid profile was obtained [30]. However, the models performed with the fingerprint profile coming from the HTGC–MS analysis gave models with more robustness than in the HPLC and the results were slightly improved and without being necessary to release FA composition and perform gas chromatography after methylation.

4. Conclusions

In this paper a pattern recognition method such as PLS-DA is applied on the liquid and gas chromatographic fingerprints, for the prediction of the geographical origin of palm oil samples, and their results are compared. Regarding the analytical methods, a new approach for the analysis of TAGs in liquid chromatography has been developed, improving the already existing methods in bibliography. For instance, a different detector such as CAD for liquid chromatography is used instead of the often used RID or ELSD and the acquisition time has been improved. In addition, both chromatographic methods provide significant advantage over conventional LC and GC, since they do not require extra sample treatment or derivatization procedures prior to injection, only the dilution of the palm oil.

On the other hand, according to our knowledge, the geographical origin of palm oils with the use of the raw chromatograms and chemometrics tools had not been studied yet. These results show that it is possible to establish classification models for palm oil samples from either liquid or gas chromatographic raw data based on their TAG profile since they present different features as well as it has been previously reported by the FA and VOCs fingerprinting [30]. In addition partial least square-discriminant analysis seems to be an appropriate classification-discriminant method for geographical authentication of palm oil. The proposed method provides a rapid tool for palm oil classification according to geographical origin and could serve as a technique to verify the labelling compliance of the oil.

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References

[1] R. Sambanthamurthi, K. Sundram, Y. Tan, Prog. Lipid Res. 39 (2000) 507–558.
[2] P. Wolmarans, Ann. Nutr. Metab. 55 (2009) 244–272.
[3] M.B. Wahid, S.N. Akmar Abdullah, I.E. Henson, Plant Prod. Sci. 8 (3) (2005) 288–297.
[4] F. Ulberth, M. Buchgraber, Eur. J. Lipid Sci. Technol. 102 (11) (2000) 687–694.
[5] E. Barcelos, P. Amblard, J. Berthaud, M. Seguin, Pesqui. Agropecu. Bras. 37 (8) (2002) 1105–1111.
[6] D.K. Sathish, C. Mohankumar, Indian J. Biotechnol. 6 (2007) 354–358.
[7] C.C. Lim, V. Rao, J. Oil Palm Res. 17 (2005) 136–144.
[8] A. Tres, G. van der Veer, M. Alewijn, E. Kok, S.M. van Ruth, in: S.A. Penna (Ed.), Classical Methodology and State-of-the-Art TechniquesNova Science Publishers, Inc., New York, 2011, pp. 1–44.
[9] C.W. Chen, C.L. Chong, H.M. Ghazali, O.M. Lai, Food Chem. 100 (2007) 178–191.
[10] P.T. Gee, Eur. J. Lipid Sci. Technol. 109 (2007) 373–379.
[11] W.L. Siew, in: F. Gunstone (Ed.), Vegetable oils in Food Technology: Composition, Properties and UsesBlackwell Publishing, CRC Press, LLC, Boca Raton, 2002, pp. 59–97.
[12] M. Buchgraber, F. Ulberth, H. Emons, E. Anklam, Eur. J. Lipid Sci. Technol. 106 (2004) 621–648.
[13] COI/T.20/Doc. No 20/Rev.2., Method of Analysis, Difference between Actual and Theoretical Content of Triacylglycerols With ECN 42, International Olive Council, Madrid, 2008.
[14] AOCS Official Methods Ce 5b-89 Triglycerides in Vegetable Oils by HPLC, American Oil Chemists' Society, 2011.
[15] J.P. Hutchinson, J. Li, W. Farrell, E. Groeber, R. Szucs, G. Dicinoski, P.R. Haddad, J. Chromatogr. A 1217 (47) (2010) 7418–7427.
[16] R.A. Moreau, Lipid Technol. 21 (8/9) (2009) 191–194.
[17] T. Vehovec, A. Obreza, J. Chromatogr. A 1217 (2010) 1549–1556.
[18] T. Gòrecki, F. Lynen, R. Szucs, P. Sandra, Anal. Chem. 78 (2006) 3186–3192.
[19] M. Lísa, F. Lynen, M. Holčapek, P. Sandra, J. Chromatogr. A 1176 (2007) 135–142.
[20] P. de la Mata Espinosa, J.M. Bosque Sendra, L. Cuadros Rodríguez, Food Anal. Methods 4 (4) (2011) 574–581.
[21] 70-8310-Application Note, Simultaneous Analysis of Glycerides (mono, di, and triglycerides) and Free Fatty Acids in Palm Oil, ESA, Dionex Corporation, Chelmsford, MA 01824-4171, USA.
[22] M.T. Rodríguez-Estrada, N. Frega, G. Lercker, Grasas Aceites 53 (2002) 76–83.
[23] A. Dieffenbacher, W.D. Pocklington, IUPAC method 2.323, in: C. Paquot, A. Hautfenne (Eds.), IUPAC Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th ed., Blackwell, Oxford, 1987.
[24] N.K. Andrikopoulos, I.G. Giannakis, V. Tzamtzis, J. Chromatogr. Sci. 39 (2001) 137–145.
[25] H. Lik Nang Lau, C. Wei Puah, Y. May Choo, A. Ngan Ma, C. Hock Chuah, Lipids 40 (2005) 523–528.
[26] T. Cserháti, E. Forgács, Z. Deyl, I. Miksik, Biomed. Chromatogr. 19 (2005) 183–190.
[27] J.M. Bosque Sendra, L. Cuadros Rodríguez, C. Ruiz Samblás, A.P. de la Mata, Anal. Chim. Acta 724 (2012) 1–11.
[28] T. Řezanka, H. Řezanková, Anal. Chim. Acta 398 (1999) 253–261.
[29] J.M.N. Marikkar, H.M. Ghazali, Y.B. Che Man, T.S.G. Peiris, O.M. Lai, Food Chem. 91 (2005) 5–14.
[30] A. Tres, C. Ruiz-Samblas, G. van der Veer, S.M. van Ruth, Food Chem. 137 (2013) 142–150.
[31] C. Ruiz Samblás, A. González Casado, L. Cuadros Rodríguez, F.P. Rodríguez García, Talanta 82 (2010) 255–260.
[32] D.L. Massart, B.G.M. Vaneginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 2007.
[33] F. Savorani, G. Tomasi, S.B. Engelsen, J. Magn. Reson. 202 (2010) 190–202.
[34] M. Forina, M. Casale, P. Oliveri, Comprehensive Chemometrics: Chemical and Biochemical Data Analysis Vol. 4: Applications, in: S.D. Brown, R. Tauler, B. Walczak (Eds.), Elsevier, Amsterdam, The Netherlands, 2008, pp. 75–128.
[35] F. Marini, Current Anal. Chem. 6 (2010) 72–79.
[36] Agilent Poroshell 120 Columns for HPLC y UHPLC, Agilent Technologies, Inc., 5990-5951EN, 2012.
[37] F. Marini, A.L. Magrì, R. Bucci, F. Balestrieri, D. Marini, Chemometr. Intell. Lab. 80 (2006) 140–149.