NMR-based and chemometric approaches applicable to adulteration studies for assessment of the botanical origin of edible oils

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Abstract The health benefits of edible oils, especially promoted by $\omega 3$ and $\omega 9$ fatty acids, have been associated with their botanical origin. In order to investigate fatty acid profiles, we assessed Brazil nut, chia, linseed, sesame (toasted and raw), and soybean oils by ^1H nuclear magnetic resonance (^1H NMR) and chemometrics. PCA plots revealed important relationships between chemical composition and botanical origin for reference and commercial samples. Strong evidence of commercial Brazil nut oil adulteration was confirmed using a spiking procedure. Our findings show that NMR and chemometrics are successful tools for correlating fatty acid profile with botanical origin, which can be suitable for detecting sample adulteration.

Keywords Fatty acids · Edible oils · Quality control · NMR · Chemometric analysis

Introduction

Edible vegetable oils are considered an important source of essential nutrients such as mono- and polyunsaturated fatty acids. The health benefits of a rich and balanced unsaturated oil intake, especially promoted by $\omega 3$ and $\omega 9$ fatty acids (Hammad et al. 2016; Jampolis et al. 2016; Kim et al.

2014; Lands 2014), have led the food industry to associate the botanical origin of high aggregate value edible oils with their chemical composition.

Edible vegetable oils consist mainly of fatty acids such as oleic, linoleic, linolenic, myristic, palmitic, and stearic acids, esterified to a glycerol unit (Barison et al. 2010; Somerville et al. 2001). There is a significant differentiation between the proportions of these majority fatty acids among different plants (Castejón et al. 2014; Dubois et al. 2007; Ryan et al. 2006). Thus, assessment of the fatty acid profile can be useful to determine the authenticity of edible vegetable oils (Troya et al. 2015).

The chemical profile of fatty acids is usually assessed by chromatography, ultraviolet–visible spectrophotometry, Fourier-transform infrared spectroscopy, fluorimetry, and mass spectrometry, among other techniques (Santana et al. 2016). However, nuclear magnetic resonance (NMR) has been established as a powerful technique for evaluation of fatty acids because it mainly involves direct analysis of the samples, avoiding toxic organic solvents and long preparation times (Castejón et al. 2014). NMR can provide information about purity, quantitative analysis, geographic origin of samples, degree of degradation, structural elucidation, chemical profile evaluation, and detection of sample adulteration within a short period of time (Alonso-Salces et al. 2011; Barison et al. 2010; Fang et al. 2013; Longobardi et al. 2012; Popescu et al. 2015; Siciliano et al. 2013). Moreover, chemometric methods have been used for NMR data reduction, achieving better system visualization and emphasizing the differences and similarities of samples.

Hence, edible vegetable oils of different botanical origin, such as Brazil nut (highest price), chia, linseed, sesame, and soybean (lowest price), were evaluated by ^1H NMR and chemometric analysis in order to compare their

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chemical profiles and investigate conformity in commercial samples.

Materials and methods

Samples

Commercial edible oils were compared with those extracted by us from in natura seeds (named as reference samples). Brazil nut, chia, linseed, sesame (toasted and raw), and soybean commercial edible oils were purchased from the local market.

The extraction of reference samples was performed according to a method slightly adapted from that described by Miraliakbari and Shahidi 2008. Around 30 g of each type of seed was powdered and subjected to 24-h extraction by percolation with approximately 40 mL of n-hexane. The percolate was collected, and the extraction was repeated three times. The extracts were combined, filtered (14- μ m paper filter), and submitted to liquid–liquid extraction with 50 mL of water in order to remove the natural water contained in the seeds. After solvent removal under reduced pressure, each extracted reference oil was centrifuged

(1120 g, 5 min), and the supernatant was kept in amber glass under refrigeration.

Four different brands of commercial edible oils were applied in this study, with the exception of Brazil nut oil, for which two brands were investigated. One Brazil nut oil brand was inspected through acquisition of two more batches.

In order to check for possible adulteration, a reference sample of Brazil nut oil was spiked with 25%, 50% and 75% (v/v) soybean oil.

NMR

The samples were prepared using 300 μ L of oil and 200 μ L of deuterated chloroform (CDCl_3 , D 99.8%) with 0.03% v/v tetramethylsilane (TMS). Five replicates from each brand/reference and type of oil were analyzed. ^1H NMR spectra were acquired at 20 °C on a Bruker DPX300 NMR spectrometer operating at 7.05 T equipped with a 5-mm dual channel (^1H and ^{13}C) direct detection probe, observing ^1H at 300.13 MHz. Spectra were obtained applying a 90° excitation pulse with 64 scans, collecting 64 k data points covering a spectral width of 16 ppm and a relaxation delay of 1 s. Spectra were processed by applying an exponential

Fig. 1 Representative ^1H NMR spectral profile of edible vegetable oil (linseed oil) and assignments (color figure online)

multiplication to free induction decays (FID) by a factor of 0.3 Hz. Phases and baselines were manually corrected, and all spectra were referenced to the TMS signal at 0.00 ppm.

Multivariate data analysis

The spectra were binned into equal buckets of 0.03 ppm by AMIX software. The special integration mode was applied, while the spectral scaling was set to the biggest bucket. Spectral ranges were 6.5–0.5 ppm, excluding 4.9–4.75 and 3.5–3.08 ppm to minimize the effect of noise.

Principal component analysis (PCA) was used as the unsupervised multivariate method to perform data reduction and observe the general model overview and possible trends (Beebe et al. 1998; Kortensniemi et al. 2015; Miller and Miller 2010).

Results and discussion

NMR analyses were quickly performed with minimal pretreatment and complete sample recovery. All NMR assignments for fatty acids are well described in the literature (Barison et al. 2010; Castejón et al. 2014) and are illustrated in Fig. 1.

The PCA score performed for reference samples showed chia and linseed oils on the negative PC1 axis, with 86.3% of explained variance, distant from sesame (both toasted and raw), soybean, and Brazil nut reference oils on the positive PC1 axis. However, Brazil nut oil was distinguished from sesame and soybean oils on the more positive PC2 axis (Fig. 2).

The buckets at 0.97 and 0.94 ppm were the most important loadings responsible for allocating chia and linseed oils to negative PC1 scores (Fig. 2), corresponding to the methyl group of the ω 3 (0.97 ppm, triplet) (Figure S1 – Electronic Supplementary Material). ^1H NMR spectra for chia and linseed reference samples also revealed a deshielding effect in the allylic (2.10–1.95 ppm) and bis-allylic hydrogens (2.9–2.7 ppm) and olefinic/ β -glyceril hydrogens (5.4–5.2 ppm) (Figure S1 – Electronic Supplementary Material), due to the higher content of double bonds in ω 3. According to the literature, chia and linseed oils have a high content of linolenic acid (approximately 50%) (Dubois et al. 2007), corroborating the loadings highlighted on the negative PC1 axis, which can be attributed to this fatty acid.

The other reference samples (soybean, Brazil nut, and sesame) were allocated to the positive PC1 axis due to the importance of loadings at 0.89, 1.25 and 1.27 ppm (Fig. 2), which can be associated with common signals for all types of fatty acids. On the positive PC2 axis, Brazil nut oil was distinguished from sesame and soybean oils due to a higher

Fig. 2 PCA score and loading plots for ^1H NMR spectra of reference oils (color figure online)

content of saturated fatty acids, as indicated by the loading at 1.25 and 1.27 ppm. Sesame (both toasted and raw) and soybean oils were allocated on the negative PC2 axis since they have a lower content of saturated and ω 3 fatty acids. Toasted and raw sesame oils were not distinguished in this PCA, indicating a high similarity of the fatty acids, even when seeds underwent a toasting treatment before oil extraction (Fig. 2).

When commercial oils were included in the sample set, all four brands of chia, linseed, sesame, and soybean oils were correctly grouped near their reference samples (Fig. 3). In contrast, the Brazil nut samples were the most dispersed group, with only one commercial sample near the reference, while the others showed an anomalous behavior, close to sesame and soybean groups.

It is well known that cheap oils, such as soybean oil, are commonly employed as adulterants (Troia et al. 2015). Considering the atypical behavior of some commercial samples of Brazil nut oil, its reference oil was spiked with

Fig. 3 PCA score and loading plots for ^1H NMR spectra of commercial and reference oils. *Arrow and ellipse indicate adulterated Brazil nut commercial oils overlapped with soybean commercial oil (color figure online)

different amounts of soybean oil, and this new data set was analyzed by PCA (Fig. 4).

In Fig. 4, it can be seen that the samples spiked with different amounts of soybean oil were allocated to different regions in the score plots. As expected, the high percentage of soybean oil displaced them towards soybean oils. Accordingly, the anomalous commercial samples were grouped closer to the soybean oil samples, even closer than the samples spiked with 75% soybean oil, which was the highest concentration analyzed. Therefore, our results showed that the anomalous commercial Brazil nut oils were probably adulterated with 100% soybean oil because of the same value of PC1.

These findings pointed out fraudulent behavior in all batches of one of the brands of Brazil nut oil evaluated. However, this same brand commercializes conforming sesame oil, indicating the importance of chemical analysis

Fig. 4 PCA score and loading PCA plots for ^1H NMR spectra of Brazil nut samples with addition of 25%, 50%, and 75% soybean oil (color figure online)

coupled to reference samples to corroborate detection of the adulteration of botanical origin.

Conclusion

Detecting strong evidence of adulteration with soybean oil in edible oil composition is a clear example that NMR and chemometrics are suitable tools for assessing the chemical properties of edible oils with high aggregate value. Moreover, samples can be investigated in a short timeframe, with minimum pretreatment and complete sample recovery. The ^1H NMR chemical profiles of vegetable edible oils were clearly differentiated by PCA depending on their botanical origin and chemical composition, which can also be applied to detect anomalous behavior and adulteration cases.

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References

- Alonso-Salces RM, Holland MV, Guillou C (2011) ^1H -NMR fingerprinting to evaluate the stability of olive oil. *Food Control* 22:2041–2046
- Barison A, Silva CWP, Campos FR, Simonelli F, Lenz CA, Ferreira AG (2010) A simple methodology for the determinations of fatty acid composition in edible oils through ^1H NMR spectroscopy. *Magn Reson Chem* 48:642–650
- Beebe KR, Pell RJ, Seasholtz MB (1998) *Chemometrics: a practical guide*. Wiley, New York
- Castejón D, Mateos-Aparicio I, Molero MD, Cambero MI, Herrera A (2014) Evaluation and optimization of the analysis of fatty acid types in edible oils by ^1H NMR. *Food Anal Methods* 7:1285–1297
- Dubois V, Breton S, Linder M, Fanni J, Parmentier M (2007) Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *Eur J Lipid Sci Technol* 109:710–732
- Fang G, Goh JY, Tay M, Lau HF, Li SFY (2013) Characterization of oils and fats by ^1H NMR and CG/MS fingerprinting: classification, prediction and detection of adulteration. *Food Chem* 138:1461–1469
- Hammad S, Pu S, Jones PJ (2016) Current evidence supporting the link between dietary fatty acids and cardiovascular disease. *Lipids* 51:507–517
- Jampolis MB, Rothkopf MM, Li Z, Diamond SJ, Allen K, Abdelhadi RA, Kahana DD, McClave SA (2016) Principles of healthful eating. *Curr Nutr Rep* 5:180–190
- Kim K, Nam YA, Kim HS, Hayes W, Lee B (2014) α -Linolenic acid: nutraceutical, pharmacological and toxicological evaluations. *Food Chem Toxicol* 70:163–178
- Kortesniemi M, Vourien AL, Sinkkonen J, Yang B, Rajala A, Kallio H (2015) NMR metabolomics of ripened and developing oilseed rape (*Brassica napus*) and turnip rape (*Brassica rapa*). *Food Chem* 172:63–70
- Lands B (2014) Historical perspectives on the impact of n-3 and n-6 nutrients on health. *Prog Lipid Res* 55:17–29
- Longobardi F, Ventrella A, Napoli C, Humpfer E, Schütz B, Schäfer H, Kontominas MG, Sacco A (2012) Classification of olive oils according to geographical origin using ^1H NMR fingerprinting combined with multivariate analysis. *Food Chem* 130:177–183
- Miller JN, Miller JC (2010) *Multivariate analysis. Statistic and chemometrics for analytical chemistry*, 6th edn. Pearson, Gosport, pp 221–250
- Miraliakbari H, Shahidi F (2008) Antioxidant activity of minor components of three nut oils. *Food Chem* 111:421–427
- Popescu R, Costinel D, Dinca OR, Marinescu A, Stefanescu I, Ionete RE (2015) Discrimination of vegetable oils using NMR spectroscopy and chemometrics. *Food Control* 48:84–90
- Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM (2006) Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts. *Int J Food Sci Nutr* 57:219–228
- Santana FB, Gontijo LC, Mitsutake H, Mazivila S Jr, Souza LM, Borges-Neto W (2016) Non-destructive fraud detection in rosehip oil MIR spectroscopy and chemometrics. *Food Chem* 209:228–233
- Siciliano C, Belsito E, De Marco R, Di Gioia ML, Leggio A, Liguori A (2013) Quantitative determination of fatty acid chain composition in pork meat products by high resolution ^1H NMR spectroscopy. *Food Chem* 136:546–554
- Somerville C, Browse J, Jaworski JG, Ohlrogge JB (2001) *Lipids*. In: Buchanan BB, Gruissem W, Jones RL (eds) *Biochemistry & molecular biology of plants*, 1st edn. American Society of Plant Physiologist, Rockville, pp 456–527
- Troya F, Lerma-García MJ, Herrero-Martínez JM, Simó-Alfonso EF (2015) Classification of vegetable oils according to their botanical origin using n-alkane profiles established by CG-MS. *Food Chem* 167:36–39