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PREDICTION OF GEOGRAPHICAL ORIGIN OF PALM OILS (ElaeisGuineensis) USING QUALITY PARAMETERS AND CHEMOMETRICS

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ABSTRACT

This study presents a footprint for geographical discrimination of palm oil using its quality characteristics. A total of 60 oil samples; 20 from each region (North N, South S and Central C) of Ondo State Nigeria, were analyzed for their quality characteristics. Principal Component Analysis (PCA) and Orthogonal Projection to Latent Structure Discriminant Analysis (OPLS-DA) regression multivariate data analytical techniques were used to elaborate the data. The models were validated by independent prediction sets and cross validation. The results of chemical data were satisfactory with PCA creating distinctive clusters of samples based on their regional differences. Significantly high carotene content, free fatty acids (FFA), acid value (AV) and peroxide (PV) helped distinguish Central palm oils. K extinction values, color density and chlorophyll content were quality parameters peculiar to North oil samples. Discriminant class modeling of all the measured variables generated >85% correct regional prediction with K_{270nm} ,ΔK, AV and FFAhaving the highest coefficients of determination. The results cannot be considered exhaustive owing to the limited sample size. But it serves as a preliminary study showing the potential of this method.

Key words: Palm oils, quality parameters, regional prediction PCA, OPLS-DA

INTRODUCTION

The economic and nutritional contributions of palm oil to the World's oils and fats industries are highly significant exceeding soya oil by a wide margin (Mancini et al. 2015). Its global popularity is as a result of a number of competitive advantages over other vegetable oils. These include lower cost of production and suitability in various food applications such as frying, easily amenable and modifiable chemical composition for margarines and shortenings production (Mba, Dumont and Ngadi, 2015). Therefore, intensive research in the area of nutritional and quality authentication of palm oil is a necessity. Nutritionally, palm oil is relatively high in saturated fatty acid (SFA) counterbalanced with monounsaturated and polyunsaturated in addition to other important minor bioactive phytonutrients (Odia, Ofori and Maduka, 2015). These, minor compounds include: carotenoids, tocopherols, chlorophyll, sterols, squalene, phospholips and about 1% polyphenols (Mba, Adewale, Dumont and Ngadi, 2014). Each of these chemical parameters undergoes variations relative to geographical origin of the palm fruits from which the oil is obtained. There are three major quality factors by which the oil palm bunch is represented i.e. oil content, ripeness and free fatty acid (FFA) level (Makky, 2016) and these are not all-inclusive in conveying the differences in palm oil produced in different regions. Hence, a comprehensive determination of several independent chemical variables may better reveal the intrinsic quality differences and peculiarity of palm oils obtained from different geographical locations.

Like every other agricultural produce, variables influencing the quality uniqueness of palm oils include, geographical location, cultivar, agronomical practices and production methods. The meaning of origin is not only limited to provenance, it includes prevailing natural factors, cultural practices and other historical attributes contributing to the relationship between food and place (William and Jen, 2017). This relationship links the quality of the product with it geographical location. For example, the quality characteristics of some vegetable oils have been vastly linked with their regions of production (Uncu and Ozen, 2016; Treset al., 2013; Karabagiaset al., 2013). Generally, high reputation and prestige are conferred on products obtained from regions known for higher desirable quality attributes. Information on these specific quality attributes peculiar to palm oils obtained from such region could influence consumer preferences and perceptions. Consumers are gradually becoming keen in their willingness to pay slightly higher price for better quality when properly informed (García-González and Aparicio, 2010). Therefore, regions reputed for better quality palm oils are likely to attract higher market share both locally and abroad. In addition to the existing quality assessments of palm oils, rapid geographical discrimination could be adopted by standard regulatory body as a confirmatory test to further boost consumers' confidence.

There are some studies on detection of adulteration, and quality authentication of palm oil with satisfactory results in literature (Basri et al. 2017; Mba et al. 2014). Similarly, there are few other studies on chromatographic and spectroscopic determination of either minor or major components of palm oil in literature with remarkable results (Azemanet al. 2015; Che Man et al. 2009; Mohet al. 1999). Apart from a recent study that confirmed geographical origin of palm oil using HPLC (Obisesanet al. 2017) there is no study in literature where geographical origin of palm oil is discriminated using its quality parameters. Therefore, the objective of this study is to show the possibility of differentiating palm oils produced within the same state into regions, using their quality characteristics in conjunction with classical multivariate data elaboration. There are a limited number of regions where environmentally sustainable palm oils are produced according to Treset al. (2013). Therefore, developing a verifiable and repeatable method ofdetermining geographical origins of palm oil has a potential for authentication of "sustainable palm oil"; which is gradually becoming a global issue.

MATERIALS AND METHODS

Palm oil samples

Sixty crude palm oil samples obtained from three regions of Ondo State Nigeria (North, Central and South) were evaluated. Twenty samples from each region and the samples were collected from four different production mills under semi-mechanized production processes. Samples obtained from Ile-Oluji/Okeigbo area constitute the South (S), and those from Akungba as North (N) and that of Akure as Central (C). These geographical locations are comparatively small compared to the entire production regions in the country. The samples were collected immediately after production, kept in dark glass bottles and stored in a cool dry place prior to analysis.

Chemical analysis

Free fatty acids, Acid Value, Peroxide value and K specific extinction coefficients determinations

According to official methods of American Oil Chemist' Society (AOCS, 1990), FFA, AV, PV and K values were measured as average of three analyses per sample. Free acidity (0.503AV) indicative of the fatty acid content, expressed as oleic acid (%) was determined by titrating the oil solution (ethanol: ethyl ether, 1:1) with 0.1N KOH and phenolphthalein was used as indicator. Peroxide value (PV) expressed as equivalents of active oxygen per kg of oil (meqO_2/kg), was determined by the reaction of oil mixture (Chloroform, acetic acid and palm oil) with potassium iodide in the absence of light. The iodine liberated was titrated with 0.1N sodium thiosulfate solution using 1% starch solution as an indicator. Spectrophotometric indices also known as specific extinction coefficients, K_{270} and K_{232} and ΔK were measured as the absorption values of oil solution (cyclohexane and palm oil) at 232 and 270 nm wavelengths respectively, using UV-vis spectrophotometer (Shimadzu UV-1800 Kyoto: Japan) with 1 cm path length.

Chlorophyll and carotenoid determination

Modified method of Harborne, (1980) was used for the determination of chlorophyll and carotenoid contents of the samples. Palm oil sample (100 mg) was mixed with 10 mL 80% acetone and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was made up to 10 mL using 80% ethanol. The optical intensity (absorbance) was taken at 480 nm for carotenoids, at 645nm and 652nm for chlorophyll in UV-vis spectrophotometer (Shimadzu UV-1800 Kyoto: Japan) Total chlorophyll and carotenoid contents were estimated using the equations below:

Total carotenoid content (mg/kg) $= [4*A_{480nm}*V*1000]/\text{Sample weight}$

Total chlorophyll content (mg/kg) $= [20.2*(A_{645nm}) + 8.02*(A_{663nm})*V]/1000*W$

Where:

A: absorbance of specific wavelength

V: final volume of chlorophyll extract in 80% acetone

W: weight of the oil sample

Color Density

Spectroscopic method described by Wroistad (1993) was used in the color determination. Palm oil sample (1 mL) was diluted with 25 mL methanol in a beaker and stirred for 30 min using Magnetic stirrer to enable proper color extraction. The mixture was allowed to stand for 10 min and centrifuged. Optical density (OD) or absorbances of the supernatant was taken at 420 and 520 nm wavelength using UV-vis spectrophotometer (Shimadzu UV-1800 Kyoto: Japan). The analyses were done in triplicate. Color density was recorded as the sum of the absorbances of the two wavelengths as thus:

$$\text{Color Density} = A_{420nm} + A_{520nm}$$

Data processing and analysis

The significance of geographical differences between the oil samples with respect to their chemical paratemers were determined by One-Way Analysis of Variance (ANOVA) at 95% confidence level (Minitab 16.0, Minitab Inc., State College, USA). In building the multivariate calibration and validation models, data matrix (60 x 10) consisting of 60 palm oil samples (n observations) and 10 measured variables (K variables) was prepared. The variables comprise of: free fatty acids, chlorophyll and carotene, color density, peroxide and acid values, K-extinction (K_{232} and K_{270}) and R-value (K_{232}/K_{270}). The most widely applied linear chemometric techniques is the unsupervised PCA (Principal Component Analysis). It is a trends, patterns and outliers recognition method that linearly transform data matrix. The transformation leads to the maximum preservation of as many variance in the original data as possible in lower dimensionality space called principal components (PC) (Worley and Power, 2013). This linear data decomposition facilitates simpler and unbiased interpretation of the data sets. Calibration and validation class modelings were built using OPLS-DA (Orthogonal Projection to Latent Structure Discriminant Analysis).The technique depends on previously defined membership class information (Y) of each observation (palm oil) relative to the chemical X matrices. The class memberships were coded in the matrix form of Y as thus: class 1 (Central), class 2 (North) and class 3 (South) based on the the oil regional differences. OPLS-DA modifies the classical PLS with the incorporation of an inbuilt orthogonal signal correction (OSC) filter that enables effective separation of X variations into Y-predictive (related to class information) and Y-uncorrelated (orthogonal or unrelated to class information) (Jolayemi et al., 2017). The same randomly selected external validation sets consisting of 15 oil samples (5 C, 5 N, and 5 S) were used to varify the models predictiveness. In addition to this, inbuilt cross validation method with seven concellation groups (7 CV) was performed to further establish the robustness of the model and avoid overfitting. SIMCA software (v. 13 Umerics, Umea, Sweden) was used for all the multivariate statistical analyses and the output parameters were recorded. These parameters include the number of significant PCs used (PC_p+PC_o where p and o represent predictive and orthogonal components, respectively) in the case of OPLS-DA, determination coefficient for calibration (R^2_{cal}), cross validation (R^2cv) were also reported

RESULTS AND DISCUSSION

Univariate data evaluation

The results of the chemical and quality parameters of palm oil samples are given in Table 1. The acidity of the oils from the three regions considered varied between 6.71 – 9.52%, which is slightly outside the expected range ($\leq$ 5.00%) for crude palm oil according to PORAM (2013) and CODEX 210 (2011) quality assessment criteria. Oxidation of unsaturated fatty acids is the main reaction responsible for the degradation of lipids and this forms the basis for the analytical quality assessment of palm oils. PV value measures the extent of accumulation of primary oxidative product called “hydroperoxides” which has not actually been converted to secondary products responsible for actual deterioration of the oils and fats. Therefore, palm oils samples of higher PV (> 4.60 meqO_2/kg) may not necessary be of low quality, but suggests low oxidative stability of the Central palm oils. High PV of Central oils may be due to fatty acid distribution. However, all the palm oil samples are within acceptable minimum level (15 meq O_2/kg) by CODEX 210 (2011). Oils are mixture of triacyglerols that can be hydrolyzed enzymatically or chemically to generate a mixture of FFA, glycerol, mono and diacyglycerols. The factors that mostly influence the rate of these reactions are related to environmental and processing conditions such as high temperature, moisture and oxygen availability and exposed surface area (Choe and Min, 2007). These rate-determining

factors cannot be completely controlled in semi-mechanized palm oil extraction processes. The comparatively high FFA and AV values of the samples may be a reflection of the difference betweenindustrial and semi-intensive processing environments as earlier stated by De Almeida et al. (2013).

The specific absorptions at 232 nm (K_{232}) and 270 nm (K_{270}) are related to the content of conjugated dienes and trienes commpounds present in oils, respectively. K values are useful tools in providing a quick read-out for oils quality comparison, but it does not provide information on the actual polyunsaturated fatty acids responsible for the diene and triene compounds. Therefore, apart from FFA and AV, there is no significant correlation between any pair of PV, FFA and Kvalues in establishing for facts, the impact of regional differences on the quality characteristics of the palm oil samples. However, free acidity values (FFA and AV) and PV of Central oil samples were significantly higher compared to other regions (Table 1). On the contrary, there was no significant regional influence on K_{232} while, K_{270}, ΔK and R-value were the same for North and Central oil samples. Low R-value and high K_{270} indicate the presence of more secondary oxidation products in the oils than primary (Multon, 1997). Therefore, oils of North and Central regions are more susceptible to oxidative rancidity.

The most widely distributed pigments present in palm oil is carotene with over 60% of it being beta-carotene with potential vitamin A precursor and high radical scavenging capacity (Rufinoet al. 2010). Apart from the nutritional importance of this pigment, it contributes to the visual appeal of palm oil and may influence the degree of consumer acceptability (Moyanoet al. 2010). Significantly higher carotene content was obtained for palm oils from the Central region and followed by North. However, all the samples are within the minimum amount of carotene required for high quality unbleached palm oils (500 – 2000 mg/kg) (CODEX 210, 2011). The variation in these values may be due to agronomical factors such as fruit cultivars, climatic conditions and extraction procedures. Chlorophyll contents of the oil samples are relatively low with North samples having the highest chlorophyll content (0.20 – 0.34 mg/kg). The same trend was observed for color density as well.

Table 1: Quality variation of palm oils based on regional differences

Quality	Regions		
parameters	Central	North	South
Free fatty acid (%)	8.98 ± 0.54^{a}	8.17 ± 0.20^{b}	7.12 ± 0.41^{c}
Acid value(%)	17.96 ± 1.08^{a}	16.34±0.40b	14.24 ± 0.82^{c}
Peroxide value(meqO2/kg)	3.97 ± 0.71^{a}	2.23 ± 0.38^{b}	0.99 ± 0.15^{c}
K_{232nm}	0.27 ± 0.04^{a}	0.27 ± 0.05^{a}	0.25 ± 0.04^{a}
K_{270nm}	0.16 ± 0.07^{a}	0.18 ± 0.03^{a}	0.09 ± 0.02^{b}
ΔK	0.12 ± 0.06^{a}	0.14 ± 0.03^{a}	0.05 ± 0.02^{b}
R-value	1.95 ± 0.75^{b}	1.53 ± 0.22^{b}	2.85 ± 0.80^{a}
Carotene (mg/kg)	737.83 ± 53.49^{a}	608.80 ± 42.42^{b}	501.70 ± 17.56^{c}
Chlorophyll (mg/kg)	0.08 ± 0.02^{b}	0.20 ± 0.07^{a}	0.03 ± 0.01^{c}
Color density	2.04 ± 0.58^{b}	2.44 ± 0.54^{a}	2.03 ± 0.39^{b}

*Means that do not share a letter (superscript) are significantly different at $P \leq 0.05$

Multivariate data evaluation

Principal component analysis (PCA)

PCA model of quality parameters data with 3 PC and 79% total explained variance produced three clusters based on regional differences between the palm oil samples with S oils completely distinguished from others, forming a distinct cluster on the left side of control eclipse (Figure 2a). The variable most responsible for the separations of South oils as revealed in the loading plot is R-value indicative of how distinctive the region is compared to North and Central (Figure 2b). Even though maximum class separation is not the explicit objective of PCA, a close to perfect class separation was obtained from the score plots of both chemical data matrix. A slight overlap was observed between N and C at the positive axis of the PC 2 probably indicating some chemical similarities. High values of K values, chlorophyll, and color helped describe the projections of N palm oils while, carotene, acidities and peroxide values are responsible for the clustering of C oils. The information conveyed by PCA creates the basis by which the secondary discriminant analysis OPLS are validated as will be shown later.

Table 2 OPLS percentage of correct regional classification of palm oils

OPLS-DA	Members	Central	North	South	%CC
Central	15	15	0	0	100
North	15	2	13	0	87
South	15	0	0	15	100
Total	*45*	*17*	*13*	*15*	*96*

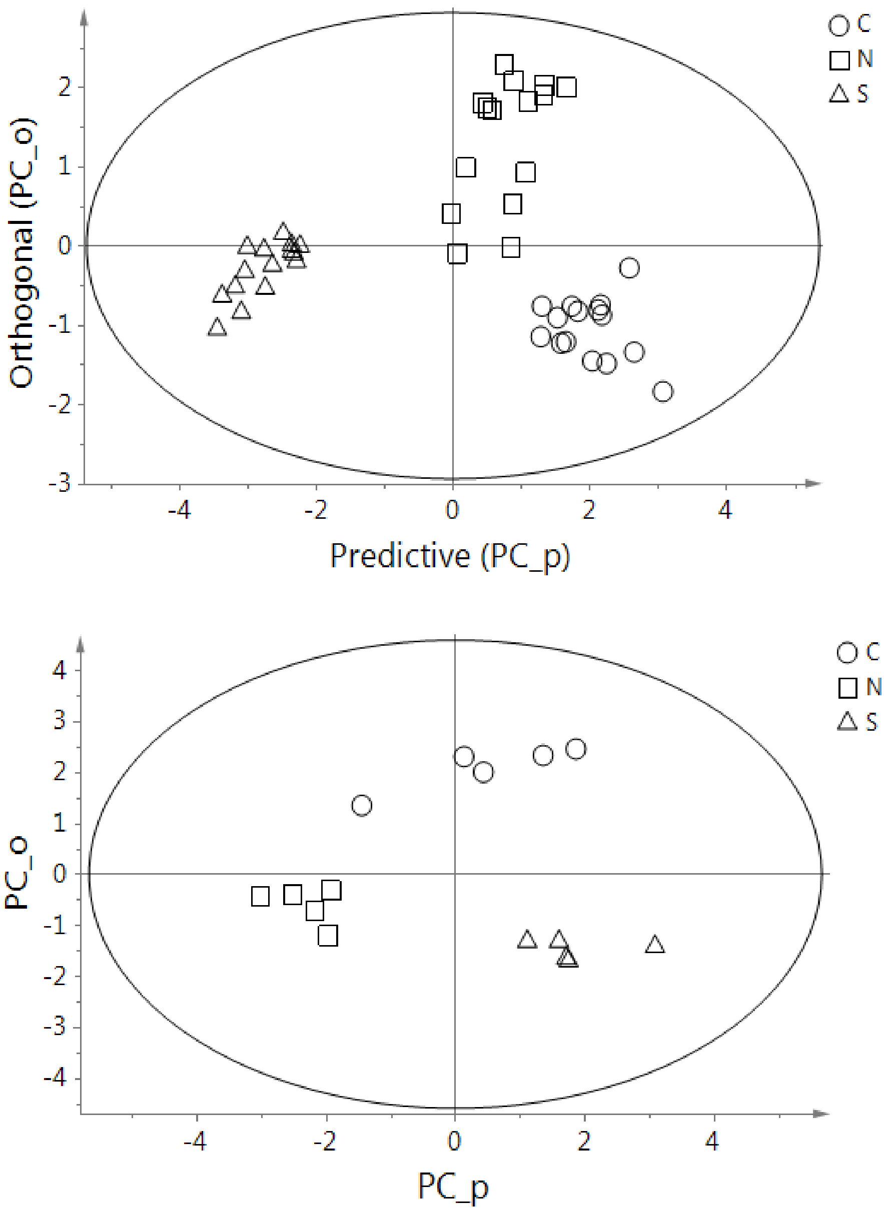
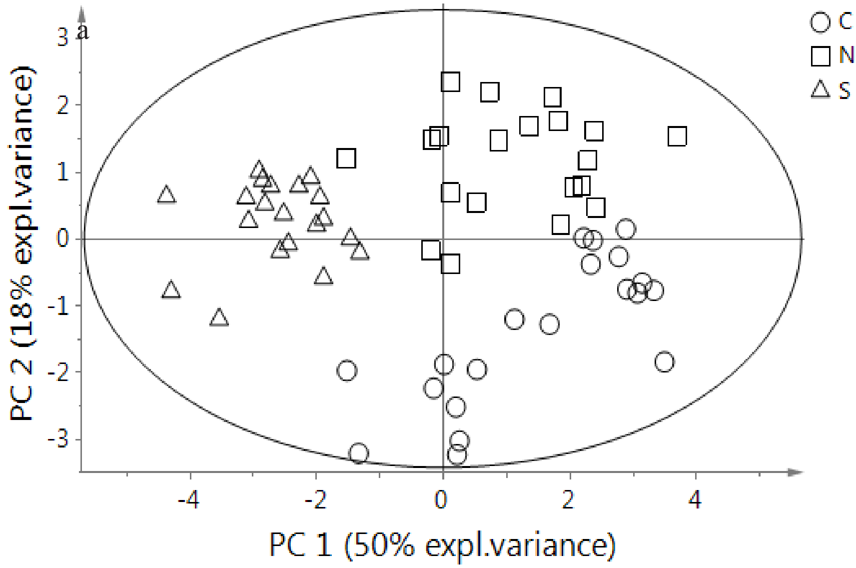


Figure 2: OPLS-DA calibration (a) and validation (b) model score plots of quality parametersof palm oils at different regions (Central C, North N, and South S)



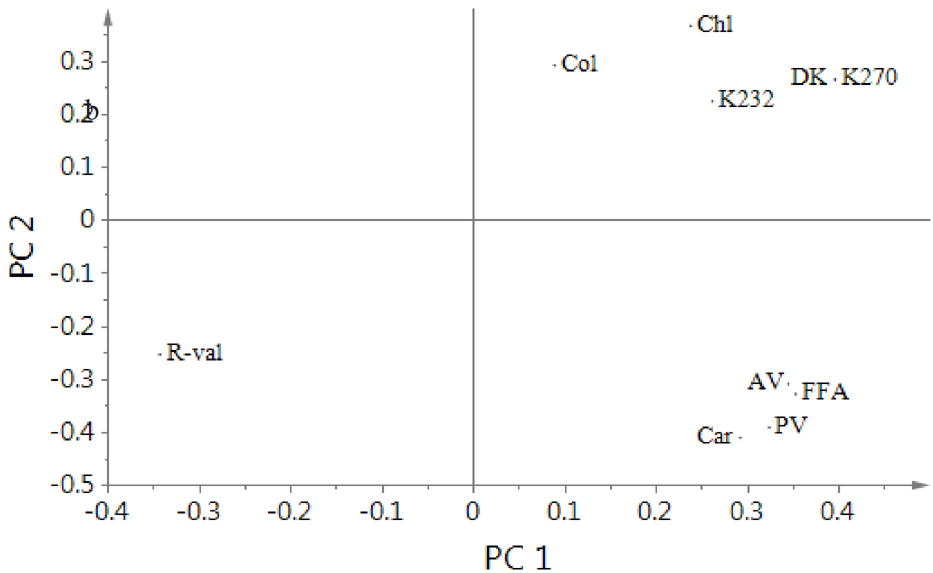


Figure 1: PCA model results: (a) score plot and (b) loading plot of quality parameters data of palm oils of different regions (Central C, North N, and South S)

OPLS discriminant and prediction analysis

Being a natural exploratory analysis, PCA shows a good distinction, but cannot be used for classification of samples into their geographical regions. Thus, class modeling technique that allows initial allocation of samples in classes, prior to modeling is required. Therefore, in order to predict correct regional classes of palm oils using chemical data matrix, OPLS discriminant analysis was adopted. The performance of this technique was verified in terms of percentage of correct classification (Table 2) and coefficients of determination for each parameter (Table 3). The models were fitted for training (calibration) and prediction (validation) and the calibrations score plots are presented in Figure 2A and 2B, respectively. There was a complete resolution of palm oils regional class-overlapping especially in the predictive direction, as a result of separation of orthogonal variation to improve the discriminatory capacity of OPLS-DA. This inbuilt error-filtering advantage improves class modeling ability of OPLS-DA (Bylesjoet al. 2006). There were two and one misclassified N oils in the C class for calibration and validation sets respectively in the model, thereby producing correct classifications of 96% and 87% in each case as shown in Table 2 and Figure 2B. It is noteworthy to state that; discriminative outcome for C and S palm oil samples were 100%.

In the OPLS prediction of different geographical origin of palm oil with respect to individual quality parameters, Table 3 shows high coefficients of determinations for all the parameters except color density and chlorophyll contents. Chlorophyll had low regional predictive capacity as compared to carotene and this can be attributed to its comparatively low quantity in palm oil. Other vegetable oil has been reported with higher coefficient of prediction with respect to chlorophyll than carotenoids (Oguz&Ozen, 2016). K_{270nm}, ΔK, AV and FFA were the most important quality variables capable of revealing the geographical origin of palm oils. This is partially in agreement with the observation of Makky, (2016) who confirmed the significance of FFA in quality differences of palm oils. The carotene contents were significant; having up to 77% correct regional prediction. This is very similar to that of PV and K_{232nm}

Table 3: OPLS coefficients of determination for the prediction of quality parameters

Parameter	PC_p+PC_o	R^2_{cal}	R^2_{pred}	R^2cv
Free fatty acid (%)	1+2	0.98	0.74	0.93
Acid value (%)	1+3	0.83	0.99	0.99
Peroxide value (meqO_2/kg)	1+2	0.74	0.81	0.74
K_{232nm}	1+3	0.83	0.74	0.62
K_{270nm}	1+2	0.77	0.98	0.97
ΔK	1+2	0.77	0.98	0.97
R-value	1+4	0.9	0.88	0.84
Carotene(mg/kg)	1+3	0.84	0.77	0.64
Chlorophyll (mg/kg)	1+3	0.87	0.43	0.26
Color density	1+1	0.68	0.25	0.11

**PC_p+PC_o*: number of principal components (predictive+orthogonal), R^2_{cal}: determination coefficient of calibration model, R^2_{pred}: determination coefficient of prediction, R^2cv: determination coefficient of leave-one-out cross validation model

CONCLUSIONS

For the first time, application of quality characteristics in geographical differentiation of palm oil was demonstrated. The models showed high potentials for regional recognition of palm oil when quality parameters of the oils were elaborated using PCA and OPLS discriminant analysis. The performance of the models in terms of calibration and external prediction, percent correct classification, and coefficients of determinations (calibration and cross-validation) are reasonably satisfactory. The projection of observations in the score ellipses in both unsupervised and supervised chemmometric models based on determined variables, were quite predictive. Therefore, the data is a valuable discriminating tool effective in correctly classifying palm oils into their separate production regions with little intersection among class members. Finally, this analytical approach could represent a valid tool for the prevention of palm oil quality misrepresentation; a form of food frauds that may be prevalent in the country of high production.

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