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Analytical Methods

High-resolution ^{1}H NMR profiling of triacylglycerols as a tool for authentication of food from animal origin: Application to hen egg matrix

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ABSTRACT

Metabolomics of complex biological matrices conducted by means of ^{1}H NMR leads to spectra suffering from severe signal overlapping. Previously, we have developed a high-resolution spectral treatment method to help solving this issue in ^{1}H NMR of triacylglycerols. In this work, we tested the potential of the developed method in the characterization and authentication of food products from animal origin using egg yolk as a model matrix. The approach consisted in a spectral deconvolution guided by the precision obtained on the deconvoluted peaks after reference lineshape adjustment of spectra. Thus, 135 peaks were quantitated and successfully used as biomarkers of origin, of hens breed, and of farming system. This required multivariate statistical analyses for classification. The same pool of variables allowed construction of multivariate quantitation models for individual fatty acids. Furthermore, minute amounts of conjugated fatty acids were quantitated and used as fingerprints of samples from backyard and free-range farming.

1. Introduction

Hen eggs are consumed worldwide and are known to be healthy source of nutrients such as vitamins, minerals, proteins, and fats. Development of mechanized farming systems and introduction of Genetically Modified Organisms (GMO) prompted consumers to look for authentic foodstuffs like organic or free-range eggs. Because of their scarcity, such products are sold at higher prices that make them targeted by food fraudsters.

According to Joint Research Center of the European Commission, several cases of egg frauds have been reported during 2018 and 2019 pertaining to mislabeling, lack of product documentation regarding their traceability, and contamination (European Commission, 2018 and 2019). We quote herein passing off conventional eggs as free-range or organic and selling them at higher prices.

Methods intended for verification of farming systems or product origins are increasingly requested. In the past decades, many studies focused on discrimination of organic and conventional food products using various analytical techniques combined with chemometrics such as isotope ratio monitoring by Mass Spectrometry (irm-MS) (Coletta et al., 2012; del Amor, Navarro, & Aparicio, 2008; Erich et al., 2015; Hohmann et al., 2015; Rogers, 2009), Nuclear Magnetic Resonance (NMR) (Ackermann et al., 2019; Erich et al., 2015; Hohmann et al., 2015), Fourier-Transform Infra-Red (FTIR) (Hohmann et al., 2015; Puertas & Vázquez, 2019), and Fourier-transform Raman spectroscopy (de Oliveira Mendes, Porto, Almeida, Fantini, & Sena, 2019).

In particular, ^{1}H NMR emerged as a powerful tool in metabolomics for product authentication and characterization (Godelmann et al., 2013; Kortesniemi et al., 2016; Monakhova et al., 2013; Spiteri et al., 2016). ^{1}H NMR also allowed to detect minor components that turned out to be important discriminators such as terpenes in olive oil (Merchak et al., 2017a, 2017b) and milk (Fernandez, Astier, Rock, Coulon, & Berdague, 2004) or to have health promoting effects such as Conjugated Linoleic (CLA) and Linolenic (CLnA) Acids. Moreover, a high-resolution ^{1}H NMR profiling of triacylglycerols (TAG) enhanced the olive oil classification and permitted individual quantitation of fatty acids by means of a precision guided spectral deconvolution approach (Hajjar, Merchak, Daniel, Rizk, Akoka, & Bejjani, 2020).

The objective of our work was to evaluate the potential of this ^{1}H NMR profiling of TAG in the characterization and classification of food products from animal origin. In this respect, TAG of hen egg yolk was chosen as a model matrix and samples from different origins were considered. Multivariate analyses of spectral variables were conducted to reveal biomarkers of origin, of farming system, and of hens breed as

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well as to construct prediction models for individual fatty acid contents within TAG.

2. Materials and methods

2.1. Egg samples

Fresh hen eggs were either purchased from local grocery stores or collected from non-commercial farms. Different farming systems were identified according to the hens' nutrition and welfare. First, conventional cages where birds were raised for large-scale egg commercialisation and were automatically supplied with a controlled mixture of grains; from this category, white and brown eggs –laid by two commonly raised hen breeds (see Section 3.4)– were obtained. Second, free-range where hens were raised in barns and fed with grains, yet they had access to outdoors for a part of the day. Third, backyard cages where hens roamed freely outdoors during the day and were supplied with a mixture of grains and a high amount of human food leftovers. Last, organic farming where hens were fed organic non-GMO diet consisting of mainly grains and a small amount of organic agriculture leftovers, and were given access to outdoors for a part of the day. In total, 34 egg samples were collected from 5 different farms (A, B, C, D, and E) as described in Supplemental Table S-1 where more details about diets are available. The same egg yolks were used in previous studies (Hajjar, Rizk, Akoka, & Bejjani, 2019; Hajjar, Rizk, Bejjani, & Akoka, 2020).

2.2. Chemicals

Extra pure ethanol, extra pure acetone, and petroleum ether (boiling range 35–60 °C, ACS basic) were purchased from Scharlab; diethylether (GPR rectapur) was purchased from VWR chemicals; and deuterated chloroform was purchased from Deutero GmbH. Whatman Purasil silica gel (60A, 230–400 Mesh ASTM) was used for column chromatography.

2.3. Triacylglycerols extraction

Triacylglycerols in egg yolks were extracted in a previous study using a procedure especially developed for this aim (Hajjar, Rizk, Bejjani, & Akoka, 2020). Eggshell was carefully broken and yolk was separated from egg white using a yolk extractor. Yolk was homogenized after removal of vitellus membrane and chalaza. Absolute ethanol (36 mL) was added to 10 g of yolk and mixed for 10 min (Palacios & Wang, 2005). The mixture was then filtered and the residue was washed several times with 10 mL fractions of ethanol:petroleum ether (1:1 v/v) until complete extraction of lipids, assessed by Thin Layer Chromatography (TLC). Solvents were evaporated under vacuum at 45 °C, extracted lipids were mixed with 18 mL of petroleum ether, acetone (36 mL) was added, and the mixture was kept for 40 min at −20 °C for phospholipids precipitation (Gładkowski, Chojnacka, Kiełbowicz, Trziszka, & Wawrzeńczyk, 2012). The liquid phase was decanted off and phospholipids were washed with cold acetone (−20 °C). Acetone-soluble fractions were pooled and solvents were evaporated under vacuum at 40 °C. The residue, consisting of egg yolk neutral lipids (mainly TAG and cholesterol), was dissolved in 10 mL of 10% diethylether in petroleum ether and passed through a chromatographic column prepared with 6 g of silica gel. 70 mL of the aforementioned mixture of solvents were used to elute TAG. Solvents were evaporated under vacuum at 35 °C and isolated TAG samples were stored at −20 °C without any further purification steps.

2.4. Gas chromatographic analysis

Samples were previously analyzed according to the following procedure (Hajjar, Rizk, Bejjani, & Akoka, 2020). Fatty acid methyl esters were prepared by shaking 200 mg of TAG in 3 mL of hexane with 0.4 mL of 2 N methanolic potassium hydroxide (European Commission, 1977; International Olive Council, 2015). A 1:10 dilution in hexane was performed. An Agilent Technologies chromatograph equipped with a flame ionization detector and a fused-silica capillary column (SGE-054616, 30 m × 0.32 mm i.d., BPX70 0.25 μm) was used in this study with helium as carrier gas at flow of 1.0 mL/min. Oven temperature was programmed as follows: at 160 °C for 15 min, to 200 °C at a rate of 10 °C/min, at 200 °C for 10 min. A volume of 1 μL was injected with a split ratio of 50:1 and an injector temperature of 200 °C. The detector temperature was set at 270 °C. Two injections were performed for each sample with a total elution time of 58 min. Mass percentages of each fatty acids within egg yolk were determined by dividing its peak area by the total peak area of detected fatty acids in each sample Supplemental Table S-2. Standard fatty acid methyl esters, purchased from sigma Aldrich, were used to determine their retention time.

2.5. NMR experiments

2.5.1. Acquisition

Triacylglycerols of egg yolk (167 mg) were dissolved in 560 μL of $CDCl_3$ and the mixture was transferred to a 5 mm NMR tubes (Fauhl, Reniero, & Guillou, 2000). One-dimensional ^1H NMR spectra (Supplemental Fig. S-1.a) were recorded on a 400 Bruker Avance II spectrometer operating at 400.13 MHz using the following conditions: probe temperature 25 °C, time domain size 64 K, pulse angle 30°, pulse width 27.4 μs, spectral width 9 ppm, acquisition time 9.1 s, relaxation delay 1 s, 4 dummy scans and 32 scans. The longest longitudinal relaxation time T_1 (3.19 s) was observed for the methyl group of the linolenic acid as measured by the inversion-recovery method. For each sample, seven spectra were recorded leading to a global experiment time of 43 min.

2.5.2. Spectral processing

The recorded Free Induction Decays (FID) was zero-filled to 128 K. An exponential apodization function was applied inducing a line broadening of 0.3 Hz prior to the Fourier transformation. Spectra were manually phased and a polynomial (n = 5) baseline correction was automatically applied. Reference Lineshape Adjustment (RLA) was applied for each spectrum using Bruker TOPSPIN software. $CHCl_3$ signal was used as reference to compute the error function $\varepsilon^r(t)$ that will be used to adjust the experimental FID according to the following equation (Metz, Lam, & Webb, 2000; Morris, Barjat, & Horne, 1997):

$$FID_{adj}(t) = \frac{FID_{exp}(t)}{\varepsilon^r(t)} \quad (1)$$

where $FID_{exp}(t)$ is the experimental recorded FID and $FID_{adj}(t)$ is the adjusted FID that henceforth replaces $FID_{exp}(t)$. Spectral deconvolution was directed by the precision obtained on the deconvoluted peaks after reference lineshape adjustment of spectra; *i.e.* the $CHCl_3$ peak shape was adjusted until a spectral resolution between 19 and 20% was reached. It was expressed in percent as intensity of the minimum between signals at 4.288 and 4.299 ppm relative to intensity of signal at 4.299 ppm (Merchak et al., 2017a, 2017b).

Each region (aliphatic, allylic, diallylic, vinylic, methyl of non-ω3 fatty acids, a methyl of cholesteryl esters, and methyl of linolenic acid, Supplemental Fig. S-1) was then calibrated and deconvoluted by adding the minimum number of peaks allowing the best fit (Hajjar, Merchak, Daniel, Rizk, Akoka, & Bejjani, 2020). Intensities of deconvoluted peaks were normalized relative to the sum of peak intensities related to the methylene at position 2 (the sum of peak intensities was set to 600); corresponding areas were normalized similarly (Supplemental Fig. S-1 and Table S-3). For samples in which signals of CLA and CLnA were perceivable, corresponding peak intensities were measured and used to calculate their percentages within TAG (See Section 3.1. and Supplemental Table S-4).

2.6. Chemometrics

Data obtained from ^{1}H NMR and gas chromatography analyses of egg yolks TAG were subjected to the following statistical treatments using TANAGRA data mining software (Rakotomalala, 2005). First, an exploratory analysis was performed by means of Principal Component Analysis (PCA). Then, Canonical Discriminant Analysis (CDA) and Linear Discriminant Analysis (LDA) were used for the classification of egg samples. A backward elimination approach was used to select variables for classification models based on their Wilks' λ values. In each case, performance and robustness of the model were assessed using LDA-*Error rate* (LDA-*Er*) and Leave-One-Out *Error rate* (LOO-*Er*) as indicators, respectively. One-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the means of egg groups.

Partial Least Square Regression (PLSR) was used for construction of fatty acid quantitation models based on ^{1}H NMR variables as predictors and fatty acids mass percentages determined by Gas Chromatography (GC) as targets PLSR models were built by eliminating non-relevant variables according to their standardized regression coefficient while adjusting the number of components (h) based on the cross-validation test (by randomly leaving out 10% of the training samples). Maximum cumulative Q^2 (Q^2_{cum}) and Predicted Residual Error Sum of Squares (PRESS) were used as robustness indicators (Gauchi & Chagnon, 2001; Hawkins, Basak, & Mills, 2003). Coefficient of determination R^2 afforded a measure of how well the amount of a given fatty acid can be predicted. Adjusted R^2 was used to compare models constructed with different number of predictors (Schinka, Velicer, & Weiner, 2003). External validation was applied to assess prediction power of models using the coefficient of determination for the test set (Pred-R^2) as indicator (Roy & Roy, 2008). The best model was selected based on performance (R^2 and Adjusted R^2) and validation (Q^2_{cum}, PRESS, and Pred-R^2) parameters (Merchak, Silvestre, Loquet, Rizk, Akoka, & Bejjani, 2017).

3. Results and discussion

Reference lineshape adjusted spectra of TAG extracted from egg yolk samples were deconvoluted using Lorentz fitting function. As previously mentioned, spectral regions corresponding to specific protons of TAG as well as a signal corresponding to methyl protons of cholesteryl esters were separately considered. In each case, peaks were iteratively added following the developed approach described in our previous study (Hajjar, Merchak, Daniel, Rizk, Akoka, & Bejjani, 2020). As a result, 135 peaks were deconvoluted and their corresponding intensities and areas were determined (Supplemental Fig. S-1). Only variables obtained with a precision lower than 10% were retained. Otherwise, peaks were combined with their appropriate neighbors in order to reach this precision. As shown in Supplemental Table S-3, 82 intensities and their corresponding areas were retained and used as predictors in chemometrics.

3.1. Quantitation of conjugated polyene acids

The main difference between conventional and other farming systems is the access to outdoor space where hens are expected to eat not only grains but insects and seeds from vegetation. It was noticed that food products derived from grass-fed animals contained more CLA and CLnA than those derived from grain-fed animals (Daley, Abbott, Doyle, Nader, & Larson, 2010). Therefore, CLA and CLnA in egg yolk could be potential biomarkers of the type of hens farming system. In order to quantitate these compounds present in minute amounts within egg yolk triacylglycerols, their barely detectable signals (5.87–5.95 and 6.21–6.31 ppm for CLA; 5.98–6.07 and 6.40–6.45 ppm for CLnA; Fig. 1) (Manzano Maria, Colnago, Aparecida Forato, & Bouchard, 2010) were considered before reference lineshape adjustment of spectra. This was to avoid the decrease of signal/noise ratios (Metz, Lam, & Webb, 2000). For CLA, intensities of peaks at 5.91 and 6.26 ppm were determined and their average I_{CLA} calculated. Likewise, I_{CLnA} was calculated based on intensities of peaks at 6.02 and 6.42 ppm. Intensity ratios I_{CLA}/I_α and I_{CLnA}/I_α (I_α is the intensity of the peak at 2.31 ppm within the signal of protons at position 2 of global fatty acids) were then determined and converted into molar percentages. Conversion factors were determined based on the quantitation of CLA and CLnA using signal integrals instead of peak intensities (only samples relatively rich in CLA and CLnA were used to this end).

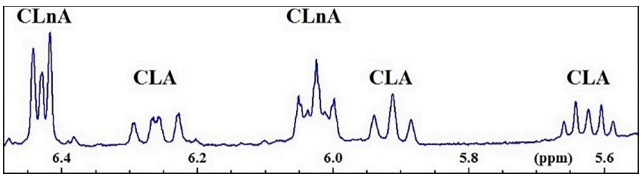

Fig. 1. Signals corresponding to vinylic protons of Conjugated Linoleic and Linolenic Acids (CLA and CLnA, respectively).

Thus, molar percentages of CLA and CLnA were added to the list of predictors used to construct classification models of samples.

It was remarkable that samples of groups B (backyard) and E (free-range) were higher in CLA and CLnA than other groups (Supplemental Table S-4). This was probably related to birds feed composition (Supplemental Table S-1) since human food leftovers that might contain ruminant meats, dairy products, and pomegranate seeds were added to hens of groups B and E. Ruminant based leftovers and pomegranate seeds are sources of CLA and CLnA, respectively. It is known that an increase of CLA in hens diet induced an increase of their percentage in lipids of egg yolks, making eggs potential sources of conjugated acids (Chamruspollert & Sell, 1999; Du, Ahn, & Sell, 1999; Jones, Ma, Robinson, Field, & Clandinin, 2000; Szymczyk & Pisulewski, 2003).

However, since feed composition of groups B and E was the most varied among all the studied groups, and was free of antibiotics, the hypothesis that these hens had intestinal bacterial flora capable of converting linoleic and linolenic acids into CLA and CLnA, on one hand, and converting CLnA into CLA, on the other hand, was also conceivable (Dhar Dubey et al., 2019). Indeed, molar percentages of CLA and CLnA were found to be strongly correlated (Pearson's $r(10) = 0.923$, $p = 0.0001$) over the 10 samples containing both these fatty acids (Supplemental Table S-4), which is in favor of this hypothesis. Previous studies demonstrated the ability of specific *Bifidobacterium* strains to convert linoleic and *alpha*-linolenic acids into CLA and CLnA, respectively (Gorissen et al., 2010; Fontes, Pimentel, Rodríguez-Alcalá, & Gomes, 2018).

3.2. Classification of samples according to their origin and to the corresponding farming system

PCA was used as an unsupervised analysis to verify whether there was a classification trend of egg samples based on intensities of deconvoluted peaks as well as molar percentages of CLA and CLnA. This revealed that organic eggs were distinguishable from other samples (Supplemental Fig. S-2 and Table S-5). Furthermore, a supervised analysis based on CDA and LDA allowed construction of classification models for organic (group A) and non-organic (the other groups) samples as well as for conventional cages (groups C and D), free-range (group E), and backyard cages (group B) samples. On the first hand, a complete separation ($F(1,32) = 334.11$; $p < 0.00001$; LDA-*Er* = 0%) between organic and non-organic samples was achieved with only one variable, SumLNi (sum of intensities of the 5 deconvoluted peaks used to fit methyl protons of linolenic acid signal). TAG in organic samples were found to be higher in linoleic acid (Fig. 2.a). On the other hand, samples from non-organic farming systems (*i.e.*, conventional cages, free-range, and backyard cages) were discriminated using 9 predictors with LDA-

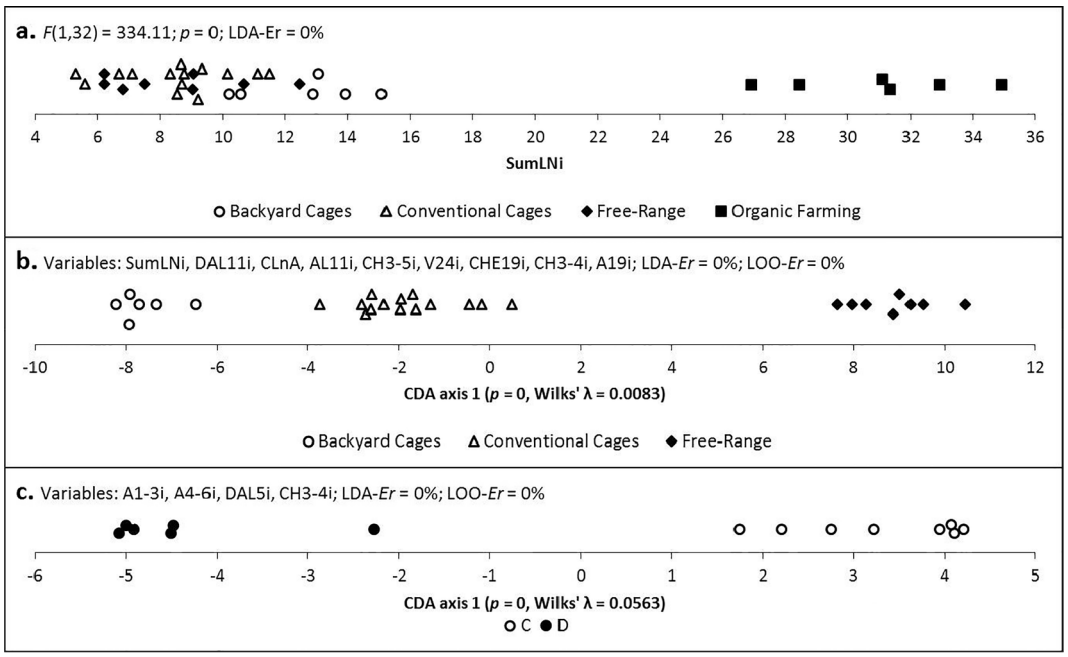

Fig. 2. Discrimination of organic and non-organic eggs (a); classification of non-organic eggs according to the hens farming system (b); and classification of eggs laid by hens raised in conventional cages according to their origin (c). $p = 0$, given by one-way ANOVA, means that it is less than 0.0001. The error rates given by Linear Discriminant Analysis (LDA-*Er*) and Leave-One-Out cross-validation test (LOO-*Er*) were used as classifier performance and robustness indicators, respectively. Variables used in classifications are reported in decreasing order of importance based on their Wilks' λ value. See Supplemental Table S-3 for peak labels.

Er and LOO-*Er* both equal to 0% (Fig. 2.b). In addition, eggs laid by hens raised in conventional cages were perfectly classified according to their origin (group C or D) using a CDA model based on 4 variables (LDA-*Er* and LOO-*Er* of 0%) (Fig. 2.c). We mention herein that Partial Least Squares (PLS) based discriminant analyses (PLS-DA and PLS-LDA) afforded less robust classification models.

3.3. Classification of samples according to the hens' breed

Classification of samples according to the hens' breed was also investigated. In order to exclude extrinsic factors that may affect eggs composition, we considered egg samples from the same origin but from different hen breeds. Thus, we considered samples of groups C and D separately, both corresponding to two hybrid hen breeds (mainly crosses of the purebred Rhode Island Red and White). The two different hybrid breeds laid eggs of different eggshell colors (brown and white). A single variable (A18i, Supplemental Fig. S-1.d and Table S-3.a) allowed the discrimination between samples of these breeds within each group (Supplemental Fig. S-3). A18i is the intensity of a deconvoluted peak in the aliphatic region of the spectra. It probably corresponds to saturated fatty acids (mainly palmitic and stearic) since their percentages within egg yolk TAG were higher in white eggs from both groups, as given by GC results (Table 1). Since both eggs of groups C and D were laid by hens raised in conventional cages, they were combined and regrouped as white and brown eggs. Also in this case, as shown in Fig. 3.a, variable A18i perfectly classified samples according to the eggshell color ($F(1,12) = 75.57$; $p < 0.00001$). Given that A18i afforded much better classification than did palmitic (P), stearic (S), and saturated fatty acids (SFA), it is most likely that this peak is correlated to the distribution of saturated fatty acids on the *sn*-1,3 and *sn*-2 positions of the glycerol moiety in triacylglycerols. Furthermore, we considered samples of group E corresponding to four hens of different breeds (designated with respect to the eggshell color as indicated in Fig. 3.b). Samples were collected over 2 days (2 eggs from each bird). As shown in Fig. 3.b, variable SumLNi (representing linolenic acid) allowed a perfect discrimination of samples according to the hen breed within this group ($F(3,4) = 24.02$; $p = 0.0051$). No other NMR variable or fatty acid percentage was able to afford such separation between these samples. It is noteworthy that such separation in this case was not necessarily due to the hen breeds. This could be due to intrinsic or extrinsic factors differentiating birds, even those of the same breed.

Table 1
Mass percentages of Palmitic (P), Stearic (S), and total Saturated Fatty Acids (SFA) determined by GC within egg yolk triacylglycerols.

Group	P %		S %		SFA %	
	White	Brown	White	Brown	White	Brown
C	27.43	26.24	6.54	3.10	34.40	29.79
	27.20	25.70	5.53	3.82	33.19	30.01
	27.87	24.51	5.99	4.78	34.35	29.82
	25.51	25.80	7.08	4.49	33.06	30.76
D	30.50	25.55	5.10	3.96	36.11	30.01
	31.13	25.50	4.26	3.78	35.91	29.90
	31.67	27.24	5.23	5.12	37.50	32.91
Mean	**28.76**	**25.79**	**5.68**	**4.15**	**34.93**	**30.46**

3.4. Quantitation of individual fatty acids within egg yolk TAG using ^{1}H NMR

GC was used as a reference analytical method for the quantitation of fatty acids within egg yolk TAG Supplemental Table S-2. It allowed to quantitate the following fatty acids listed in decreasing order of their mass percentages: oleic, palmitic, linoleic, stearic, vaccenic, palmitoleic, hypogeic, myristic, linolenic, gondoic, and margaric acids. Relative mass percentages of fatty acids thus determined were used as dependent variables (targets) in the construction of PLSR models for their prediction with deconvoluted peak areas and intensities from ^{1}H NMR spectra (Table S-2) as input variables. This approach allowed to construct quantitation models for oleic (O), stearic (S), vaccenic (V), palmitoleic (Po), hypogeic (H), and myristic (My) acids (Eqs. (2)–(7) in Supplemental Table S-6). Parameters corresponding to the performance and the robustness of each model are shown in Fig. 4.

Following the same approach, we did not reach a robust model for

Fig. 3. Classification of egg samples according to the hens' breed. Variable A18i was the breed predictor for samples of groups C and D (a). Within group F, samples were discriminated using SumLNi (b). $p = 0$, given by one-way ANOVA, means that it is less than 0.0001. The error rate given by Linear Discriminant Analysis (LDA-*Er*) was used as classifier performance indicator. See Supplemental Table S-3 for peak labels.

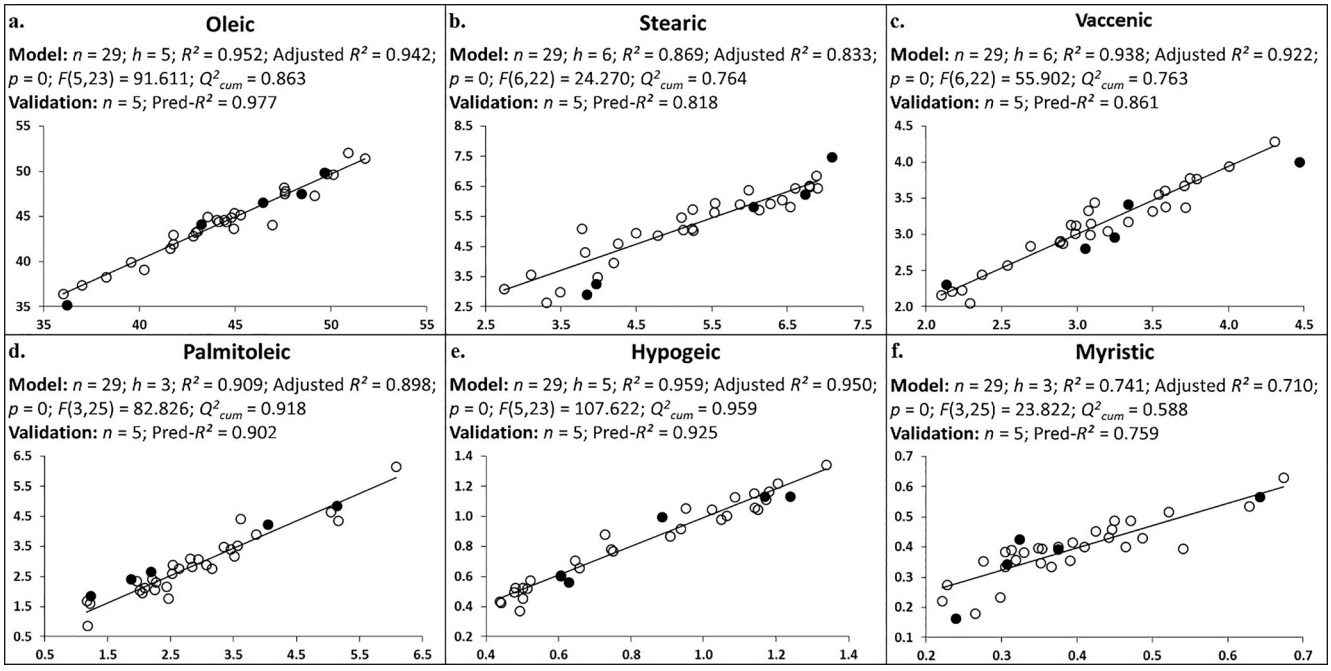

Fig. 4. Correlations between fatty acid percentages obtained by GC (x-axis) and those predicted using the high-resolution ^{1}H NMR method (y-axis). ◦ Training samples, • test samples for model validation, $p = 0$ means that it is less than 0.00001.

the prediction of palmitic acid (P) percentage within yolk TAG. However, since palmitic and stearic acids were the most abundant Saturated Fatty Acids (SFA) in this matrix, and aiming for a better prediction of palmitic acid, a model for the quantitation of SFA was built instead (Eq. (8) in Supplemental Table S-6 & Supplemental Fig. S-4). Then, mass percentage of palmitic acid was calculated by subtracting the predicted mass percentage of stearic acid from that of SFA. Predicted values of palmitic acid were highly correlated with values obtained by GC (training samples: $n = 29$, $R^2 = 0.921$, $p < 0.00001$; test samples: $n = 5$, $R^2 = 0.906$, $p = 0.0127$).

Moreover, linoleic acid (L) was directly quantitated using variable AL3i at 2.037 ppm (Supplemental Table S-3.a and Fig. S-1.e). The same variable was used for quantitation of L in olive oil (Hajjar, Merchak, Daniel, Rizk, Akoka, & Bejjani, 2020). This variable, obtained with a reproducibility of 0.37%, highly correlated with L over the 34 samples considered in the present study ($L = 0.437 \times AL3i - 1.707$, $n = 34$, $R^2 = 0.998$, $p < 0.00001$). Similarly, linolenic acid (Ln) was quantitated using the sum of intensities of its methyl protons ($Ln = 0.039 \times SumLNi - 0.143$, $n = 33$, $R^2 = 0.986$, $p < 0.00001$). In this case, a sample was excluded from the population since its Ln content was undetectable by GC.

4. Conclusion

A reliable and fast ^{1}H NMR-based metabolomics was conducted for the authentication of hen eggs as a model food matrix of animal origin. Reference lineshape adjustment of resulting spectra followed by their deconvolution allowed discovering efficient variables for discrimination of samples according to their origin. Such predictors can be used to detect food fraud. The presence of conjugated acids in specific samples was closely related to the hens rearing system and feed composition. Almost all fatty acids were quantitated using variables from deconvoluted ^{1}H NMR spectra of triacylglycerols. Therefore, this approach can be applied for authentication of foodstuffs of animal and vegetable origins due to the ubiquitous occurrence of triacylglycerols. More precisely, dairy products authentication using this mean can be interesting owing to their particular fatty acid profile comprising butyric, caproleic, and conjugated linoleic acids.

CRediT authorship contribution statement

Ghina Hajjar: Investigation, Data curation, Formal analysis, Writing - original draft. **Lenny Haddad:** Investigation, Formal analysis. **Toufic Rizk:** Project administration, Funding acquisition. **Serge Akoka:** Conceptualization, Supervision, Resources, Writing - review & editing, Funding acquisition. **Joseph Bejjani:** Conceptualization, Methodology, Validation, Supervision, Resources, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130056.

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